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(71) Applicant: **WEINER, David** [US/US]; 717 Beacom Lane, Merion, PA 19066 (US).

(72) Inventors; and
(71) Applicants: **PATEL, Ami** [US/US]; 1 Rainey Court, Philadelphia, PA 19103 (US). **YAN, Jian** [US/US]; 503 Sheffield Drive, Wallingford, PA 19086 (US).

(74) Agent: **NGUYEN, Quang, D.** et al.; Riverside Law, LLP, Glenhardie Corporate Center, 1285 Drummers Lane, Suite 202, Wayne, PA 19087 (US).

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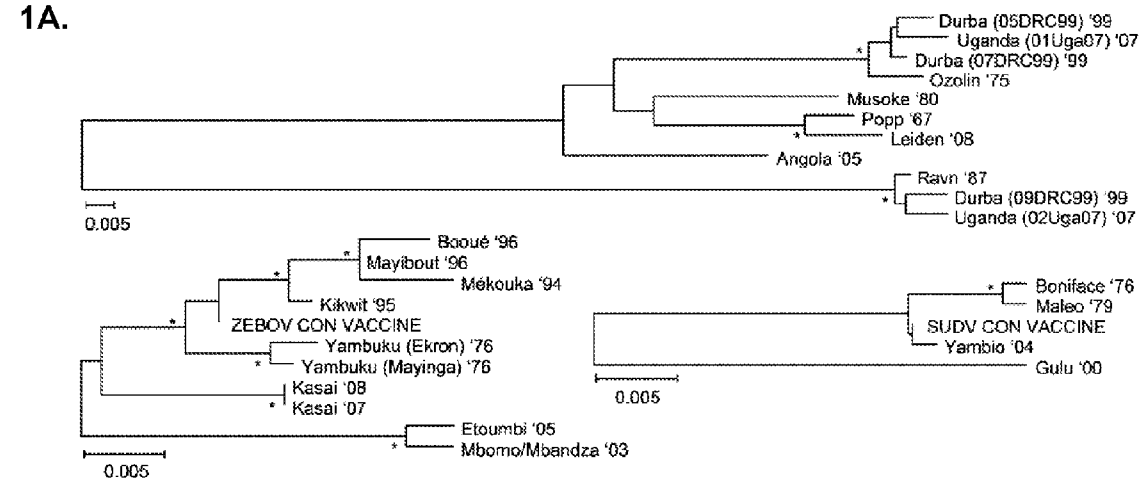
(54) **Title:** FILOVIRUS CONSENSUS ANTIGENS, NUCLEIC ACID CONSTRUCTS AND VACCIENS MADE THEREFROM, AND METHODS OF USING SAME



(57) **Abstract:** Nucleic acid molecules and compositions comprising one or more nucleic acid sequences that encode a consensus Ebolavirus glycoproteinimmunogens are disclosed. The coding sequences optionally include operable linked coding sequence that encode a signal peptide. Immunomodulatory methods and methods of inducing an immune response against Ebolavirus are disclosed. Method of preventing Ebolavirus and methods of treating individuals infected with Ebolavirus are disclosed. Consensus Ebolavirus proteins are disclosed.

[Continued on next page]

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FILOVIRUS CONSENSUS ANTIGENS, NUCLEIC ACID CONSTRUCTS AND VACCINES MADE THEREFROM, AND METHODS OF USING SAME

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 62/332,372, filed May 5, 2016, U.S. Provisional Application No. 62/402,519, filed September 30, 2016, and U.S. Provisional Application No 62/483,979, filed April 11, 2017, each of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to vaccines for inducing immune responses and preventing filovirus infection and/or treating individuals infected with filovirus, particularly infection by Ebolavirus. The present invention relates to consensus Ebolavirus proteins and nucleic acid molecules which encode the same.

BACKGROUND OF THE INVENTION

The *Filoviridae* are non-segmented, single stranded RNA viruses which contain two divergent genera, *Marburgvirus* (MARV) and *Ebolavirus* (EBOV). Members from each can cause severe and highly lethal hemorrhagic fever disease to which there is no cure or licensed vaccine (Bradfute S.B., et al. (2011) Filovirus vaccines. Hum Vaccin 7: 701-711; Falzarano D., et al. (2011) Progress in filovirus vaccine development: evaluating the potential for clinical use. Expert Rev Vaccines 10: 63-77; Fields B.N., et al. (2007) Fields' virology. Philadelphia: Lippincott Williams & Wilkins. 2 v. (xix, 3091, I-3086 p.); Richardson J.S., et al. (2009) Enhanced protection against Ebola virus mediated by an improved adenovirus-based vaccine. PLoS One 4: e5308; and Towner J.S., et al. (2006) Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. J Virol 80: 6497-6516).

Due to lethality rates of up to 90% they have been described as "one of the most virulent viral diseases known to man" by the World Health Organization. The US Centers for Disease Control and Prevention has classified them as 'Category A Bioterrorism Agents' due in part to their potential threat to national security if weaponized (Burki T.K. (2011) USA focuses on Ebola vaccine but research gaps remain. Lancet 378: 389). These 'high priority' agents could in

theory be easily transmitted, result in high mortality rates, cause major public health impact and panic, and require special action for public health preparedness (CDC (2011) Bioterrorism Agents/Diseases. Atlanta: Centers for Disease Control and Prevention).

The haemorrhagic fever diseases are acute infectious with no carrier state, although they are easily transmissible among humans and nonhuman primates by direct contact with contaminated bodily fluids, blood, and tissue (Feldmann H., et al. (2003) Ebola virus: from discovery to vaccine. Nat Rev Immunol 3: 677-685). During outbreak situations, reuse of medical equipment, health care facilities with limited resources, and untimely application of prevention measures escalate transmission of the disease, allowing amplification of infections in medical settings.

Since the natural reservoirs of these zoonotic pathogens are likely to be African bats and pigs (Kobinger G.P., et al. (2011) Replication, pathogenicity, shedding, and transmission of Zaire ebolavirus in pigs. J Infect Dis 204: 200-208), the latter possibly being more of an amplifying host, the manner in which the virus first appears at the start of an outbreak is thought to occur through human contact with an infected animal. Unpredictable endemic surfacing in the Philippines, potentially Europe, and primarily Africa of this disease further constitutes a major public health concern (Outbreak news. (2009) Ebola Reston in pigs and humans, Philippines. Wkly Epidemiol Rec 84: 49-50).

Experiments have been performed to determine the capacity of the vaccine for inducing protective efficacy and broad CTL including experiments in rodent preclinical studies. (Fenimore PW, *et al.* (2012). Designing and testing broadly-protective filoviral vaccines optimized for cytotoxic T-lymphocyte epitope coverage. *PLoS ONE* 7: e44769; Hensley LE, *et al.* (2010). Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebolavirus Species. *PLoS Pathog* **6**: el000904; Zahn R, *et al* (2012). Ad35 and ad26 vaccine vectors induce potent and cross-reactive antibody and T-cell responses to multiple filovirus species. *PLoS ONE* 7: e44115; Geisbert TW, Feldmann H (2011). Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. *J Infect Dis* **204 Suppl 3:** S1075-1081; and Grant-Klein RJ, Van Deusen NM, Badger CV, Hannaman D, Dupuy LC, Schmaljohn CS (2012). A multiagent filovirus DNA vaccine delivered by intramuscular electroporation completely protects mice from ebola and Marburg virus challenge. *Hum Vaccin Immunother* **8;** Grant-Klein

RJ, Altamura LA, Schmaljohn CS (2011). Progress in recombinant DNA-derived vaccines for Lassa virus and filoviruses. *Virus Res* **162:** 148-161**)**.

Vaccine-induced adaptive immune responses have been described in numerous preclinical animal models (Blaney JE, *et al.* (2011). Inactivated or live-attenuated bivalent vaccines that confer protection against rabies and Ebola viruses. *J Virol* **85**: 10605-10616; Dowling W, *et al.* (2007). Influences of glycosylation on antigenicity, immunogenicity, and protective efficacy of ebola virus GP DNA vaccines. *J Virol* **81**: 1821-1837; Jones SM, *et al.* (2005). Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med* **11:** 786-790; Kalina WV, Warfield KL, Olinger GG, Bavari S (2009). Discovery of common marburgvirus protective epitopes in a BALB/c mouse model. *Virol J 6:* 132; Kobinger GP, *et al.* (2006). Chimpanzee adenovirus vaccine protects against Zaire Ebola virus. *Virology* **346:** 394-401; Olinger GG, *et al.* (2005). Protective cytotoxic T-cell responses induced by Venezuelan equine encephalitis virus replicons expressing Ebola virus proteins. *J Virol* **79**: 14189-14196; Rao M, Bray M, Alving CR, Jahrling P, Matyas GR (2002). Induction of immune responses in mice and monkeys to Ebola virus after immunization with liposome-encapsulated irradiated Ebola virus: protection in mice requires CD4(+) T cells. *J Virol* **76**: 9176-9185; Rao M, Matyas GR, Grieder F, Anderson K, Jahrling PB, Alving CR (1999). Cytotoxic T lymphocytes to Ebola Zaire virus are induced in mice by immunization with liposomes containing lipid A. *Vaccine* **17**: 2991-2998; Richardson JS, *et al.* (2009). Enhanced protection against Ebola virus mediated by an improved adenovirus-based vaccine. *PLoS One* **4**: e5308; Vanderzanden L, *et al* (1998). DNA vaccines expressing either the GP or NP genes of Ebola virus protect mice from lethal challenge. *Virology* **246:** 134-144; Warfield KL, *et al.* (2005). Induction of humoral and CD8+ T cell responses are required for protection against lethal Ebola virus infection. *J Immunol* **175:** 1184-1191; Jones SM, *et al.* (2007). Assessment of a vesicular stomatitis virus-based vaccine by use of the mouse model of Ebola virus hemorrhagic fever. *J Infect Dis* **196 Suppl2:** S404-412 Grant-Klein RJ, Van Deusen NM, Badger CV, Hannaman D, Dupuy LC, Schmaljohn CS (2012). A multiagent filovirus DNA vaccine delivered by intramuscular electroporation completely protects mice from ebola and Marburg virus challenge. *Hum Vaccin Immunother* **8**.; Geisbert TW, *et al.* (2010). Vector choice determines immunogenicity and potency of genetic vaccines against Angola Marburg virus in nonhuman primates. *J Virol* **84**: 10386-10394.) Viral vaccines have shown promise and include mainly the recombinant adenoviruses and vesicular stomatitis viruses. Non-infectious

strategies such as recombinant DNA and Ag-coupled virus-like particle (VLP) vaccines have also demonstrated levels of preclinical efficacy and are generally considered to be safer than virus-based platforms. Virus-specific Abs, when applied passively, can be protective when applied either before or immediately after infection (Gupta M, Mahanty S, Bray M, Ahmed R, Rollin PE (2001). Passive transfer of antibodies protects immunocompetent and immunodeficient mice against lethal Ebola virus infection without complete inhibition of viral replication. *J Virol* **75:** 4649-4654; Marzi A, *et al.* (2012). Protective efficacy of neutralizing monoclonal antibodies in a nonhuman primate model of Ebola hemorrhagic fever. *PLoS ONE* 7: e36192; Parren PW, Geisbert TW, Maruyama T, Jahrling PB, Burton DR (2002). Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. *J Virol* **76:** 6408-6412; Qiu X, *et al.* (2012). Ebola GP-Specific Monoclonal Antibodies Protect Mice and Guinea Pigs from Lethal Ebola Virus Infection. *PLoSNegl Trop Dis* **6**: el575; Wilson JA, *et al.* (2000). Epitopes involved in antibody-mediated protection from Ebola virus. *Science* **287**: 1664-1666; Sullivan NJ, *et al.* (2011). CD8(+) cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. *Nat Med* **17**: 1128-1131; Bradfute SB, Warfield KL, Bavari S (2008). Functional CD8+ T cell responses in lethal Ebola virus infection. *J Immunol* **180:** 4058-4066; Warfield KL, Olinger GG (2011). Protective role of cytotoxic T lymphocytes in filovirus hemorrhagic fever. *J Biomed Biotechnol* **2011:** 984241). T cells have also been shown to provide protection based on studies performed in knockout mice, depletion studies in NHPs, and murine adoptive transfer studies where efficacy was greatly associated with the lytic function of adoptively-transferred CD8+ T cells. However, little detailed analysis of this response as driven by a protective vaccine has been reported.

Countermeasure development will ultimately require an improved understanding of protective immune correlates and how they are modulated during infection. This proves difficult when infected individuals who succumb to filoviral disease fail to mount an early immune response. These fast-moving hemorrhagic fever diseases result in immune dysregulation, as demonstrated by the lack of a virus-specific Ab response and a great reduction in gross T cell numbers, leading to uncontrolled viral replication and multi-organ infection and failure. Conversely, survivors of Ebola virus (EBOV) disease exhibit an early and transient IgM response, which is quickly followed by increasing levels of virus-specific IgG and CTL. These observations suggest that humoral and cell-mediated immune responses play a role in conferring

protection against disease. These data are also supported by numerous preclinical efficacy studies demonstrating the contribution of vaccine-induced adaptive immunity to protection against lethal challenge. However, mounting evidence has demonstrated a critical role for T cells in providing protection where efficacy was greatly associated with the functional phenotype of CD8+ T cells. While these recent studies highlight the importance of T cells in providing protection, their precise contributions remain uncharacterized and controversial. Furthermore, little detailed analysis of this response driven by a protective vaccine has been reported.

SUMMARY OF THE INVENTION

Isolated nucleic acid molecues comprising on or more nucleic acid sequences encoding a a first consensus Zaire ebolavirus envelope glycoprotein immunogen (ZEBOVCON), a nucleic acid encoding a second consensus Zaire ebolavirus envelope glycoprotein immunogen (ZEBOVCON2), or a nucleic acid encoding a ZEBOV Guinea 2014 Outbreak envelope glycoprotein immunogen (ZEBOVGUI) are provided.

In one embodiment, the the ZEBOVCON comprises an amino acid sequence that is at least 95% homologous to SEQ ID NO:1, a fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:1, an amino acid sequence that is at least 99% homologous to SEQ ID NO:1, a fragment of an amino acid sequence that is at least 99% homologous to SEQ ID NO:1, an amino acid sequence of SEQ ID NO:1, or a fragment of SEQ ID NO:1.

In one embodiment, the ZEBOVCON2 comprises an amino acid sequence that is at least 95% homologous to SEQ ID NO:68, a fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:68, an amino acid sequence that is at least 99% homologous to SEQ ID NO: 68, a fragment of an amino acid sequence that is at least 99% homologous to SEQ ID NO: 68, an amino acid sequence of SEQ ID NO: 68, or a fragment of SEQ ID NO: 68.

In one embodiment, the ZEBOVGUI comprises an amino acid sequence that is at least 95% homologous to SEQ ID NO:67, a fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:67, an amino acid sequence that is at least 99% homologous to SEQ ID NO: 67, a fragment of an amino acid sequence that is at least 99% homologous to SEQ ID NO: 67, an amino acid sequence of SEQ ID NO: 67, or a fragment of SEQ ID NO: 67.

In some embodiments, the fragments comprise at least 600 amino acids, at least 630 amino acids, or at least 660 amino acids.

In one embodiment, ZEBOVCON is linked to an IgE signal peptide. In one embodiment, ZEBOVCON2 is linked to an IgE signal peptide. In one embodiment, ZEBOVGUI is linked to an IgE signal peptide.

In one embodiment, the nucleic acid encoding ZEBOVCON comprises a nucleic acid sequence at least 95% homologous SEQ ID NO:69, or a fragment thereof. In one embodiment, the nucleic acid encoding ZEBOVGUI comprises a nucleic acid sequence at least 95% homologous SEQ ID NO:72, or a fragment thereof. In one embodiment, the nucleic acid encoding ZEBOVCON2 comprises a nucleic acid sequence at least 95% homologous SEQ ID NO:70, or a fragment thereof.

In one embodiment, the nucleic acid encoding ZEBOVCON comprises a nucleic acid transcribed from a DNA sequence at least 95% homologous SEQ ID NO:69, or a fragment thereof. In one embodiment, the nucleic acid encoding ZEBOVGUI comprises a nucleic acid transcribed from a DNA sequence at least 95% homologous SEQ ID NO:72, or a fragment thereof. In one embodiment, the nucleic acid encoding ZEBOVCON2 comprises a nucleic acid transcribed from a DNA sequence at least 95% homologous SEQ ID NO:70, or a fragment thereof.

A composition comprising one or more nucleic acid sequence encoding one or more of ZEBOVCON, ZEBOVCON2 and ZEBOVGUI is also provided. In one embodiment, the composition comprises two or more nucleic acid sequence encoding two or more of ZEBOVCON, ZEBOVCON2 and ZEBOVGUI.In one embodiment, the composition comprises two nucleic acid molecules. In one embodiment, the composition comprises three or more nucleic acid sequence encoding ZEBOVCON, ZEBOVCON2 and ZEBOVGUI.In one embodiment, the composition comprises three nucleic acid molecules.

The invention also provides novel sequence for producing immunogens in mammailian cells or viral vectors.

A composition comprising a nucleic acid sequence that encodes a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a nucleic acid sequence that encodes a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, and a nucleic acid sequence that encodes a

Marburg marburgvirus Angola 2005 envelope glycoprotein immunogen is provided. The amino acid sequence of the consensus *Zaire ebolavirus* envelope glycoprotein immunogen may be SEQ ID NO:1 (ZEBOV CON), a fragment of SEQ ID NO:1, an amino acid sequence that is homologous to SEQ ID NO:1, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:1. Amino acid sequences that are homologous to SEQ ID NO:1 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:1. Fragments of SEQ ID NO:1 or fragments of amino acid sequences that are homologous to SEQ ID NO:1 are typically 600 or more, 630 or more, or 660 or more amino acids. The amino acid sequence of the consensus *Sudan ebolavirus* envelope glycoprotein immunogen may be SEQ ID NO:2 (SUDV CON), a fragment of SEQ ID NO:2, an amino acid sequence that is homologous to SEQ ID NO:2, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:2. Amino acid sequences that are homologous to SEQ ID NO:1 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:2. Fragments of SEQ ID NO:2 or fragments of amino acid sequences that are homologous to SEQ ID NO:2 are typically 600 or more, 630 or more, or 660 or more amino acids. The amino acid sequence of the *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen may be SEQ ID NO:3 (MARV ANG), a fragment of SEQ ID NO:3, an amino acid sequence that is homologous to SEQ ID NO:3, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:3. Amino acid sequences that are homologous to SEQ ID NO:3 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:3. Fragments of SEQ ID NO:3 or fragments of amino acid sequences that are homologous to SEQ ID NO:3 are typically 600 or more, 637 or more, or 670 or more amino acids. The amino acid sequence may optionally comprise a leader sequences such as the IgE leader.

A composition comprising a nucleic acid sequence that encodes a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a nucleic acid sequence that encodes a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a nucleic acid sequence that encodes a *Marburg marburgvirus* first consensus envelope glycoprotein immunogen, a nucleic acid sequence that encodes a *Marburg marburgvirus* second consensus envelope glycoprotein immunogen, and a nucleic acid sequence that encodes a *Marburg marburgvirus* third consensus envelope glycoprotein immunogen is also provided. The amino acid sequence of the consensus

Zaire ebolavirus envelope glycoprotein immunogen may be SEQ ID NO:1 (ZEBOV CON), a fragment of SEQ ID NO:1, an amino acid sequence that is homologous to SEQ ID NO:1, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:1. Amino acid sequences that are homologous to SEQ ID NO:1 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:1. Fragments of SEQ ID NO:1 or fragments of amino acid sequences that are homologous to SEQ ID NO:1 are typically 600 or more, 630 or more, or 660 or more amino acids. The amino acid sequence of the consensus *Sudan ebolavirus* envelope glycoprotein immunogen may be SEQ ID NO:2 (SUDV CON), a fragment of SEQ ID NO:2, an amino acid sequence that is homologous to SEQ ID NO:2, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:2. Amino acid sequences that are homologous to SEQ ID NO:1 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:2. Fragments of SEQ ID NO:2 or fragments of amino acid sequences that are homologous to SEQ ID NO:2 are typically 600 or more, 630 or more, or 660 or more amino acids. The amino acid sequence of the *Marburg marburgvirus* first consensus envelope glycoprotein immunogen may be SEQ ID NO:4 (MARV RAV), a fragment of SEQ ID NO:4, an amino acid sequence that is homologous to SEQ ID NO:4, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:4. Amino acid sequences that are homologous to SEQ ID NO:4 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:4. Fragments of SEQ ID NO:4 or fragments of amino acid sequences that are homologous to SEQ ID NO:4 are typically 600 or more, 637 or more, or 670 or more amino acids. The amino acid sequence of the *Marburg marburgvirus* second consensus envelope glycoprotein immunogen may be SEQ ID NO:5 (MARV OZO), a fragment of SEQ ID NO:5, an amino acid sequence that is homologous to SEQ ID NO:5, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:5. Amino acid sequences that are homologous to SEQ ID NO:5 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:4. Fragments of SEQ ID NO:5 or fragments of amino acid sequences that are homologous to SEQ ID NO:5 are typically 600 or more, 637 or more, or 670 or more amino acids. The amino acid sequence of the *Marburg marburgvirus* third consensus envelope glycoprotein immunogen may be SEQ ID NO:6 (MARV MUS), a fragment of SEQ ID NO:6, an amino acid sequence that is homologous

to SEQ ID NO:6, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:6. Amino acid sequences that are homologous to SEQ ID NO:6 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:6. Fragments of SEQ ID NO:6 or fragments of amino acid sequences that are homologous to SEQ ID NO:6 are typically 600 or more, 637 or more, or 670 or more amino acids. The amino acid sequence may optionally comprise a leader sequences such as the IgE leader. In some embodiments, the composition further comprises a nucleic acid sequence that encodes the *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen. The amino acid sequence of the *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen may be SEQ ID NO:3 (MARV ANG), a fragment of SEQ ID NO:3, an amino acid sequence that is homologous to SEQ ID NO:3, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:3. Amino acid sequences that are homologous to SEQ ID NO:3 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:3. Fragments of SEQ ID NO:3 or fragments of amino acid sequences that are homologous to SEQ ID NO:3 are typically 600 or more, 637 or more, or 670 or more amino acids. The amino acid sequence may optionally comprise a leader sequences such as the IgE leader.

Also provided is a composition comprising a nucleic acid sequence that encodes a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, and a nucleic acid sequence that encodes a consensus *Sudan ebolavirus* envelope glycoprotein immunogen.. The amino acid sequence of the consensus *Zaire ebolavirus* envelope glycoprotein immunogen may be SEQ ID NO:1 (ZEBOV CON), a fragment of SEQ ID NO:1, an amino acid sequence that is homologous to SEQ ID NO:1, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:1. Amino acid sequences that are homologous to SEQ ID NO:1 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:1. Fragments of SEQ ID NO:1 or fragments of amino acid sequences that are homologous to SEQ ID NO:1 are typically 600 or more, 630 or more, or 660 or more amino acids. The amino acid sequence of the consensus *Sudan ebolavirus* envelope glycoprotein immunogen may be SEQ ID NO:2 (SUDV CON), a fragment of SEQ ID NO:2, an amino acid sequence that is homologous to SEQ ID NO:2, or a fragment of an amino acid sequence that is homologous to SEQ ID NO:2. Amino acid sequences that are homologous to SEQ ID NO:1 are typically 95% or more, 96% or more, 97%

or more, 99% or more, or 99% or more, homologous to SEQ ID NO:2. Fragments of SEQ ID NO:2 or fragments of amino acid sequences that are homologous to SEQ ID NO:2 are typically 600 or more, 630 or more, or 660 or more amino acids. The amino acid sequence may optionally comprise a leader sequences such as the IgE leader.

A composition comprising a nucleic acid sequence that encodes a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a nucleic acid sequence that encodes a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, and a nucleic acid sequence that encodes a *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen is provided. The nucleic acid sequence that encodes a consensus *Zaire ebolavirus* envelope glycoprotein immunogen may be SEQ ID NO:64, a fragment of SEQ ID NO:64, a nucleic acid sequence that is homologous to SEQ ID NO:64, or a fragment of a nucleotide sequence that is homologous to SEQ ID NO:64. Nucleic acid sequences that are homologous to SEQ ID NO:64 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:64. Fragments of SEQ ID NO:64 or fragments of amino acid sequences that are homologous to SEQ ID NO:64 typically encode 600 or more, 630 or more, or 660 or more amino acids of the consensus *Zaire ebolavirus* envelope glycoprotein immunogen encoded by SEQ ID NO:64. The nucleic acid sequence that encodes a consensus *Sudan ebolavirus* envelope glycoprotein immunogen may be SEQ ID NO:65, a fragment of SEQ ID NO:65, a nucleic acid sequence that is homologous to SEQ ID NO:65, or a fragment of a nucleotide sequence that is homologous to SEQ ID NO:65. Nucleic acid sequences that are homologous to SEQ ID NO:65 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:65. Fragments of SEQ ID NO:65 or fragments of amino acid sequences that are homologous to SEQ ID NO:65 typically encode 600 or more, 630 or more, or 660 or more amino acids of the consensus *Sudan ebolavirus* envelope glycoprotein immunogen encoded by SEQ ID NO:65. The nucleic acid sequence that encodes *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen may be SEQ ID NO:66, a fragment of SEQ ID NO:66, a nucleic acid sequence that is homologous to SEQ ID NO:66, or a fragment of a nucleotide sequence that is homologous to SEQ ID NO:66. Nucleic acid sequences that are homologous to SEQ ID NO:66 are typically 95% or more, 96% or more, 97% or more, 99% or more, or 99% or more, homologous to SEQ ID NO:66. Fragments of SEQ ID NO:66 or fragments of amino acid sequences that are

homologous to SEQ ID NO:66 typically encode 600 or more, 630 or more, or 670 or more amino acids of the *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen encoded by SEQ ID NO:66. The nucleic acid sequences may optionally include sequences that encode leader sequences such as the IgE leader linked to the sequences encoding the immunogens.

Each of the different nucleic acid sequences may be on a single nucleic acid molecule, may each be on a separate nucleic acid molecules or various permutations. Nucleic acid molecules may be plasmids.

The composition may be formulated for delivery to an individual using electroporation.

The composition may further comprise nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

The composition may be used in methods of inducing an immune response against a filovirus. The filovirus may be selected from the group consisting of: Marburgvirus, Ebolavirus Sudan and Ebolavirus Zaire.

Methods of treating an individual who has been diagnosed with filovirus comprising administering a therapeutically effective amount of the composition to an individual are provided. The filovirus may be selected from the group consisting of: Marburgvirus, Ebolavirus Sudan and Ebolavirus Zaire.

Method of preventing filovirus infection in an individual are provided. The methods comprise administering a prophylactically effective amount of the composition to an individual. The filovirus may be selected from the group consisting of: Marburgvirus, Ebolavirus Sudan and Ebolavirus Zaire.

Compositions comprising two or more proteins selected from the group consisting of: a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen, a first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, a second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and a third consensus *Marburg marburgvirus* envelope glycoprotein immunogen are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C refer to the polyvalent-vaccine construction strategy and expression experiments in Example 1. Figure 1A shows phylogenetic trees for MGP (top), SGP (lower right), and ZGP (lower left). Significant support values are indicated (*) as verified by bootstrap analysis. A consensus strategy was adopted for the ZGP and SGP immunogens (CON VACCINE). Scale bars signify distance of amino acids per site and analyses were conducted using *MEGA* version 5 software. GP transgenes were commercially synthesized, genetically optimized, and subcloned into modified pVAX1 mammalian expression vectors. Antigen expression was analyzed following transfection of HEK 293T cells by Western immunoblotting and FACS. Western immunoblotting results are shown in Figure 1B and FACS in Figure 1C. For a comparative control, rVSV expressing MGP, SGP, or ZGP was run concurrently with each GP sample and species-specific anti-GP1 mAbs were used for detection. Size is indicated (kDa). For FACS, transfected cells were indirectly stained with mouse-derived GP-specific serum reagents followed by extensive washing and goat anti-mouse IgG and MHC class I. Western immunoblotting and FACS experiments were repeated at least three times with similar results. Significance for unrooted phylogenetic trees was determined by maximum-likelihood method and verified by bootstrap analysis and significant support values (≥80%; 1,000 bootstrap replicates) were determined by *MEGA* version 5 software.

Figures 2A-2H show results from experiments in Example 1 in which complete protection against MARV and ZEBOV challenge was observed. Animal survival data is shown in Figure 2A and Figure 2E. Figure 2A shows trivalent vaccinated animals survived post MARV challenge while control animals all died by day 10. Figure 2E shows trivalent vaccinated animals survived post ZEBOV challenge while control animals all died by day 7. Data for % change in body weight for vaccinated and control animal are displayed in Figure 2B for vaccinated challenged with MARV. The y axis indicates change in body weight as shown in Figure 2F. The light solid line is for Trivalent vaccinated animals. The light dashed line is for TriAVE, the average results of the Trivalent vaccinated animals. The dark solid line is for control animals. The dark dashed line is for Control AVE, the average result for the control animals. The light solid lines and light dashed lines remain steady on the graph in the days post challenge showing no significant weight loss among vaccinated animals. The dark solid lines and dark dashed lines

decline on the graph from days 0-9 post challenge ending with the dagger denote animals that succumbed to disease by day 10. Data for % change in body weight for vaccinated and control animal are displayed in Figure 2F for vaccinated challenged with ZEBOV. The y axis shows change in body weight as a percent. The light solid line is for Trivalent vaccinated animals. The light dashed line is for TriAVE, the average results of the Trivalent vaccinated animals. The dark solid line is for control animals. The dark dashed line is for Control AVE, the average result for the control animals. The light solid lines and light dashed lines remain steady on the graph in the days post challenge showing no significant weight loss among vaccinated animals. The dark solid lines and dark dashed lines decline on the graph from days 0-6 post challenge ending with the dagger denote animals that succumbed to disease before day 8. (n=3 for gpMARV and n=6 for gpZEBOV). Binding Abs (Figure 2C and Figure 2G) and NAbs (Figure 2D and Figure 2H) were measured in serum from vaccinated animals before (Pre) and after the first (1X) and second (2X) immunizations. Analysis was conducted on pooled serum (Figure 2H). $*p < 0.1$; $***p < 0.001$; $****p < 0.0001$.

Figures 3A-3C show results from Example 1 demonstrating induction of neutralizing Abs. B cell responses were assessed in mice (n=5/group) 20 days following each of two vaccinations, spaced three weeks between injections with 40 μg of E-DNA vaccination. Figure 3A shows serum GP-specific IgG responses from vaccinated (solid lines) mice or pre-bled (dotted lines) mice were measured by ELISA. The data is summarized in Figures 3B. All responses from pEBOS- and pEBOZ-immunized animals were measured against sucrose-purified ZGP since SGP was not available for this study. IgG responses from pMARV-immunized mice were measured against MARV-Ozolin GP or with negative control sucrose-purified Nipah G protein, Neutralization activity of serum samples was measured against ZEBOV-EGFP, SUDV-Boniface and MARV-Angola in a BSL-4 facility and NAb titers are shown in Figure 3C. NAbs against SUDV-Boniface were assayed based on cytopathic effect (CPE) on CV-1 cells and those against MARV-Angola were assayed using an immunofluorescent assay. Averages are shown in Figure 3B and Figure 3C and error bars represent SEM. Group analyses were completed by matched, two-tailed, unpaired *t* test. Experiments were repeated at least two times with similar results and $*p < 0.1$; $**p < 0.01$;***p<0.001.

Figures 4A-4D shows refer to broad T cells responses generated by vaccination. In Figure 4A H-2^b (light bars) and H-2^d (dark bars) mice (n=5/group) were immunized twice with either pMARV, pEBOS or pEBOZ DNA, and IFNγ responses were measured by IFNγ ELISPOT assay. Splenocytes harvested 8 days after the second immunization were incubated in the presence of individual GP peptides (15-mers overlapping by 9 amino acids) and results are shown in stacked bar graphs. Epitope-containing peptides were identified (≥10 AVE spots AND ≥80% response rate), confirmed by flow cytometry and characterized in the population of total activated IFNγ+ and CD44+ CD4+ and/or CD8+ T cells (Tables 1-6), and peptide numbers of positive inducers are indicated above the bars. Peptides containing CD4+ epitopes alone, CD8+ epitopes alone (*), and dual CD4+ and CD8+ epitopes (**) are numbered. Putative shared and/or partial epitopes were explored for contiguous positive peptide responses (Tables 1-6). Figure 4B shows amino acid similarity plots comparing GP sequences from MARV, SUDV, and ZEBOV viruses displayed in Figure 1A. Figure 4C is a diagram showing putative domains within the ZEBOV GP (GenBank #VGP_EBOZM). SP, signal peptide; RB, receptor binding; MUC, mucin-like region; FC, furin cleavage site; TM, transmembrane region. In Figure 4D, total subdominant (darker shade) and immunodominant (lighter shade) T cell epitopic responses are displayed as a percentage of the total IFNγ response generated by each vaccine. Experiments were repeated at least two times with similar results.

Figures 5A-5D show data from experiment assessing protective 'single-dose' vaccination induced neutralizing Abs and CTL. H-2^k mice (n=10/group) were vaccinated once i.m. with pEBOZ E-DNA and then challenged 28 days later with 1,000 LD_{50} of mZEBOV in a BSL-4 facility. Mice were weighed daily and monitored for disease progression. Animal survival data in Figure 5A. Vaccinated animals survived challenge while control animals died by day 7. Figures 5B shows data for % change in body weight in challenged animals. Data from immunized animals is shown as a solid light line; the average data for immunized animals is shown as a dashed light line. Data from control animals is shown as a solid dark line; the average data for control animals is shown as a dashed dark line. The light solid lines and light dashed lines remain steady within the range of about 85%-120% on the graph in the days post challenge showing no significant weight loss among vaccinated animals. The dark solid lines and dark dashed lines decline on the graph from days 0-6 post challenge ending with the dagger denote animals that

succumbed to disease by day 7. NAbs measured prior to challenge; the data shown in Figure 5C. T cell responses after a single pEBOZ immunization as measured by FACS are summarized as AVE % of total CD44+/IFNγ+ CD4+ (dark) or CD8+ (light) cells in Figure 5D. T_hl-type effector markers were assessed (TNF and T-bet) and data for CD44+/IFNγ+ CD4+ and CD8+ T cells were compared with total T cell data which was as follows: For Total Cells: TNF 2.9±0.8, Tbet 13.0±1.1. For CD4+/CD44+/IFNγ+ Cells: TNF 61.4±3.1, Tbet 72.6±2.0. For CD8+/CD44+/IFNγ+ Cells: TNF 33.0±3.3, Tbet 992.1±1.4 (*$p < 0.1$; ***$p < 0.001$; ****$p < 0.0001$). Group analyses were completed by matched, two-tailed, unpaired *t* test and survival curves were analyzed by log-rank (Mantel-Cox) test. Experiments were performed twice with similar results and error bars represent SEM.

Figure 6 shows a GP-specific T cell gating disclosed in Example 1.

Figures 7A and 7B show that vaccination experiments in Example 1 generated robust T cells.

Figures 8A and 8B show T cell induction by 'single-dose' vaccination disclosed in Example 1.

Figure 9 depicts the vaccination strategy against Ebola. Ebola viral glycoproteins are the major target for vaccines. Currently, three vaccines are currently in clinical trials which are immunogenic protective in non-human primates (NHPs) and have single dose protection. However, these vaccines develop anti-vector immunity, show adverse reactions in human clinical trials, have uncertain duration of memory response and may not be suitable for all populations.

Figure 10 refers to the EBOV glycoprotein vaccine construction and formulation strategy and expression experiments in Example 7.

Figure 11 shows results from experiments in Example 7 demonstrating a single immunization of DNA vaccine is immunogenic in mice.

Figure 12 shows results from Example 7 demonstrating a single immunization is fully protective in mice against lethal mouse-adapted Ebola virus challenge.

Figure 13 shows results from Example 7 demonstrating individual GP DNA vaccine constructs induce robust memory responses in mice.

Figure 14 shows results from Example 7 demonstrating efficacy of GP DNA vaccines in non-human primates.

Figure 15 shows results from Example 7 demonstrating GP DNA vaccine formulations are immunogenic in NHPs.

Figure 16 shows results from Example 7 demonstrating GP DNA formulation vaccines protect against lethal Zaire Ebola virus (Makona) challenge.

Figure 17 shows EBOV-001 phase I clinical (NCT02464670) study strategy.

Figure 18 shows results from Example 7 demonstrating EBOV-001 seroconversion in the phase I clinical trial.

Figure 19 shows results from Example 7 demonstrating induction of Ebola GP Specific T-Cell Responses (ELISpot) in Representative Patients.

Figure 20 shows results from Example 7 demonstrating a comparison with other Ebola vaccine platforms currently in clinical trials.

Figure 21 shows the cohort descriptions and cohort demographics for the ELISA experiments from Example 8.

Figure 22 shows results from Example 8 demonstrating ELISA Titers by Cohort and Time point for cohorts 1-3.

Figure 23 shows results from Example 8 demonstrating ELISA Titers by Cohort and Time point for cohorts 4 and 5.

Figure 24 shows results from Example 8 demonstrating an ELISA Summary for all cohorts.

Figure 25 shows the cohort descriptions and cohort demographics for the ELISpot experiments from Example 8.

Figure 26 shows results from Example 8 demonstrating subject responses by peptide pool for cohort 1.

Figure 27 shows results from Example 8 demonstrating subject responses by peptide pool for cohort 2.

Figure 28 shows results from Example 8 demonstrating subject responses by peptide pool for cohort 3.

Figure 29 shows results from Example 8 demonstrating subject responses by peptide pool for cohort 4.

Figure 30 shows results from Example 8 demonstrating subject responses by peptide pool for cohort 5.

Figure 31 shows results from Example 8 demonstrating ELISpot summary by cohort.

Figure 32 shows results from Example 8 demonstrating ELISpot summary by cohort.

Figure 33 shows results from Example 8 demonstrating analysis of vaccine responders for all subjects.

Figure 34 shows results from Example 8 demonstrating analysis of vaccine responders for subjects with baseline outliers removed.

Figure 35 shows the cohort descriptions and cohort demographics for the ICS experiments from Example 8.

Figure 36 shows results from Example 8 demonstrating Wilcoxon Paired analysis based on cohort for ICS experiments.

Figure 37 shows results from Example 8 demonstrating ICS analysis for cohort 3 (INO4201 ID) Cytokines in CD4+ T cells.

Figure 38 shows results from Example 8 demonstrating ICS analysis for cohort 3 Cytokines in CD8+ T cells.

Figure 39 shows results from Example 8 demonstrating a detailed analysis of vaccine responders for each subject in cohorts 1-3.

Figure 40 shows results from Example 8 demonstrating a detailed analysis of vaccine responders for each subject in cohorts 4-5.

Figure 41 shows results from Example 8 demonstrating median responses by cohort and pool.

Figure 42 shows results from Example 8 demonstrating mean responses by cohort and pool.

Figure 43 shows results demonstrating that bivalent and trivalent DNA vaccines elicit long-term immune responses *in vivo* in cynomolgus macaques. Serum and peripheral blood mononuclear cells (PBMCs) were collected 2-weeks post-each DNA injection and monthly following final injection. Total IgG endpoint titers against Guinea-GP and Mayinga-GP were assayed by ELISA. Cellular immune responses against Ebola GP following DNA immunization.

PMBCs were collected from NHPs and assayed by ELISPOT-IFNγ for T cells responses following stimulation with pools of GP peptides.

Figure 44, comprising Figure 44A through Figure 44C, shows results demonstrating protection in cynomolgous macaques with bivalent and trivalent DNA vaccines against lethal EBOV challenge. Figure 44A) Survival. Animals were challenged with 1000TCID50 of lethal Guinea Makona C07 EBOV and survival was monitored for 28 days post-challenge, Figure 44B) Clinical score. Clinical signs of disease were monitored throughout the course of infection. Figure 44C) Viral load. Viremia during the course of infection was assayed from blood by TCID50 assay.

Figure 45 shows a quantification ELISA using the ADI human anti-Zaire Ebola virus glycoprotein IgG kit to assess the amount of ZEBOV specific IgG antibody in the sera of each vaccinated subject at study entry and 2 weeks post each immunization. Statistical analyses were performed using two tailed Wilcoxon Sign Rank test.

Figure 46 shows an Interferon Gamma ELISpot response. PBMCs were isolated from vaccinated subjects at study entry and 2 weeks post each immunization and stimulated with Ebola peptides spanning the full length GP in an IFNg ELISpot assay. The graph represents EBOV-GP specific spot forming units per million PBMCs. Lines within boxes represent Median response. Individual dots represent outlier data.

Figure 47 depicts experimental results demonstrating long-term immunogenicity with Ebola GP DNA vaccine formulations in non-human primates (NHPs). Strong antibody responses are observed greater than 6 months post-vaccination.

Figure 48 depicts the Memory study of intramuscular delivery of EBOV GP DNA vaccine. NHPs were immunized over a 3 month period with bivalent or trivalent EBOV GP DNA vaccine formulations. The immune responses were followed over a 12 months following the final dose. A 1 year boost was given at month 13.

Figure 49 depicts the total IgG endpoint titers of animals receiving a trivalent DNA GP formulation. An increase in total IgG antibody response was observed following the 1-year boost.

Figure 50 depicts the total IgG endpoint titers of animals receiving a bivalent or trivalent DNA GP formulation. An increase in total IgG antibody response was observed following the 1-year boost.

Figure 51 depicts the ELISPOT of trivalent DNA GP formulations. Increase IFNγ ELISPOT responses following the 1 year boost. Magnitude of boost is not as high in the 3 injection group.

Figure 52 depicts the ELISPOT of bivalent or trivalent DNA GP formulations. Increase IFNγ ELISPOT responses following the 1 year boost. There was a remarkably strong boost in single immunization group.

Figure 53 depicts a summary of IFNγ ELISPOT results. 17/19 animals had increased T cell responses following the boost. 1/19 animals maintained the same level of T cell responses. 1/19 animals had worse T cell responses following the boost, however this animal was consistently high over the past year in the 3 injection group.

Figure 54 depicts an exemplary IFNγ ELISPOT for animals in the single immunization group at the 12 month time point, before the 1-year boost.

Figure 55 depicts an exemplary IFNγ ELISPOT for animals in the single immunization group after the 1-year boost.

Figure 56 depicts the results of the IM memory study. The EBOV GP DNA vaccines elicit long term immune response with a strong recall following a 1 year boost. Intradermal delivery studies are carried out to study the immune response of intradermal EBOV GP DNA vaccines.

Figure 57 depicts a summary of the EBOV DNA vaccine clinical trial (EBOV-001). Healthy volunteers receive a 3 dose regimen of INO-4201, INO-4202, INO-4212 or INO-4212 and INO-9021.

Figure 58 depicts a comparison of binding antibodies in EBOV-001 to rVSV EBOV. All EBOV-001 cohorts had significant increases at both weeks 6 and 14 compared to week 0.

Figure 59 depicts a summary of the Intradermal Delivery of EBOV GP DNA vaccine study. Cohorts were added to explore dosing, dose regimens and use of IL-12 DNA as an immuno-adjuvant.

Figure 60 depicts the results of ID cohorts at week 14. Sereoreactivity was observed in 125/127 (98.4%) subjects in all ID cohorts.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

In one aspect of the invention, it is desired that the consensus antigen provides for improved transcription and translation, including having one or more of the following: low GC content leader sequence to increase transcription; mRNA stability and codon optimization; eliminating to the extent possible cis-acting sequence motifs (i.e., internal TATA-boxes).

In some aspects of the invention, it is desired to generate a consensus antigen that generates a broad immune response across multiple strains, including having one or more of the following: incorporate all available full-length sequences; computer generate sequences that utilize the most commonly occurring amino acid at each position; and increase cross-reactivity between strains.

Diversity among the *Filoviridae* is relatively high. Intensive efforts have been aimed at developing a universal and broadly-reactive filovirus vaccine that would ideally provide protection against multiple species responsible for the highest human case-fatality rates. However, this proves difficult due to the relative high level of diversity among the *Filovirida*. The EBOV are currently classified into five distinct species, *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SUDV), *Reston ebolavirus* (RESTV), *Bundibugyo ebolavirus* (BDBV) and *Taï Forest ebolavirus* (TAFV; formerly *Cote d'Ivoire ebolavirus),* the first two responsible for the highest lethality rates and the most likely candidates for weaponization. Diversity is lower among the Marburg viruses (MARV) of which can also be up to 90% lethal. Currently, there is only one classified species, *Marburg marburgvirus* (formerly *Lake Victoria marburgvirus),* although a recent amendment proposes that it contain two viruses including the Ravn virus (RAVV). Adding to the complexity for polyvalent-vaccine development, the MARV and EBOV are highly divergent, in which there exists about 67% divergence at the nucleotide level. Furthermore, phylogenetic diversity among the filoviral GP is also very high (82% overall). These allude to the potential of the filoviruses to evolve, as demonstrated by the recent emergence of BDBV in 2007. Therefore, due to relative divergence among the *Filoviridae,* we

hypothesized that development of an effective polyvalent-filovirus vaccine will likely require a cocktail of immunogenic components.

A synthetic polyvalent-filovirus DNA vaccine against *Marburg marburgvirus* (MARV), *Zaire ebolavirus* (ZEBOV), and *Sudan ebolavirus* (SUDV) was developed. The novel polyvalent-filovirus vaccine comprised by three DNA plasmids encoding the envelope glycoprotein (GP) genes of *Marburg marburgvirus* (MARV), *Sudan ebolavirus* (SUDV) or *Zaire ebolavirus* (ZEBOV), adopting the multiagent approach. As a filoviral vaccine candidate, an enhanced DNA (DNA)-based platform exhibits many advantages given recent advances in genetic optimization and delivery techniques (Bagarazzi ML, *et al.* (2012). Immunotherapy Against HPV16/18 Generates Potent TH1 and Cytotoxic Cellular Immune Responses. *Sci Transl Med* **4***:* 155ral38; Kee ST, Gehl J, W. LE (2011). *Clinical Aspects of Electroporation,* Springer, New York, NY.; Hirao LA, *et al.* (2011). Multivalent smallpox DNA vaccine delivered by intradermal electroporation drives protective immunity in nonhuman primates against lethal monkeypox challenge. *J Infect Dis* **203**: 95-102). As such, each GP was genetically-optimized, subcloned into modified mammalian expression vectors, and then delivered using *in vivo* electroporation (EP).

Preclinical efficacy studies were performed in guinea pigs and mice using rodent-adapted viruses, while murine T cell responses were extensively analyzed using a novel modified assay described herein. T cell responses were extensively analyzed including the use of a novel method for epitope identification and characterization described herein. This model provides an important preclinical tool for studying protective immune correlates that could be applied to existing platforms.

Vaccination in preclinical rodent studies was highly potent, elicited robust neutralizing antibodies (NAbs) and CTL expressing T_h1-type markers, and completely protected against MARV and ZEBOV challenge. Comprehensive T cell analysis as extensively analyzed using a novel modified assay described herein (Shedlock DJ, *et al.* (2012). Vaccination with synthetic constructs expressing cytomegalovirus immunogens is highly T cell immunogenic in mice. *Hum Vaccin Immunother* **8**: 1668 - 1681) revealed cytotoxic T lymphocytes of great magnitude, epitopic breadth, and T_h1-type marker expression. In total, 52 novel T cell epitopes from two different mouse genetic backgrounds were identified (19 of 20 MARV epitopes, 15 of 16 SUDV, and 18

of 22 ZEBOV) and occurred primarily in highly conserved regions of their respective glycoproteins (GPs). These data represent the most comprehensive report of preclinical glycoprotein epitopes to date.

In developing a strategy to provide protection against multiple species responsible for the highest human case-fatality rates, we focused on MARV, SUDV, and ZEBOV. Due to their relative divergence, we hypothesized that development of a polyvalent-filovirus vaccine would require a cocktail of components that can be quickly and easily adapted in response to future outbreak strains and/or species. While overall diversity among the EBOV is about 33%, amino acid identity increases substantially when SUDV and ZEBOV are analyzed separately (~94% identity within each species). Therefore, as shown in Figure 1A, a two component strategy for coverage of the most lethal EBOV, one plasmid GP vaccine for SUDV and another for ZEBOV was designed. Since GP diversity among each species was relatively low (5.6% for SUDV and 7.1% for ZEBOV), consensus immunogens were developed increase inter-species coverage, a strategy shown previously to enhance protection among divergent strains of influenza and HIV. These GP sequences were consensus for all reported outbreak sequences (GenBank) as determined by alignment using Vector NTI software (Invitrogen, CA, USA). Non-consensus residues, 4 amino acids each in SUDV (95, 203, 261, and 472) and ZEBOV (314, 377, 430, and 440), were weighted towards Gulu and Mbomo/Mbanza, respectively. Gulu was chosen since it was responsible for the highest human case-fatality rate of any *Filoviridae* outbreak (n=425), while Mbomo/Mbanza was chosen since they were the most recent and lethal outbreaks with published sequence data. The consensus GP for SUDV (SUDV CON VACCINE) and ZEBOV (ZEBOV CON VACCINE) were phylogenetically intermediary their parentally aligned strains.

Identification of proteins in Figure 1A are as follows: MARV Durba (05DRC99) '99: ABE27085; Uganda (01Uga07) '07: ACT79229; Durba (07DRC99) '99: ABE27078; Ozolin '75: VGP_MABVO; Musoke '80: VGP_MABVM; Popp '67: VGP_MABVP; Leiden '08: AEW11937; Angola '05: VGP_MABVA; Ravn '87: VGP_MABVR; Durba (09DRC99) '99; ABE27092; Uganda (02Uga07) '07: ACT79201. SUDV: Boniface '76: VGP_EBOSB; Maleo '79 : VGP_EBOSM; Yambio '04 : ABY75325; Gulu '00 : VGP_EBOSU. ZEBOV: Booue '96: AAL25818; Mayibout '96: AEK25495; Mekouka '94: AAC57989, VGP_EBOG4; Kikwit '95: VGP_EBOZ5; Yambuku (Ekron) '76: VGP_EBOEC; Yambuku (Mayinga) '76: VGP_EBOZM;

Kasai '08: AER59712; Kassai '07: AER59718; Etoumbi '05: ABW34742; Mbomo/Mbandza '03: ABW34743.

A sequence listing provided herewith contains a list of 72 sequences including the following

SEQ ID NO:1 is the amino acid sequence of ZEBOV CON (CONGP1), which is a consensus *Zaire ebolavirus* envelope glycoprotein immunogen.

SEQ ID NO:2 is the amino acid sequence of SUDV CON, which is a consensus *Sudan ebolavirus* envelope glycoprotein immunogen.

SEQ ID NO:3 is the amino acid sequence of MARV or MARV ANG, which the amino acid sequence of the *Marburg marburgvirus* Angola 2005 envelope glycoprotein and a *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen.

SEQ ID NO:4 is the amino acid sequence of MARV CON1, which is the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen.

SEQ ID NO:5 is the amino acid sequence of MARV CON2, which is the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen.

SEQ ID NO:6 is the amino acid sequence of MARV CON3, which is the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen.

SEQ ID NOs:7-25 are peptides derived from MARV ANG.

SEQ ID NO:26-41 are peptides derived from SUDV CON.

SEQ ID NO: 42-62 are peptides derived from ZEBOV CON.

SEQ ID NO:63 is the sequence of the IgE signal peptide: MDWTWILFLVAAATRVHS.

SEQ ID NO:64 is the nucleotide sequence insert in plasmid pEBOZ which encodes consensus *Zaire ebolavirus* envelope glycoprotein immunogen.

SEQ ID NO:65 is the nucleotide sequence insert in plasmid pEBOS which encodes consensus *Sudan ebolavirus* envelope glycoprotein immunogen.

SEQ ID NO:66 is the nucleotide sequence insert in plasmid pMARZ ANG which encodes the *Marburg marburgvirus* Angola 2005 envelope glycoprotein.

SEQ ID NO:67 is the amino acid sequence of ZEBOVGUI (GuineaGP), which is a consensus *Zaire ebolavirus* envelope glycoprotein immunogen isolated from the 2014 Outbreak in Guinea.

SEQ ID NO:68 is the amino acid sequence of ZEBOVCON2 (CONGP2), which is a second consensus *Zaire ebolavirus* envelope glycoprotein.

SEQ ID NO:69 is the nucleotide sequence insert in plasmid pZEBOVGUI which encodes consensus *Zaire ebolavirus* envelope glycoprotein immunogen isolated from the 2014 Outbreak in Guinea.

SEQ ID NO:70 is the nucleotide sequence insert in plasmid pEBOZCON2 which encodes a second consensus *Zaire ebolavirus* envelope glycoprotein.

SEQ ID NO:71 is the nucleotide sequence of plasmid pEBOZCON2 which encodes a second consensus *Zaire ebolavirus* envelope glycoprotein.

SEQ ID NO:72 is the nucleotide sequence insert in plasmid pZEBOVGUI which encodes a *Zaire ebolavirus* envelope glycoprotein immunogen isolated from the 2014 Outbreak in Guinea.

In some embodiments, the strategy employs coding sequences for three filovirus immunogens selected from: MARV, SUDV, ZEBOV, ZEBOVGUI and ZEBOVCON2. MARV immunogen is the glycoprotein of the Angola 2005 isolate. For SUDV ZEBOV, ZEBOVGUI, and ZEBOVCON2 concensus glycoprotein sequences were designed.

In some embodiments, the strategy employs coding sequences for five filovirus immunogens. Three MARV immunogens are provided. Consensus glycoprotein Ozolin, Musoke, or Ravn derived from three clusters, were designed. These three MARV immunogens are targets for immune responses together the SUDV, ZEBOV or ZEBOVCON2, ZEBOVGUI consensus glycoprotein sequences that were designed.

In some embodiments, the strategy employs coding sequences for six filovirus immunogens. Four MARV immunogens are provided: three consensus glycoproteins derived from three clusters were designed. These three MARV immunogens are targets for immune responses together the SUDV, ZEBOV and ZEBOVCON2, ZEBOVGUI consensus glycoprotein sequences that were designed, the MARV immunogen is the glycoprotein of the Angola 2005 isolate.

As a candidate for filoviral vaccines, DNA vaccines exhibit a multitude of advantages including rapid and inexpensive up-scale production, stability at room temperature, and ease of transport, all of which further enhance this platform from an economic and geographic perspective.

Due to the synthetic nature of the plasmids, Ag sequences can be quickly and easily modified in response to newly emergent species and/or expanded to include additional vaccine components and/or regimen for rapid response during outbreak settings. For example, the MARV strategies herein can be easily expanded for greater coverage by the co-administration of additional plasmids encoding consensus MARV GP (MGP) immunogens for other phylogenetic clusters.

While 'first-generation' DNA vaccines were poorly immunogenic, recent technological advances have dramatically improved their immunogenicity in clinical trials. Optimization of plasmid DNA vectors and their encoded Ag genes have led to increases in *in vivo* immunogenicity. Cellular uptake and subsequent Ag expression are substantially amplified when highly-concentrated plasmid vaccine formulations are administered with *in vivo* electroporation, a technology that uses brief square-wave electric pulses within the vaccination site to drive plasmids into transiently permeabilized cells. In theory, a cocktail of DNA plasmids could be assembled for directing a highly-specialized immune response against any number of variable Ags. Immunity can be further directed by co-delivery with plasmid molecular adjuvants encoding species-specific cytokine genes as well as 'consensus-engineering' of the Ag amino acid sequences to help bias vaccine-induced immunity towards particular strains. This strategy has been shown to enhance protection among divergent strains of influenza virus and HIV. Due in parts to these technological advancements, immunization regimens including these DNA vaccines are highly versatile and extremely customizable.

1. Definitions.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

a. Adjuvant

"Adjuvant" as used herein may mean any molecule added to the DNA plasmid vaccines described herein to enhance antigenicity of the one or more consensus filovirus immunogens encoded by the DNA plasmids and encoding nucleic acid sequences described hereinafter.

b. Antibody

"Antibody" may mean an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')2, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody may be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

c. Coding Sequence

"Coding sequence" or "encoding nucleic acid" as used herein may mean refers to the nucleic acid (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence may also comprise a DNA sequence which encodes an RNA sequence. The coding sequence may further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered. In some embodiments, the coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide.

d. Genetic Construct

Genetic construct" as used herein refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein, such as an immunogen. The genetic construct may also refer to a DNA molecule which transcribes RNA. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence

that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

e. Complement

"Complement" or "complementary" as used herein may mean a nucleic acid may mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

f. Consensus or Consensus Sequence

"Consensus" or "consensus sequence" as used herein may mean a synthetic nucleic acid sequence, or corresponding polypeptide sequence, constructed based on analysis of an alignment of multiple subtypes of a particular filovirus antigen, that can be used to induce broad immunity against multiple subtypes or serotypes of a particular filovirus antigen.

Consensus *Zaire ebolavirus* envelope glycoprotein immunogen refers to SEQ ID NO:1, fragments of SEQ ID NO:1, variants of SEQ ID NO:1 and fragment of variants of SEQ ID NO:1. (ZEBOV or ZEBOV CON or ZEBOV CON VACCINE). Plasmids comprising coding sequences of SEQ ID NO:1 may be referred to as pZEBOV or pEBOZ. Coding sequences for consensus *Zaire ebolavirus* envelope glycoprotein immunogen include SEQ ID NO:64, fragments of SEQ ID NO:64, variants of SEQ ID NO:64 and fragment of variants of SEQ ID NO:64. Plasmid pEBOZ comprises SEQ ID NO:64.

Consensus *Sudan ebolavirus* envelope glycoprotein immunogen refers to SEQ ID NO:2, fragments of SEQ ID NO:2, variants of SEQ ID NO:2 and fragment of variants of SEQ ID NO:2. (SUDV or SUDV CON or SUDV CON VACCINE) Plasmids comprising coding sequences of SEQ ID NO:2 may be referred to as pSUDV or pEBOS. Coding sequences for consensus *Sudan ebolavirus* envelope glycoprotein immunogen include SEQ ID NO:65, fragments of SEQ ID NO:65, variants of SEQ ID NO:65 and fragment of variants of SEQ ID NO:65. Plasmid pEBOS comprises SEQ ID NO:65.

Marburg marburgvirus Angola 2005 envelope glycoprotein is not a consensus but a protein sequence derived from an isolate. It has sequence SEQ ID NO:3. *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen refers to SEQ ID NO:3, fragments of SEQ ID NO:3, variants of SEQ ID NO:3 and fragment of variants of SEQ ID NO:3. (MARV or MARV

ANG or MARV ANG or MARV ANG VACCINE) Plasmids comprising coding sequences of SEQ ID NO:3 may be referred to as pMARV or pMARV-ANG. Coding sequences for *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen include SEQ ID NO:66, fragments of SEQ ID NO:66, variants of SEQ ID NO:66 and fragment of variants of SEQ ID NO:66. Plasmid pMARV ANG comprises SEQ ID NO:66.

The first consensus *Marburg marburgvirus* envelope glycoprotein immunogen refers to SEQ ID NO:4, fragments of SEQ ID NO:4, variants of SEQ ID NO:4 and fragment of variants of SEQ ID NO:4. SEQ ID NO:4 is a *Marburg marburgvirus* consensus sequence from the Ravn cluster consensus (Ravn, Durba (09DRC99) and Uganda (02Uga07Y). (MARV CON1 or MARV-RAV CON or MARV-RAV CON VACCINE) Plasmids comprising coding sequences of SEQ ID NO:4 may be referred to as pMARV-RAV.

The second consensus *Marburg marburgvirus* envelope glycoprotein immunogen refers to SEQ ID NO:5, fragments of SEQ ID NO:5, variants of SEQ ID NO:5 and fragment of variants of SEQ ID NO:5. SEQ ID NO:5 is a *Marburg marburgvirus* consensus sequence from the Ozolin cluster consensus (Ozolin, Uganda (01Uga07), and Durba (05 and 07DRC99)). (MARV CON2 or MARV-OZO CON or MARV-OZO CON VACCINE) Plasmids comprising coding sequences of SEQ ID NO:5 may be referred to as pMARV-OZO.

The third consensus *Marburg marburgvirus* envelope glycoprotein immunogen refers to SEQ ID NO:6, fragments of SEQ ID NO:6, variants of SEQ ID NO:6 and fragment of variants of SEQ ID NO:6. SEQ ID NO:6 is a *Marburg marburgvirus* consensus sequence from the Musoke cluster consensus (Musoke, Popp, and Leiden). (MARV CON1 or MARV-MUS CON or MARV-MUS CON VACCINE) Plasmids comprising coding sequences of SEQ ID NO:6 may be referred to as pMARV-MUS.

Consensus *Zaire ebolavirus GP* envelope glycoprotein immunogen refers to SEQ ID NO:67, fragments of SEQ ID NO:67, variants of SEQ ID NO:67 and fragment of variants of SEQ ID NO:67. (ZEBOVGUI or ZEBOVGUI VACCINE). Plasmids comprising coding sequences of SEQ ID NO:67 may be referred to as pZEBOVGUI or pEBOZGUI. Coding sequences for consensus *Zaire ebolavirus Guinea 2014* envelope glycoprotein immunogen include SEQ ID NO:69, fragments of SEQ ID NO:69, variants of SEQ ID NO:69 and fragment of variants of SEQ ID NO:69. Plasmid pEBOZGUI comprises SEQ ID NO:69.

A second consensus *Zaire ebolavirus* envelope glycoprotein immunogen refers to SEQ ID NO:68 fragments of SEQ ID NO:68, variants of SEQ ID NO:68 and fragment of variants of SEQ ID NO:68. (ZEBOV2 or ZEBOVCON2 or ZEBOVCON2 VACCINE) Plasmids comprising coding sequences of SEQ ID NO: 68 may be referred to as pEBOVCON2. Coding sequences for second consensus *Zaire ebolavirus* envelope glycoprotein immunogen include SEQ ID NO:70, fragments of SEQ ID NO:70, variants of SEQ ID NO:70 and fragment of variants of SEQ ID NO:70. Plasmid pEBOVCON2 comprises SEQ ID NO:70.

g. Constant Current

"Constant current" as used herein to define a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

h. Current Feedback or Feedback

"Current feedback" or "feedback" as used herein may be used interchangeably and may mean the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback may be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop may be instantaneous as it is an analog closed-loop feedback.

i. Decentralized Current

"Decentralized current" as used herein may mean the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

j. Electroporation

"Electroporation," "electro-permeabilization," or "electro-kinetic enhancement" ("EP") as used interchangeably herein may refer to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

k. Feedback Mechanism

"Feedback mechanism" as used herein may refer to a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value, preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism may be performed by an analog closed loop circuit.

l. Fragment

"Fragment" may mean a polypeptide fragment of a filovirus immunogen that is capable of eliciting an immune response in a mammal against filovirus by recognizing the particular filovirus antigen. The filovirus envelope glycoprotein immunogen may optionally include a signal peptides and/or a methionine at position 1, proteins 98% or more homologous to the consensus sequences set forth herein, proteins 99% or more homologous to the consensus sequences set forth herein, and proteins 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. A fragment may or may not for example comprise a fragment of a filovirus immunogen linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide or IgG signal peptide.

Fragments of any of ZEBOV CON, SUDV CON, MARV ANG, MARV-RAV CON, MARV-OZO CON, MARV-MUS CON, ZEBOVGUI or ZEBOVCON2 or variants thereof, in

each case with or without signal peptides and/or a methionine at position 1, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length ZEBOV CON, SUDV CON, MARV ANG, MARV-RAV CON, MARV-OZO CON, MARV-MUS CON, ZEBOVGUI or ZEBOVCON2 or variants thereof. Fragments refer to fragments polypeptides 100% identical to the sequences ZEBOV CON, SUDV CON, MARV ANG, MARV-RAV CON, MARV-OZO CON, MARV-MUS CON, ZEBOVGUI or ZEBOVCON2 in each case with or without signal peptides and/or a methionine at position 1. Fragments also refer to fragments of variants, i.e. polypeptides that 95% or more, 98% or more, or 99% or more homologous to the sequences ZEBOV CON, SUDV CON, MARV ANG, MARV-RAV CON, MARV-OZO CON, MARV-MUS CON, ZEBOVGUI or ZEBOVCON2 , in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the filovirus immunogens set forth in SEQ ID NOs: 1-6, 67-68 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of SEQ ID NOs: 1-6, 67-68 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide or IgG signal peptide. The fragment may comprise fragments of SEQ ID NOs: 1-6, 67-68including the N terminal methionine. Fragments also refer to fragments of a polypeptide that is 95% or more, 98% or more, or 99% or more homologous to the sequence disclosed in SEQ ID NOs: 1-6, 67-68. If a signal peptide is present it is not included when calculating percent homology.

In some embodiments, fragments of SEQ ID NOs: 1-6, 67-68 or variants thereof may comprise 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 610, 620, 630, 640, 650, 660, 670 or more contiguous amino acids of any of SEQ ID NOs:1-6 or variants thereof. In some embodiments, fragments of SEQ ID NOs:1-6 or variants thereof may comprise 15, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 610, 620,

630, 640, 650, 660, 670, 675 or less contiguous amino acids of any of SEQ ID NOs: 1-6, 67-68 or variants thereof.

"Fragment" may also mean a fragment of a nucleic acid sequence that encodes a filovirus immunogen, the nucleic acid fragment encoding a fragment of filovirus immunogen that is capable of eliciting an immune response in a mammal against filovirus by recognizing the particular filovirus antigen. Fragments of nucleic acid fragment encoding a filovirus immunogen or variants thereof, in each case with or without signal peptides and/or a methionine at position 1, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length nucleic acid sequence that encodes a filovirus immunogen or variants thereof. The fragment may comprise a fragment of nucleotide sequence that encodes polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the filovirus immunogens set forth in SEQ ID NOs: 1-6, 67-68 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, fragment of nucleotide sequence that encodes a fragment of SEQ ID NOs: 1-6, 67-68 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide or IgG signal peptide. Coding sequences of a signal peptide sis present it is not included when calculating percent homology. In some embodiments, fragment of nucleotide sequence that encodes fragments of SEQ ID NOs:1-6 or variants thereof may comprises sequences that encode 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 610, 620, 630, 640, 650, 660, 670 or more contiguous amino acids of any of SEQ ID NOs: 1-6, 67-68 or variants thereof. In some embodiments, fragment of nucleotide sequence that encodes fragments of SEQ ID NOs:1-6, 67-68 or variants thereof may sequences that encode comprise 15, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 610, 620, 630, 640, 650, 660, 670, 675 or less contiguous amino acids of any of SEQ ID NOs:1-6, 67-68 or variants thereof.

In some embodiments, fragments are fragments of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72. Fragments of SEQ ID NO:64,

SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72, in each case with or without signal peptides and/or a methionine at position 1, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length nucleic acid sequence that encodes a filovirus immunogen or variants thereof. The fragment of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 may comprise a fragment of nucleotide sequence that encodes polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the filovirus immunogens encoded by of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. Fragments of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 may comprises sequences that encode 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 610, 620, 630, 640, 650, 660, 670 or more contiguous amino acids of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 or variants thereof. Fragments of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 may comprises sequences that encode 15, 20, 30, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 610, 620, 630, 640, 650, 660, 670, 675 or less contiguous amino acids of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 or variants thereof. In some embodiments, fragments are fragments of RNA transcribed from or encoded by SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72

m. Identical

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of

matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

n. Impedance

"Impedance" as used herein may be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

o. Immune Response

"Immune response" as used herein may mean the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of one or more filovirus consensus antigen via the provided DNA plasmid vaccines. The immune response can be in the form of a cellular or humoral response, or both.

p. Nucleic Acid

"Nucleic acid" or "oligonucleotide" or "polynucleotide" as used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine,

guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

q. Operably Linked

"Operably linked" as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

r. Promoter

"Promoter" as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

s. Stringent Hybridization Conditions

"Stringent hybridization conditions" as used herein may mean conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent conditions may be selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a

defined ionic strength pH. The T_m may be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions may be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal may be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

t. Substantially Complementary

"Substantially complementary" as used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

u. Substantially Identical

"Substantially identical" as used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

v. Variant

"Variant" used herein with respect to a nucleic acid may mean (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

"Variant" with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hyrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. Variants are preferably homologous to SEQ ID NO:1-6,67-68 by 95% or more, 96% or more, 97% or more, 98% or more or 99% or more.

"Variant" with respect to a nucleic acid sequence that encodes the same specific amino acid sequence differs in nucleotide sequence by use of different codons. Variants of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 that

encode the same amino acid sequence as those encoded by SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 may be any degree of homology, preferably 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more or 99% or more. Variants of RNA transcribed by SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 that encode the same amino acid sequence as those encoded by SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 may be any degree of homology, preferably 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more or 99% or more.Variant may also be variants of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 that encode protein which are variants of the proteins encoded by SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity, typically the amino acid sequences are homologous by 95% or more, 96% or more, 97% or more, 98% or more or 99% or more. Variant may also be variants of RNA transcribed by SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 that encode protein which are variants of the proteins encoded the RNA transcribed by SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:72 with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity, typically the amino acid sequences are homologous by 95% or more, 96% or more, 97% or more, 98% or more or 99% or more

w. Vector

"Vector" used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial, viral vector, artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

2. Proteins

Provided herein are filovirus immunogens that can be used to induce broad immunity against multiple subtypes or serotypes of a particular filovirus antigen. Consensus filovirus antigens may include consensus amino acid sequences of Marburgvirus filovirus glycoprotein MARV RAV immunogen, consensus amino acid sequences of Marburgvirus filovirus glycoprotein MARV OZO immunogen, consensus amino acid sequences of Marburgvirus filovirus glycoprotein MARV MUS immunogen, isolate amino acid sequences of Marburgvirus filovirus glycoprotein MARV ANG immunogen, consensus amino acid sequences of Zaire ebolavirus glycoprotein ZEBOV immunogen, consensus amino acid sequences of Zaire ebolavirus glycoprotein ZEBOV2014 immunogen, isolate amino acid sequences of Zaire ebolavirus glycoprotein ZEBOVCON2 immunogen, and consensus amino acid sequences of Sudan ebolavirus glycoprotein SUDV immunogen, respectively. In some embodiments, the immunogens may comprise a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide or an IgG signal peptide.

The amino acid sequence for immunogens include SEQ ID NO:1-6,67-68 variants thereof and fragments of SEQ ID NO:1-6, 67-68 and variants thereof, optionally including a signal peptide such as for example an IgE or IgG signal peptide.

3. Coding sequences encoding Proteins

Coding sequences encoding the proteins set forth herein may be generated using routine methods. Composition comprising a nucleic acid sequence that encodes a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, comprising a nucleic acid sequence that encodes a second consensus Zaire ebolavirus envelope glycoprotein immunogen, a nucleic acid sequence that encodes a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen envelope glycoprotein immunogen, a nucleic acid sequence that encodes a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a nucleic acid sequence that encodes a *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen are provided and a nucleic acid sequence that encodes a first consensus *Marburg marburgvirus* envelope glycoprotein immunogen , a nucleic acid sequence that encodes a second consensus *Marburg marburgvirus* envelope glycoprotein immunogen, and a nucleic acid sequence that encodes a third consensus

Marburg marburgvirus envelope glycoprotein immunogen can be generated based upon the amino acid sequences disclosed.

Nucleic acid sequence may encodes a full length consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a full length second consensus Zaire ebolavirus envelope glycoprotein immunogen, a full length consensus *Consensus Zaire ebolavirus Guinea envelope* glycoprotein immunogen envelope glycoprotein immunogen, a full length consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a full length *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen, a full length first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, a full length second consensus *Marburg marburgvirus* envelope glycoprotein immunogen, or a full length third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Nucleic acid sequences may comprise a sequence that encodes SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:67, or SEQ ID NO:68. Nucleic acid sequence may comprise SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69 SEQ ID NO:70, or SEQ ID NO:72. In one embodiment the nucleotide sequence comprises an RNA sequence transcribed from a DNA sequence described herein. For example, nucleic acids may comprise an RNA sequence transcribed by the DNA sequence of SEQ ID NOs: 64, 65, 66, 69, 70 or 72, a fragment thereof or a variant thereof. Nucleic acid sequence may optionally comprise coding sequences that encode a signal peptide such as for example an IgE or IgG signal peptide.

Nucleic acid sequence may encode a fragment of a full length consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a fragment of a full length second consensus Zaire ebolavirus envelope glycoprotein immunogen, a fragment of a full length *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen envelope glycoprotein immunogen, a fragment of a full length consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a fragment of a full length *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen, a fragment of a full length first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, a fragment of a full length second consensus *Marburg marburgvirus* envelope glycoprotein immunogen, or a fragment of a full length third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Nucleic acid sequence may comprise a sequence that encodes a fragment of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID

NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:67, or SEQ ID NO:68. Nucleic acid sequence may comprise a fragment of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69 SEQ ID NO:70, or SEQ ID NO:72. Fragment sizes are disclosed herein as set forth in section entitled "Fragments". Nucleic acid sequence may optionally comprise coding sequences that encode a signal peptide such as for example an IgE or IgG signal peptide.

Nucleic acid sequences may encode a protein homologous to a full length consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a full length second consensus Zaire ebolavirus envelope glycoprotein immunogen, a full length *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen envelope glycoprotein immunogen, a protein homologous to a full length consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a protein homologous to a full length *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen, a protein homologous to a full length first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, a protein homologous to a full length second consensus *Marburg marburgvirus* envelope glycoprotein immunogen, or a protein homologous to a full length third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Nucleic acid sequence may comprise a sequence that encodes a protein homologous to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:67, or SEQ ID NO:68. Nucleic acid sequence may be homologous to SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69 SEQ ID NO:70, or SEQ ID NO:72. Degrees of homology are discussed herein such as in the section referring to Variants. Nucleic acid sequence may optionally comprise coding sequences that encode a signal peptide such as for example an IgE or IgG signal peptide.

Nucleic acid sequence may encode a protein homologous to fragment of a full length consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a protein homologous to fragment of a full length second consensus Zaire ebolavirus envelope glycoprotein immunogen, a protein homologous to fragment of a full length *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen envelope glycoprotein immunogen, a protein homologous to a fragment of a full length consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a protein homologous to a fragment of a full length *Marburg marburgvirus* Angola 2005 envelope glycoprotein immunogen, a protein homologous to a fragment of a full length first consensus

Marburg marburgvirus envelope glycoprotein immunogen, a protein homologous to a fragment of a full length second consensus *Marburg marburgvirus* envelope glycoprotein immunogen, or a protein homologous to a fragment of a full length third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Nucleic acid sequence may comprise a sequence that encodes a protein homologous to a fragment of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:67, or SEQ ID NO:68. Nucleic acid sequence may comprise a fragment homologous to SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:69 SEQ ID NO:70, or SEQ ID NO:72. Degrees of homology are discussed herein such as in the section referring to Variants. Nucleic acid sequence may optionally comprise coding sequences that encode a signal peptide such as for example an IgE or IgG signal peptide.

SEQ ID NO:64 is the nucleotide sequence insert in plasmid pEBOZ which encodes consensus *Zaire ebolavirus* envelope glycoprotein immunogen.

SEQ ID NO:65 is the nucleotide sequence insert in plasmid pEBOS which encodes consensus *Sudan ebolavirus* envelope glycoprotein immunogen.

SEQ ID NO:66 is the nucleotide sequence insert in plasmid pMARZ ANG which encodes the *Marburg marburgvirus* Angola 2005 envelope glycoprotein.

SEQ ID NO:69 is the nucleotide sequence insert in plasmid pEBOZGUI which encodes consensus *Zaire ebolavirus GP* envelope glycoprotein immunogen

SEQ ID NO:70 is the nucleotide sequence insert in plasmid pEBOZCON2 which encodes a second consensus *Zaire ebolavirus* envelope glycoprotein

4. Vectors

Vectors include, but are not limited to, plasmids, expression vectors, recombinant viruses, any form of recombinant "naked DNA" vector, and the like. A "vector" comprises a nucleic acid which can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (e.g., a cell membrane, a viral lipid envelope, etc.). Vectors include, but are not limited to replicons (e.g., RNA replicons, bacteriophages) to which fragments of DNA may be attached and

become replicated. Vectors thus include, but are not limited to RNA, autonomous self-replicating circular or linear DNA or RNA (e.g., plasmids, viruses, and the like, see, e.g., U.S. Pat. No. 5,217,879), and include both the expression and non-expression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector" this includes both extra-chromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

a. Plasmid

Plasmid may comprise a nucleic acid sequence that encodes one or more of the various immunogens disclosed above including coding sequences that encode synthetic, consensus antigen capable of eliciting an immune response against filoproteins.

A single plasmid may contain coding sequence for a single filoprotein immunogen, coding sequence for two filoprotein immunogens, coding sequence for three filoprotein immunogens, coding sequence for four filoprotein immunogens, coding sequence for five filoprotein immunogens or coding sequence for six filoprotein immunogens. A single plasmid may contain a coding sequence for a single filoprotein immunogen which can be formulated together. In some embodiments, a plasmid may comprise coding sequence that encodes IL-12, IL-15 and/or IL-28.

The plasmid may further comprise an initiation codon, which may be upstream of the coding sequence, and a stop codon, which may be downstream of the coding sequence. The initiation and termination codon may be in frame with the coding sequence.

The plasmid may also comprise a promoter that is operably linked to the coding sequence The promoter operably linked to the coding sequence may be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human

muscle creatine, or human metalothionein. The promoter may also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

The plasmid may also comprise a polyadenylation signal, which may be downstream of the coding sequence. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 plasmid (Invitrogen, San Diego, CA).

The plasmid may also comprise an enhancer upstream of the coding sequence. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and WO94/016737, the contents of each are fully incorporated by reference.

The plasmid may also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. The plasmid may be pVAX1, pCEP4 or pREP4 from Invitrogen (San Diego, CA), which may comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which may produce high copy episomal replication without integration. The backbone of the plasmid may be pAV0242. The plasmid may be a replication defective adenovirus type 5 (Ad5) plasmid.

The plasmid may also comprise a regulatory sequence, which may be well suited for gene expression in a cell into which the plasmid is administered. The coding sequence may comprise a codon that may allow more efficient transcription of the coding sequence in the host cell.

The coding sequence may also comprise an Ig leader sequence. The leader sequence may be 5' of the coding sequence. The consensus antigens encoded by this sequence may comprise an N-terminal Ig leader followed by a consensus antigen protein. The N-terminal Ig leader may be IgE or IgG.

The plasmid may be pSE420 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Escherichia coli* (E.coli). The plasmid may also be pYES2 (Invitrogen, San Diego, Calif.), which may be used for protein production in Saccharomyces cerevisiae strains of yeast. The plasmid may also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which may be used for protein production in insect cells. The plasmid may also be pcDNA I or pcDNA3 (Invitrogen, San Diego, Calif.), which may be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells.

b. RNA vectors

In one embodiment, the nucleic acid is an RNA molecule. Accordingly, in one embodiment, the invention provides an RNA molecule encoding one or more of the envelope glycoprotein (GP) genes of *Marburg marburgvirus* (MARV), *Sudan ebolavirus* (SUDV) or *Zaire ebolavirus* (ZEBOV). The RNA may be plus-stranded. Accordingly, in some embodiments, the RNA molecule can be translated by cells without needing any intervening replication steps such as reverse transcription. A RNA molecule useful with the invention may have a 5′ cap (e.g. a 7-methylguanosine). This cap can enhance in vivo translation of the RNA. The 5′ nucleotide of a RNA molecule useful with the invention may have a 5′ triphosphate group. In a capped RNA this may be linked to a 7-methylguanosine via a 5′-to-5′ bridge. A RNA molecule may have a 3′ poly-A tail. It may also include a poly-A polymerase recognition sequence (e.g. AAUAAA) near its 3′ end. A RNA molecule useful with the invention may be single-stranded.

c. Linear Vectors

Also provided herein is a linear nucleic acid vaccine, or linear expression cassette ("LEC"), that is capable of being efficiently delivered to a subject via electroporation and expressing one or more desired antigens. The LEC may be any linear DNA devoid of any phosphate backbone. The DNA may encode one or more antigens. The LEC may contain a promoter, an intron, a stop codon, a polyadenylation signal. The expression of the antigen may be controlled by the promoter. The LEC may not contain any antibiotic resistance genes and/or a phosphate backbone. The LEC may not contain other nucleic acid sequences unrelated to the desired antigen gene expression.

The LEC may be derived from any plasmid capable of being linearized. The plasmid may be capable of expressing the antigen. The plasmid may be pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing the DNA and enabling a cell to translate the sequence to an antigen that is recognized by the immune system.

The LEC may be derived from any plasmid capable of being linearized. The plasmid may be capable of expressing the antigen. The plasmid may be pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing the DNA and enabling a cell to translate the sequence to a antigen that is recognized by the immune system.

d. Viral Vectors

In one embodiment, viral vectors are provided herein which are capable of delivering a nucleic acid of the invention to a cell. The expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001), and in Ausubel et al. (1997), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

5. Compositions

Compositions are provided which comprise nucleic acid molecules. The compositions may comprise a plurality of copies of a single nucleic acid molecule such a single plasmid, a plurality of copies of two or more different nucleic acid molecules such as two or more different plasmids. For example a composition may comprise plurality of two, three, four, five, six, seven,

eight, nine or ten or more different nucleic acid molecules. Such compositions may comprise plurality of two, three, four, five, six, or more different plasmids.

Compositions may comprise nucleic acid molecules, such as plasmids, that collectively contain coding sequence for a single filoprotein immunogen selected from the group consisting of one or more a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebolavirus envelope glycoprotein immunogen, a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen.

Composition comprise nucleic acid sequence that encode the combination of: a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a consensus *Sudan ebolavirus* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a second consensus Zaire ebolavirus envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the *Marburg marburgvirus* Angola 2005 envelope glycoprotein; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the second consensus Zaire ebolavirus envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebolavirus envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg*

marburgvirus envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebolavirus envelope glycoprotein immunogen the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen.

Each coding sequence for each filoprotein immunogens is preferably included on a separate plasmid.

Accordingly, compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a consensus *Sudan ebolavirus* envelope glycoprotein immunogen; a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a second consensus Zaire ebolavirus envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and the *Consensus Zaire ebolavirus Guinea*

envelope glycoprotein immunogen, may be on a single plasmid but are preferably on two separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the *Marburg marburgvirus* Angola 2005 envelope glycoprotein; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the second consensus Zaire ebolavirus envelope glycoprotein immunogen; or a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen; may be on a single plasmid or on two plasmids in any permutation but are preferably on three separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation but are preferably on five separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebolavirus envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation but are preferably on five separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen

and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation but are preferably on five separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebolavirus envelope glycoprotein immunogen the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation or on four plasmids in any permutation but are preferably on six separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation or on four plasmids in any permutation but are preferably on six separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation or on five plasmids in any permutation but are preferably on six separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebolavirus envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation or on five plasmids in any permutation or on six plasmids in any permutation but are preferably on seven separate plasmids.

Compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation or on five plasmids in any permutation or on six plasmids in any permutation but are preferably on seven separate plasmids.

Likewise, compositions that comprise nucleic acid sequence that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebolavirus envelope glycoprotein immunogen, the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen may be on a single plasmid or on two plasmids in any permutation, or on three plasmids in any permutation or on four plasmids in any permutation or on five plasmids

in any permutation or on six plasmids in any permutation or on seven plasmids in any permutation but are preferably on eight separate plasmids.

6. Vaccine

Provided herein is a vaccine capable of generating in a mammal an immune response against filovirus, particularly Marburgvirus, Ebolavirus Sudan and/or Ebolavirus Zaire. The vaccine may comprise each plasmid as discussed above. The vaccine may comprise a plurality of the plasmids, or combinations thereof. The vaccine may be provided to induce a therapeutic or prophylactic immune response.

Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a consensus *Sudan ebolavirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen and a second consensus Zaire ebola virus envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and a second consensus Zaire ebola virus envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen and the *Marburg marburgvirus* Angola 2005 envelope glycoprotein. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebola virus envelope glycoprotein immunogen and the *Marburg marburgvirus* Angola 2005 envelope glycoprotein. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen and the *Marburg marburgvirus* Angola

2005 envelope glycoprotein. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebola virus envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a *Zaire ebolavirus 2014* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope

glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebola virus envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines may be used to deliver nucleic acid molecules that encode a consensus *Zaire ebolavirus* envelope glycoprotein immunogen, a second consensus Zaire ebola virus envelope glycoprotein immunogen, the *Consensus Zaire ebolavirus Guinea* envelope glycoprotein immunogen, a consensus *Sudan ebolavirus* envelope glycoprotein immunogen, the *Marburg marburgvirus* Angola 2005 envelope glycoprotein, the first consensus *Marburg marburgvirus* envelope glycoprotein immunogen, the second consensus *Marburg marburgvirus* envelope glycoprotein immunogen and the third consensus *Marburg marburgvirus* envelope glycoprotein immunogen. Vaccines are preferably compositions comprising plasmids.

The vaccine may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may be functional molecules as vehicles, adjuvants, carriers, or diluents. The pharmaceutically acceptable excipient may be a transfection facilitating agent, which may include surface active agents, such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and more preferably, the poly-L-glutamate is present in the vaccine at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the genetic construct. In some embodiments, the DNA plasmid vaccines may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

The pharmaceutically acceptable excipient may be one or more adjuvants. An adjuvant may be other genes that are expressed from the same or from an alternative plasmid or are delivered as proteins in combination with the plasmid above in the vaccine. The one or more adjuvants may be proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL20, α-interferon (IFN- α), β-interferon (IFN-β), γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence or coding sequence that encodes the signal sequence deleted and optionally including a different signal peptide such as that from IgE or coding sequence that encodes a difference signal peptide such as that from IgE, IL-28, MHC, CD80, CD86, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-lα, MIP-1β, IL-8, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF

receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof or a combination thereof. In some embodiments adjuvant may be one or more proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL-20, IL-12, IL-15, IL-28, CTACK, TECK, MEC or RANTES. Examples of IL-12 constructs and sequences are disclosed in PCT application no. PCT/US1997/019502 and corresponding US Application Serial No. 08/956,865, and U.S. Provisional Application Serial No 61/569600 filed December 12, 2011, which are each incorporated herein by reference. Examples of IL-15 constructs and sequences are disclosed in PCT application no. PCT/US04/18962 and corresponding US Application Serial No. 10/560,650, and in PCT application no. PCT/US07/00886 and corresponding U.S. Application Serial No. 12/160,766, and in PCT application no. PCT/US10/048827, which are each incorporated herein by reference. Examples of IL-28 constructs and sequences are disclosed in PCT application no. PCT/US09/039648 and corresponding U.S. Application Serial No. 12/936,192, which are each incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. and 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of chemokines CTACK, TECK and MEC constructs and sequences are disclosed in PCT application no. PCT/US2005/042231 and corresponding U.S. Application Serial No. 11/719,646, which are each incorporated herein by reference. Examples of OX40 and other immunomodulators are disclosed in U.S. Application Serial No. 10/560,653, which is incorporated herein by reference. Examples

of DR5 and other immunomodulators are disclosed in U.S. Application Serial No. 09/622452, which is incorporated herein by reference.

The vaccine may further comprise a genetic vaccine facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

The vaccine may comprise the consensus antigens and plasmids at quantities of from about 1 nanogram to 100 milligrams; about 1 microgram to about 10 milligrams; or preferably about 0.1 microgram to about 10 milligrams; or more preferably about 1 milligram to about 2 milligram. In some preferred embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms, from about 100 to about 200 microgram, from about 1 nanogram to 100 milligrams; from about 1 microgram to about 10 milligrams; from about 0.1 microgram to about 10 milligrams; from about 1 milligram to about 2 milligram, from about 5 nanogram to about 1000 micrograms, from about 10 nanograms to about 800 micrograms, from about 0.1 to about 500 micrograms, from about 1 to about 350 micrograms, from about 25 to about 250 micrograms, from about 100 to about 200 microgram of the consensus antigen or plasmid thereof.

The vaccine may be formulated according to the mode of administration to be used. An injectable vaccine pharmaceutical composition may be sterile, pyrogen free and particulate free. An isotonic formulation or solution may be used. Additives for isotonicity may include sodium chloride, dextrose, mannitol, sorbitol, and lactose. The vaccine may comprise a vasoconstriction agent. The isotonic solutions may include phosphate buffered saline. Vaccine may further comprise stabilizers including gelatin and albumin. The stabilizing may allow the formulation to be stable at room or ambient temperature for extended periods of time such as LGS or polycations or polyanions to the vaccine formulation.

The vaccine may be stable for is stable at room temperature (25°C) for more than 1 week, in some embodiments for more than 2 weeks, in some embodiments for more than 3 weeks, in

some embodiments for more than 4 weeks, in some embodiments for more than 5 weeks, and in some embodiments for more than 6 weeks. In some embodiments, the vaccine is stable for more than one month, more than 2 months, more than 3 months, more than 4 months, more than 5 months, more than 6 months, more than 7 months, more than 8 months, more than 9 months, more than 10 months, more than 11 months, or more than 12 months. In some embodiments, the vaccine is stable for more than 1 year, more than 2 years, more than years, or more than 5 years. In one embodiment, the vaccine is stable under refrigeration (2-8°C). Accordingly, in one embodiment, the vaccine does not require frozen cold-chain. A vaccine is stable if it retains its biological activity for a sufficient period to allow its intended use (e.g., to generate an immune response in a subject). For example, for vaccines that are to be stored, shipped, etc., it may be desired that the vaccines remain stable for months to years.

7. Methods of Delivery the Vaccine

Provided herein is a method for delivering the vaccine for providing genetic constructs and proteins of the consensus antigen which comprise epitopes that make them particular effective against immunogens of filovirus, particularly Marburgvirus, Ebolavirus Sudan and/or Ebolavirus Zaire, against which an immune response can be induced. The method of delivering the vaccine or vaccination may be provided to induce a therapeutic and prophylactic immune response. The vaccination process may generate in the mammal an immune response against filovirus, particularly Marburgvirus, Ebolavirus Sudan and/or Ebolavirus Zaire. The vaccine may be delivered to an individual to modulate the activity of the mammal's immune system and enhance the immune response. The delivery of the vaccine may be the transfection of the consensus antigen as a nucleic acid molecule that is expressed in the cell and delivered to the surface of the cell upon which the immune system recognized and induces a cellular, humoral, or cellular and humoral response. The delivery of the vaccine may be used to induce or elicit and immune response in mammals against filovirus, particularly Marburgvirus, Ebolavirus Sudan and/or Ebolavirus Zaire by administering to the mammals the vaccine as discussed above.

Upon delivery of the vaccine and plasmid into the cells of the mammal, the transfected cells will express and secrete consensus antigens for each of the plasmids injected from the vaccine. These proteins will be recognized as foreign by the immune system and antibodies will

be made against them. These antibodies will be maintained by the immune system and allow for an effective response to subsequent infections by filovirus, particularly Marburgvirus, Ebolavirus Sudan and/or Ebolavirus Zaire.

The vaccine may be administered to a mammal to elicit an immune response in a mammal. The mammal may be human, primate, non-human primate, cow, cattle, sheep, goat, antelope, bison, water buffalo, bison, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, and chicken.

a. Combination Treatments

The vaccine may be administered in combination with other proteins and/or genes encoding CCL20, α-interferon, γ-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence deleted and optionally including the different signal peptide such as the IgE signal peptide, MHC, CD80, CD86, IL-28, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-lα, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof or combinations thereof. In some embodiments, the vaccine is administered in combination with one or more of the following nucleic acid molecules and/or proteins: nucleic acid molecules selected from the group consisting of nucleic acid molecules comprising coding sequence that encode one or more of CCL20, IL-12, IL-15, IL-28, CTACK, TECK, MEC and RANTES or functional fragments thereof, and proteins selected from the group

consisting of: CCL02, IL-12 protein, IL-15 protein, IL-28 protein, CTACK protein, TECK protein, MEC protein or RANTES protein or functional fragments thereof.

The vaccine may be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal, intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The vaccine may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation ("EP"), "hydrodynamic method", or ultrasound.

The plasmid of the vaccine may be delivered to the mammal by several well-known technologies including DNA injection (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. The consensus antigen may be delivered via DNA injection and along with in vivo electroporation.

b. Electroporation

Administration of the vaccine via electroporation of the plasmids of the vaccine may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA EP system (VGX Pharmaceuticals, Blue Bell, PA) or Elgen electroporator (Genetronics, San Diego, CA) to facilitate transfection of cells by the plasmid.

The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50 μs, 20 μs, 10 μs or 1 μs, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates

the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

Examples of electroporation devices and electroporation methods that may facilitate delivery of the DNA vaccines of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the DNA vaccines include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array

based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

c. Method of Preparing DNA Plasmids

Provided herein is methods for preparing the DNA plasmids that comprise the DNA vaccines discussed herein. The DNA plasmids, after the final subcloning step into the mammalian expression plasmid, can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

The DNA plasmids for use with the EP devices of the present invention can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using an optimized plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792,

which was filed on May 23, 2007. In some examples, the DNA plasmids used in these studies can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

EXAMPLES

The present invention is further illustrated in the following Example. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Example 1

METHODS

Plasmid vaccine construction

The pMARV, pEBOS, and pEBOZ plasmid DNA constructs encode full-length GP proteins. An amino acid consensus strategy was used for the pEBOS and pEBOZ, while a type-matched sequence from the 2005 Angola outbreak strain was used (GenBank #VGP_MABVR) for pMARV (Towner JS, *et al.* (2006). Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. *J Virol* **80**: 6497-6516). Consensus sequences were determined by alignment of the prevailing ZEBOV and SUDV GP amino acid sequences and generating a consensus for each. Each vaccine GP gene was genetically optimized for expression in humans (including codon- and RNA-optimization for enhancing protein expression (GenScript,

Piscataway, NJ)), synthesized commercially, and then subcloned (GenScript, Piscataway, NJ) into modified pVAX1 mammalian expression vectors (Invitrogen, Carlsbad, CA) under the control of the cytomegalovirus immediate-early (CMV) promoter; modifications include 2A>C, 3C>T, 4T>G, 241C>G, 1,942C>T, 2,876A>-, 3,277C>T, and 3,753G>C. Phylogenetic analysis was performed by multiple-alignment with ClustalW using *MEGA* version 5 software. Alternatively, GP diversity among the MARV was much higher (~70% identity) in comparison, so a consensus strategy was not adopted. For coverage of MARV, we chose to utilize the MGP sequence from the 2005 outbreak in Angola (GenBank #VGP_MABVR) since it was solely responsible for the largest and deadliest MARV outbreak to date. This sequence was greater than 10% divergent from either of its closest cluster of relative strains including Musoke, Popp and Leiden (10.6% divergence), or Uganda (01Uga07), Durba (05DRC99 and 07DRC99) and Ozolin (10.3% divergence). Altogether, a three-plasmid strategy formed the foundation for our novel trivalent polyvalent-filovirus vaccine strategy.

Transfections and immunoblotting

Human Embryonic Kidney (HEK) 293T cells were cultured, transfected, and harvested. Briefly, cells were grown in DMEM with 10% FBS, 1% Pen-strep, sodium pyruvate, and L-glutamine. Cells were cultured in 150 mm Corning dishes and grown to 70% confluence overnight in a 37° incubator with 5% CO_2. Dishes were transfected with 10 - 25 μg of *Filoviridae* pDNA using either a Calphos™ Mammalian Transfection Kit protocol (Clonetech) or Lipofectamine™ 2000 reagent (Invitrogen) per the manufacturer's protocol and then incubated for 24 - 48 h. Cells were harvested with ice cold PBS, centrifuged and washed, and then pelleted for Western immunoblot or FACS analysis. Standard Western blotting was used and GP-specific MAbs for GP1 detection were generated. Data from Western immunoblotting experiments is shown in Figure 1B. Data from FACS analysis is shown in Figure 1C.

Animals, vaccinations, and challenge

Adult female C57BL/6 (H-2^b), BALB/cJ (H-2^d), and Bl0.Br (H-2^k) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) while Hartley guinea pigs were from Charles River (Wilmington, MA). All animal experimentation was conducted following UPenn IACUC and School of Medicine Animal Facility, or NML Institutional Animal Care Committee of the PHAC and the Canadian Council on Animal Care guidelines for housing and care of laboratory

animals and performed in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of NIH after pertinent review and approval by the abovementioned institutions. UPenn and NML comply with NIH policy on animal welfare, the Animal Welfare Act, and all other applicable federal, state and local laws.

Mice were immunized i.m. by needle injection with 40 μg of plasmid resuspended in water, while guinea pigs were immunized i.d., with 200 μg of each into three separate vaccination sites. Vaccinations were immediately followed by EP at the same site. Briefly, a three-pronged CELLECTRA® adaptive constant current Minimally Invasive Device was inserted approximately 2 mm i.d. (Inovio Pharmaceuticals, Inc., Blue Bell, PA). Square-wave pulses were delivered through a triangular 3-electrode array consisting of 26-gauge solid stainless steel electrodes and two constant-current pulses of 0.1 Amps were delivered for 52 msec/pulse separated by a 1 sec delay.

For lethal challenge studies, challenges were limited to rodent-adapted ZEBOV and MARV. Guinea pigs were challenged 28 days after the final vaccination by i.p. injection with 1,000 LD_{50} of guinea pig-adapted ZEBOV (21.3 FFU/animal) (Richardson JS, Abou MC, Tran KN, Kumar A, Sahai BM, Kobinger GP (2011). Impact of systemic or mucosal immunity to adenovirus on ad-based Ebola virus vaccine efficacy in guinea pigs. *J Infect Dis* **204 Suppl 3**: S1032-1042) or 1,000 LD_{50} MARV-Angola (681 TCID50/animal). Briefly, the guinea-pig adapted MARV was made by the serial passage of wild-type MARV-Angola in outbred adult female Hartley guinea pigs. Seven days after inoculation, the animals were euthanized and livers were harvested and homogenized. This homogenate was then injected i.p. into naive adult guinea pigs and the process repeated until animals lost weight, gloss of hair, and succumbed to infection similar to EBOV adaptation in guinea pigs. For mouse lethal challenge studies (Kobinger GP, *et al.* (2006). Chimpanzee adenovirus vaccine protects against Zaire Ebola virus. *Virology* **346**: 394-401), mice were injected i.p. with 200 μl of a 1,000 LD_{50} (10 FFU/animal) of mouse-adapted ZEBOV. All animals were weighed daily and monitored for disease progression using an approved score sheet for at least 18 days for mice and 22 days for guinea pigs. All infectious work was performed in a 'Biosafety Level 4' (BSL4) facility at NML, PHAC.

ELISA and neutralization assays

Antibody (Ab) titers were determined using 96-well ELISA plates coated with either sucrose-purified MARV Ozolin GP or ZGP, or with negative control sucrose-purified Nipah G protein at a concentration of 1:2,000. Briefly, the plates were then incubated for 18 h at 4° C, washed with PBS and 0.1% Tween-20, and 100 µl/sample of the sera were tested in triplicate (at dilutions 1:100,1:400, 1:1,600, and 1:6,400 in PBS with 5% skim milk and 0.5% Tween-20). Following an incubation at 37°C for 1 h in a moist container, the plates were washed and then 100 µl of goat anti-mouse IgG-conjugated HRP antibody (Cedarlane) was added (1:2,000 dilution) and incubated for another 37°C for 1 h in a moist container. After a wash, 100 µl of the ABST (2,2'-azino-bis(3-ethylbenthiazoline-6-sulphonic acid) and peroxidase substrate (Cedarlane) was added to visualize Ab binding. Again in a moist container, the plate was incubated for 30 min at 37°C and then later read at 405 nm. Positive binding results were characterized by being > 3 SD when subtracting the positive control from the negative control serum.

The ZEBOV neutralization assay was performed. Briefly, Sera collected from immunized mice and guinea pigs were inactivated at 56°C for 45 minutes and serial dilutions of each sample (1:20,1:40, etc..., for mice and 1:50 for guinea pigs, in 50 µl of DMEM) was mixed with equal volume of ZEBOV expressing the EGFP reporter gene (ZEBOV-EGFP) (100 transducing units/well, according to EGFP expression) and incubated at 37°C for 90 minutes. The mixture was then transferred onto sub-confluent VeroE6 cells in 96-well flat-bottomed plates and incubated for 5-10 minutes at RT. Control wells were infected with equal amounts of the ZEBOV-EGFP virus without addition of serum or with non-immune serum. 100 µl of DMEM supplemented with 20% FBS was then added to each well and plates were incubated at 37°C in 5% CO_2 for 48 h.

Alternatively, neutralization of MARV-Angola 368 was assessed using an immunofluorescent assay. A primary rabbit anti-MARV Ab and secondary goat anti-rabbit IgG FITC-conjugated Ab was used for detection. Neutralizing Abs (NAbs) against SUDV Boniface were assayed based on cytopathic effect (CPE) on CV-1 cells. Cells were incubated with equal parts of immunized sera and SUDV Boniface for 10 days before subsequently fixed with 10% buffered formalin for 24 hours and examined under a light microscope. EGFP and FITC positive cells were counted in each well and sample dilutions showing >50% reduction in the number of

green cells compared to controls scored positive for NAb. Alternatively, NAbs against SUDV-Boniface were assayed based on cytopathic effect (CPE) on CV-1 cells. All infectious work was performed in the BSL4 laboratory of NML, PHAC.

Splenocyte isolation

Mice were sacrificed 8-11 days following the final immunization and the spleens were harvested. Briefly, spleens were placed in RPMI 1640 medium (Mediatech Inc., Manassas, VA) supplemented with 10% FBS, 1X Anti-anti (Invitrogen), and 1X β-ME (Invitrogen). Splenocytes were isolated by mechanical disruption of the spleen using a Stomacher machine (Seward Laboratory Systems Inc., Bohemia, NY), and the resulting product was filtered using a 40 μm cell strainer (BD Falcon). The cells were then treated for 5 min with ACK lysis buffer (Lonza, Switzerland) for lysis of RBCs, washed in PBS, and then resuspended in RPMI medium for use in ELISPOT or FACS assay.

ELISPOT assays

Standard IFNγ ELISPOT assay was performed. Briefly, 96-well plates (Millipore, Billerica, MA) were coated with anti-mouse IFN-γ capture antibody and incubated for 24h at 4°C (R&D Systems, Minneapolis, MN). The following day, plates were washed with PBS and then blocked for 2h with blocking buffer (1% BSA and 5% sucrose in PBS). Splenocytes (1-2 x 10^5 cells/well) were plated in triplicate and stimulated overnight at 37°C in 5% CO_2 and in the presence of either RPMI 1640 (negative control), Con A (positive control), or GP peptides either individually (15-mers overlapping by 9 amino acids and spanning the lengths of their respective GP) or whole pooled (2.5 μg/ml final). After 18 - 24 h of stimulation, the plates were washed in PBS and then incubated for 24h at 4°C with biotinylated anti-mouse IFN-γ mAb (R&D Systems, Minneapolis, MN). Next, the plates were washed again in PBS, and streptavidin-alkaline phosphatase (MabTech, Sweden) was added to each well and incubated for 2 h at RT. Lastly, the plates were washed again in PBS and then BCIP/NBT Plus substrate (MabTech) was added to each well for 5-30 min for spot development. As soon as the development process was complete upon visual inspection, the plate were rinsed with distilled water and then dried overnight at RT. Spots were enumerated using an automated ELISPOT reader (Cellular Technology Ltd., Shaker Heights, OH).

For comprehensive analysis of T cell breadth, standard IFNγ ELISPOT was modified herein as previously described in Shedlock DJ, *et al.* (2012). SUPRA. Identification and measurement of subdominant and immunodominant T cell epitopes were assessed by stimulating splenocytes with individual peptides as opposed to whole or matrix peptide pools; the traditional practice of pooling peptides for the sake of sample preservation, such as the use of matrix array pools, results in a reduction of assay sensitivity since total functional responses in pools containing multiple epitope-displaying peptides will effectively lower assay resolution, i.e. 'drown-out' those of lower magnitude. Thus, modified ELISPOT was performed with individual peptides (15-mers overlapping by 9 amino acids; 2.5 μg/ml final) spanning each GP immunogen. Peptides containing T cell epitopes were identified (≥10 AVE IFNγ+ spots AND ≥80% animal response rate; summarized in Tables 1-6) and then later confirmed functionally and phenotypically by FACS. No shared or partial epitopes were identified, nor did FACS data or web-based epitope prediction software suggest the presence of a CD4+ or CD8+ T cell epitope that was preserved within consecutive peptides. Here, possible shared/partial T cell epitopes were addressed for all instances of contiguous peptide responses as identified by modified ELISPOT assay. Cells were stimulated individually with each of the contiguous peptides, as well as paired in combination for direct comparison, and were defined as 'shared/partial' if the combined response was not greater than either of the two individual responses. Also, it must be noted, that the epitopic response presented herein may not have been completely comprehensive since the '15-mer overlapping by 9 amino acids' algorithm for generating peptides is biased towards complete coverage of CD8 T cell epitopes which may underestimate CD4 T cell responses due to the nature of class II-restricted epitopes being longer than 15 amino acids. Lastly, amino acid similarity plots were generated using Vector NTI software and the results are shown in Figure 4B.

Flow cytometry

Splenocytes were added to a 96-well plate (1×10^6 cells/well) and stimulated for 5-6 h with either individual peptides or 'Minimal Peptide Pools' (2.5 μg/ml final). Individual peptides stimulation was used for functional confirmation of all peptides identified by modified ELISPOT (Tables 1-6) as well as phenotypic characterization. Splenocytes and transfected 293Ts were first pre-stained with LIVE/DEAD® Fixable Violet Dead Cell Stain Kit (Invitrogen). For

splenocytes, cells were surface-stained for CD19 (V450; clone 1D3), CD4 (PE-Cy7; clone RM4-5), CD8α (APC-Cy7; clone 53-6.7) and CD44 (PE-Cy5; clone IM7) (BD Biosciences, San Jose, CA), washed three times in PBS + 1% FBS, permeabilized with BD Cytofix/Cytoperm™ kit, and then stained intracellularly with IFNγ (APC; clone XMG1.2), TNF (FITC; clone MP6-XT22), CD3 (PE-cy5.5; clone 145-2C11), and T-bet (PE; clone 4B10) (eBioscience, San Diego, CA). GP expression in transfected 293T cells was assessed 24 h post-transfection. Indirect staining was performed following a 30 min incubation at 4°C in PBS + 1% FBS containing the indicated mouse-derived GP-specific polyclonal serum reagent (1:200 dilution), each produced by pooling serum from H-2^b mice immunized three times with their respective DNA vaccine or pVAX1 empty vector control. Cells were then stained with FITC-conjugated goat anti-mouse IgG (BioLegend, San Diego, CA), washed extensively, and then stained for MHC class I (HLA-ABC; PE-Cy7; clone G46-2.6; BD). All cells were fixed in 1% paraformaldehyde. All data was collected using a LSRII flow cytometer (BD) and analyzed using FlowJo software (Tree Star, Ashland, OR). Splenocytes were gated for activated IFNγ-producing T cells that were CD3+ CD44+, CD4+ or CD8+, and negative for the B cell marker CD19 and viability dye.

Figure 6 shows a GP-specific T cell gating. Functional and phenotypic analysis for peptides containing T cell epitopes as identified by ELISPOT was performed by FACS gating of total lymphocytes, live (LD) CD3+ cells that were negative for CD 19 and LIVE-DEAD (dump channel), singlets (excludes cell doublets), CD4+ and CD8+ cells, activated cells (CD44+), and peptide-specific IFNγ-producing T cells.

Statistical analysis

Significance for unrooted phylogenetic trees was determined by maximum-likelihood method and verified by bootstrap analysis and significant support values (≥80%; 1,000 bootstrap replicates) were determined by *MEGA* version 5 software. Group analyses were completed by matched, two-tailed, unpaired *t* test and survival curves were analyzed by log-rank (Mantel-Cox) test. All values are mean ± SEM and statistical analyses were performed by GraphPad Prism (La Jolla, CA).

RESULTS

Vaccine construction and expression

Phylogenetic analysis revealed relative conservation among the EBOV GPs (94.4% for SUDV and 92.9% for ZEBOV), whereas the MARV GP (MGP) were more divergent (~70% conserved). Thus, a consensus strategy, as determined by alignment of the prevailing ZEBOV and SUDV GP amino acid sequences, was adopted for the EBOV GPs, while a type-matched strategy was used for MARV employing the 2005 Angola outbreak sequence which was solely responsible for the largest and deadliest MARV outbreak. Each GP transgene was genetically optimized, synthesized commercially, and then subcloned into modified pVAX1 mammalian expression vector. Altogether, a three-plasmid strategy formed the foundation for our novel polyvalent-filovirus vaccine strategy.

HEK 293T cells were transfected separately with each plasmid and GP expression was assessed by Western immunoblotting and FACS. A ~130 kDa protein was observed for each in cell lysates harvested 48 h post-transfection using species-specific anti-GP1 mAbs for detection Results are shown in Figure 1B. For a comparative control, recombinant vesicular stomatitis viruses (rVSV) expressing the respective GPs were loaded in concurrent lanes. Next, GP expression on the cell surface was analyzed 24 h post-transfection by indirect staining with GP-specific or control polyclonal serum by FACS. Results are shown in Figure 1C. Cell surface expression was detected for all vaccine plasmids while little non-specific binding was observed; control serum did not react with GP-transfected cells nor did the positive sera with pVAX1-transfected cells (data shown for pEBOZ). As expected for the EBOV GPs, cell surface expression sterically occluded recognition of surface MHC class I, as well as β1-integrin (Francica JR, Varela-Rohena A, Medvec A, Plesa G, Riley JL, Bates P (2010). Steric shielding of surface epitopes and impaired immune recognition induced by the ebola virus glycoprotein. *PLoS Pathog* **6**: e1001098).

Complete protection against MARV and ZEBOV challenge

To determine protective efficacy, we employed the guinea pig preclinical challenge model. Preclinical immunogenicity and efficacy studies were performed herein using the guinea pig and mouse models. The guinea pig preclinical model has been extensively used as a screening and 'proof-of-concept' tool for filoviral vaccine development. Although primary isolates of MARV and EBOV cause non-fatal illness in guinea pigs, a small number of passages in this host results in selection of variants able to cause fatal disease with pathological

features similar to those seen in filovirus-infected primates. Similarly, mice have also been widely used for filoviral vaccine development, however, unlike the guinea pig model, immunodetection reagents for assessing immunity and T cell responses are extensively available. Infection with a murine-adapted ZEBOV (mZEBOV) results in disease characterized by high levels of virus in target organs and pathologic changes in livers and spleens akin to those found in EBOV-infected primates.

Guinea pigs (n=24) were immunized i.d. two times with 200μg of each plasmid (pEBOZ, pEBOS and pMARV) into three separate vaccination sites or with pVAX1 empty vector control (n=9), and then boosted with the same vaccines one month later. Animals were challenged 28 days following the second immunization with 1,000 LD_{50} of a guinea pig-adapted MARV-Angola (gpMARV) (n=9) or ZEBOV (gpZEBOV) (n=15) in a BSL-4 facility, and then observed and weighed daily. Results are shown in Figures 2A-2H. Vaccinated animals were completely protected while control-vaccinated animals succumbed to gpMARV by 10 days post-challenge (n=3; $P = 0.0052$) or to gpZEBOV by day 7 post-challenge (n=6; $P = 0.0008$) (Figure 2A and Figure 2E). Additionally, vaccinated animals were protected from weight loss (Figure 2B and Figure 2F; $P < 0.0001$). It is likely that vaccine-induced Abs may have contributed to protection since GP-specific Abs in pooled serum exhibited a significant increase in binding (Figure 2C and Figure 2G) and neutralization (Figure 2D and Figure 2H) titers. Experiments were performed in a BSL-4 facility and repeated twice with similar results and error bars in Figures A-2H represent SEM. Group analyses were completed by matched, two-tailed, unpaired *t* test and survival curves were analyzed by log-rank (Mantel-Cox) test.

Plasmid vaccines were highly immunogenic

To better characterize immune correlates as driven by the protective DNA vaccine (plasmids pEBOZ, pEBOS and pMARV, also referred to as trivalent DNA vaccine), we next employed the mouse model which has been widely used as a screening and 'proof-of-concept' tool for filoviral vaccine development and in which extensive immunodetection reagents are available. First, B cell responses were assessed in H-2^d mice (n=5/group) 20 days following each of two vaccinations, three weeks between injections with 40 μg of respective monovalent DNA vaccine. Data from these experiments is shown in Figures 3A-3C. While little GP-specific IgG was observed in pre-bleed control samples, as shown in Figure 3A and Figure 3B, a significant

increase was detected in all animals following vaccination. Since purified SGP was not available, purified ZGP was used as a surrogate. IgG in SUDV-vaccinated mice bound ZGP, demonstrating the ability for vaccine-induced Ab generation as well as its capability for cross-species recognition. Additionally, seroconversion occurred in 100% of vaccinated animals after only one immunization, after which responses were significantly increased by homologous boost; AVE reciprocal endpoint dilution titres were boosted 22.1-fold in pMARV-immunized mice, and 3.4-fold and 8.6-fold in pEBOS- and pEBOZ-vaccinated animals, respectively. Samples were next assayed for neutralization of ZEBOV, SUDV-Boniface, and MARV-Angola in a BSL-4 facility. The results of the neutralization assay are shown in Figure 3C. Significant increases in NAb titres were detected following vaccination in all animals.

Mice from two different genetic backgrounds (H-2^d and H-2^b; n=5/group) were immunized with 40 μg of respective plasmids pEBOZ, pEBOS and pMARV, homologous boosted after two weeks, and then sacrificed 8 days later for T cell analysis Results from a novel modified ELISPOT assay to assess the comprehensive vaccine-induced T cell response, in which splenocytes were stimulated using individual peptides as opposed to matrix pools are shown in Figure 4A. DNA vaccination induced robust IFNγ+ responses that recognized a diversity of T-cell epitopes (Tables 1-6). All positive epitope-comprising peptides were subsequently gated (See Figure 6), confirmed, and further characterized by FACS. This modified ELISPOT approach proved extremely sensitive since background responses from control wells were low (7.2±0.2 IFNγ-producing SFC/10^6 splenocytes in H-2^b and 9.2±0.5 in H-2^d mice). Results as shown in Figure 4A revealed that vaccination with pMARV induced 9 measurable epitopes in H-2^b mice and 11 in H-2^d, pEBOS induced 9 and 8, and pEBOZ generated 10 and 12, in these respective strains. While five of nine (55.6%) of the epitopes from pMARV-immunized H-2^b mice were CD8+, they accounted for about 57.3% of the total MGP-specific IFNγ+ response as measured by both ELISPOT and FACS confirmation and phenotypic analysis. Similarly, only 33% and 38% of confirmed epitopes were CD8-restricted in pEBOS-immunized H-2^b and H-2^d mice, respectively. However these epitopes comprised roughly 50-90% of the total response; CD8+ T cell responses were estimated to be approximately 56% in both mouse strains while FACS estimates were 51% and 90% in H-2^b and H-2^d mice, respectively. Total CD8+ responses

were lower in pEBOZ-vaccinated animals and measured between 33% and 57% (33% for both strains by ELISPOT and 6% and 57% for H-2^b and H-2^d mice, respectively, by FACS).

A single immunodominant epitope was detected in both mouse strains receiving pEBOS where an immunodominant epitope was loosely defined as generating an IFNγ response at least two-fold over the highest subdominant epitope; pMARV induced four H-2^b-restricted immunodominant CD8+ epitopes within peptides MGP_{25-39} (#5), MGP_{67-81} (#12), $MGP_{181-195}$ (#31) and $MGP_{385-399}$ (#65), and an H-2^d-restricted CD4+ epitope in $MGP_{151-171}$ (#27). Four of these epitopes occurred within highly conserved regions of MARV GP1, including three of which were located within the putative receptor binding domain, while only one occurred within the variable mucin-like region ($MGP_{385-399}$ (#65)) as shown in Figure 4B and Figure 4C. pEBOS stimulated CD8+ epitopes occurring in SUDV GP $(SGP)_{19-33}$ (#4) and $SGP_{241-255}$ (#41) in H-2^b and H-2^d mice, respectively, both in highly conserved regions of GP1. However, pEBOZ immunization revealed three immunodominant epitopes in H-2^d mice (a CD8-restricted epitope located in the ZEBOV GP receptor binding domain $(GP)_{139-153}$ (#24), and two CD4-restricted epitopes $ZGP_{175-189}$ (#30) and $ZGP_{391-405}$ (#66)), occurring within the receptor binding domain and the mucin-like region, respectively. Only one immunodominant epitope was defined in H-2^b mice which contained both a CD4+ and a CD8+ epitope (#89) and occurred in a highly conserved region of GP2. Overall, diverse epitope hierarchies were consistent and reproducible in each vaccine group. Furthermore, as shown in Figure 4D, the subdominant response comprised a significant proportion of the total response; the total AVE subdominant response as measured by the modified ELISPOT assay was approximately 12%, 62%, and 74% in pMARV-, pEBOS- and pEBOZ-immunized H-2^b mice, respectively, while responses in H-2^d mice were 47%, 50% and 34%, respectively.

Lastly, total GP-specific T cell responses were measured by FACS using stimulation with minimal peptide pools containing only confirmed epitope-comprising peptides identified. . Robust responses were detected in each of the vaccinated animals and were, in a majority of cases, comprised by both activated CD4+ and CD8+ T cells. Responses were GP-specific, since little IFNγ production was observed with a control peptide (h-Clip), and correlated well with ELISPOT data. The only instance where immunization did not induce remarkable CTL as measured by FACS was in H-2^d mice vaccinated with pMARV in which no epitope identified by

ELISPOT was confirmed to be CD8-restricted. Altogether, these data show that each of the vaccine plasmids was highly immunogenic in mice and yielded robust GP-specific T cell responses recognizing a diverse array of T cell epitopes including immunodominant epitopes within highly conserved regions of the GP. Furthermore, the highly diverse subdominant T cell response characterized herein might have otherwise been overlooked using traditional matrix array peptide pools for epitope identification.

T cell responses were measured for reactivity against minimal peptide pools comprised by all positively identified peptides for each respective GP by FACS. Figure 7A shows DNA vaccine-induced T cell responses are shown from a representative animal and IFNγ-producing CD4+ (right) and CD8+ (left) cells are gated. FACS plots are shown. Incubation with h-CLIP peptide served as a negative control (Control). Figure 7B shows results of gated cells in Figure 7A are summarized as average % of total CD44+/IFNγ+ CD4+ or CD8+ cells and error bars represent SEM. Experiments were repeated at least two times with similar results.

'Single-dose' protection in mice

Vaccine efficacy against ZEBOV challenge was next assessed in the preclinical murine model. Mice were vaccinated only once due to strong NAb induction and protection data observed. Mice (H-2^k; n=10/group) were immunized with 40 μg of the pEBOZ DNA and protection was evaluated 28 days later by challenge with 1,000 LD_{50} of mouse-adapted ZEBOV (mZEBOV) in a BSL4 facility. While all control animals succumbed to infection by day 7 post-challenge, Figure 5A shows DNA-vaccinated mice were completely protected (P = 0.0002). In addition, as shown in Figure 5B, control mice exhibited progressive loss of body weight until death ($P < 0.0001$).

To better understand the mechanisms of DNA-induced protection in a 'single-dose' model, we next assessed NAb and T cell generation. NAbs were assessed 25 days post-vaccination, 3 days prior to challenge, and, as shown in Figure 5C, a significant ($P < 0.0001$) increase was detected in all vaccinated animals (n=10/group); reciprocal endpoint dilution titers ranged from 19 to 42, 27.3±2.5.

We next evaluated the generation of ZGP-specific T cells and increased the scope of our analysis to compare responses in mice immunized with either the pEBOZ alone, or in a trivalent formulation IFN-γ production (n=5) was assessed 11 days later by FACS using whole ZGP

peptide pools; the data is shown in Figure 5D. IFNγ-producing T cells were detected in all animals and were specific for ZGP peptides since stimulation with a control peptide did not induce cytokine production. Immunization with either the monovalent or trivalent formulation induced robust IFNγ T cell responses that, when compared, were not significantly different $P = 0.0920$).

Since CTL may be important in eliminating virus-infected cells (Warfield KL, *et al.* (2005). Induction of humoral and CD8+ T cell responses are required for protection against lethal Ebola virus infection. *J Immunol* **175:** 1184-1191; Kalina WV, Warfield KL, Olinger GG, Bavari S (2009). Discovery of common marburgvirus protective epitopes in a BALB/c mouse model. *Virol J 6:* 132; Olinger GG, *et al.* (2005). Protective cytotoxic T-cell responses induced by venezuelan equine encephalitis virus replicons expressing Ebola virus proteins. *J Virol* **79**: 14189-14196; Sullivan NJ, *et al.* (2011). CD8(+) cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. *Nat Med* **17**: 1128-1131; and Geisbert TW, *et al.* (2010). Vector choice determines immunogenicity and potency of genetic vaccines against Angola Marburg virus in nonhuman primates. *J Virol* **84**: 10386-10394), production of an additional effector cytokine, TNF, as well as a developmental restriction factor, T-box transcription factor TBX21 (T-bet), known to correlate with T_hl-type CTL immunity and cytotoxicity were measured and the results were as follows. For Total Cells: TNF 2.9±0.8, Tbet 13.0±1.1. For CD4+/CD44+/IFNγ+ Cells: TNF 61.4±3.1, Tbet 72.6±2.0. For CD8+/CD44+/IFNγ+ Cells: TNF 33.0±3.3, Tbet 992.1±1.4 ($*p < 0.1$; $***p < 0.001$; $****p < 0.0001$). We found that ~61% and ~33% of activated CD4+ and CD8+ T cells, respectively, also produced TNF in addition to IFNγ. Furthermore, a majority of IFNγ-producing T cells expressed high levels of T-bet; about 73% and 92% of CD8+ and CD4+ T cells, respectively, were CD44+ and produced IFNγ following ZGP peptide stimulation.

Figures 8A and 8B show T cell induction by 'single-dose' vaccination**.** T cell responses in H-2^k mice after a single pEBOZ immunization or a single trivalent vaccination, comprised by the three vaccine plasmids in separate sites, as measured by FACS are shown (a) and summarized (b) as AVE % of total CD44+/IFNγ+ CD4+ (purple) or CD8+ (orange) cells. Pseudocolor FACS plots are from a representative animal and IFNγ-producing CD4+ (right) and CD8+ (left) cells are gated. Incubation

with h-CLIP peptide served as a negative control (Control). Experiments were performed twice with similar results, error bars represent SEM; ns, no significance.

DISCUSSION

We report development and evaluation of a polyvalent-filoviral vaccine in preclinical rodent immunogenicity and efficacy studies. Complete protection against challenge with gpMARV and gpZEBOV was observed following two DNA vaccine doses in guinea pigs, as well as with a 'single-dose' DNA vaccine in mice against mZEBOV. To date, genetic vaccination of guinea pigs has included either injection of naked DNA (Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ (2000). Development of a preventive vaccine for Ebola virus infection in primates. *Nature* **408**: 605-609) or DNA delivered by gene gun (Dowling W, *et al.* (2006). The influences of glycosylation on the antigenicity, immunogenicity, and protective efficacy of Ebola virus GP DNA vaccines. *J Virol* **81**: 1821-1837; Vanderzanden L, *et al.* (1998). DNA vaccines expressing either the GP or NP genes of Ebola virus protect mice from lethal challenge. *Virology* **246**: 134-144; and Riemenschneider J, *et al* (2003). Comparison of individual and combination DNA vaccines for B. anthracis, Ebola virus, Marburg virus and Venezuelan equine encephalitis virus. *Vaccine* **21**: 4071-4080), however, either method required at least three vaccinations to achieve complete protection. Improved protection herein may be due to the induction of robust Abs since a single DNA vaccination generated GP-specific IgG binder titers that were comparable in magnitude to titers in protected animals following gene gun administration; DNA vaccination induced 3.85 and 2.18 log10 ZGP and MGP-specific Ab titers, respectively, after a single administration versus 2.7 and 3.0 after three gene gun vaccinations. For comparison with an alternative 'single-dose' protective strategy in guinea pigs, an Ag-coupled virus-like particle (VLP) platform generated Ab titers that were only slightly higher than observed following DNA vaccination (Swenson DL, Warfield KL, Negley DL, Schmaljohn A, Aman MJ, Bavari S (2005). Virus-like particles exhibit potential as a pan-filovirus vaccine for both Ebola and Marburg viral infections. *Vaccine* **23**: 3033-3042). Furthermore, a recombinant adenovirus (rAd) approach induced ZGP-specific NAb titers that were lower than those from a single DNA vaccination (53 reciprocal endpoint dilution titer verses 88 herein) (Kobinger GP, *et al.* (2006). Chimpanzee adenovirus vaccine protects against Zaire Ebola virus. *Virology* **346**: 394-401). Vaccination with rVSV (Jones SM, *et al* (2007). Assessment of a vesicular stomatitis virus-based vaccine by use

of the mouse model of Ebola virus hemorrhagic fever. *J Infect Dis* **196 Suppl 2**: S404-412) generated ZGP-specific Ab titers that were similar to the current platform. Altogether, these data demonstrate that DNA vaccination was capable of inducing binding and neutralizing Abs that were comparable to non-replicating viral platforms and that these data may help, in part, to explain strong guinea pig survival data herein.

The generation of NAbs by protective DNA vaccination may have benefitted by transgene-expressed mature GP structures. *In vitro* transfection studies confirmed that the vaccine-encoded GP were highly expressed, post-translationally cleaved (Figure 1B), transported to the cell surface, and sterically occluded the immunodetection of cell surface molecules (Figure 1C). Therefore, it was highly likely that the vaccine immunogens formed herein matured into hetero-trimeric spikes that would otherwise be functional upon virion assembly during infection. This may be important for the generation and display of virologically-relevant neutralizing determinants which would be subsequently critical for the induction of conformation-dependent Nabs (Dowling W, *et al.* (2007). Influences of glycosylation on antigenicity, immunogenicity, and protective efficacy of ebola virus GP DNA vaccines. *J Virol* **81**: 1821-1837; Shedlock DJ, Bailey MA, Popernack PM, Cunningham JM, Burton DR, Sullivan NJ (2010). Antibody-mediated neutralization of Ebola virus can occur by two distinct mechanisms. *Virology* **401**: 228-235). Thus, in this regard, the expression of native anchored structures may be superior to soluble derivatives in the capacity for generating NAbs (Sullivan NJ, *et al.* (2006). Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. *PLoS Med* **3**: el77; Xu L, *et al.* (1998). Immunization for Ebola virus infection. *Nat Med* **4**: 37-42).

To better characterize T cells responses as driven by a protective vaccine, we performed immunogenicity and efficacy studies in mice and determined 'single-dose' complete protection against mZEBOV with DNA vaccination (Figures 5A-5D). To date, the most effective platforms conferring complete protection in this model are VLP, either with (Warfield KL, *et al.* (2005). Induction of humoral and CD8+ T cell responses are required for protection against lethal Ebola virus infection. *J Immunol* **175:** 1184-1191; Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S (2007). Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *J Infect Dis* **196 Suppl 2**: S430-437) or without

(Sun Y, *et al.* (2009). Protection against lethal challenge by Ebola virus-like particles produced in insect cells. *Virology* **383**: 12-21) adjuvant, rAd vaccination ((Kobinger GP, *et al.* (2006) SUPRA; Choi JH, *et al.* (2012). A single sublingual dose of an adenovirus-based vaccine protects against lethal Ebola challenge in mice and guinea pigs. *Mol Pharm* **9**: 156-167; Richardson JS, *et al.* (2009). Enhanced protection against Ebola virus mediated by an improved adenovirus-based vaccine. *PLoS One* 4: e5308), or rRABV vaccination (Blaney JE, *et al.* (2011). Inactivated or live-attenuated bivalent vaccines that confer protection against rabies and Ebola viruses. *J Virol* **85**: 10605-10616). However, characterization of T cell responses were severely limited in these studies and were restricted to splenocyte stimulation with either two (Warfield KL, (2007), SUPRA) or one (Warfield KL, *et al.* (2005) SUPRA) peptides previously described to contain ZGP T cell epitopes (Warfield KL, *et al.* (2005) SUPRA. Olinger GG, *et al.* (2005) SUPRA; Kobinger GP, *et al.* (2006), SUPRA; Sun Y, *et al.* (2009). Choi, JH, *et al.* (2012). Herein, we report induction of robust and broad CTL by protective vaccination as extensively analyzed by a novel modified T cell assay (Figure 4A and Tables 1-6). In total, 52 novel T cell epitopes were identified including numerous immunodominant epitopes occurring primarily in highly conserved regions of GP. Of the 22 total ZGP epitopes identified, only 4 have been previously reported. Moreover, only one of the 20 MGP (Kalina WV, Warfield KL, Olinger GG, Bavari S (2009). Discovery of common marburgvirus protective epitopes in a BALB/c mouse model. *Virol J 6:* 132) and one of 16 SGP epitopes were previously described. As such, this the most comprehensive report of preclinical GP epitopes to date, describing GP epitopes from multiple filoviruses in two different mouse genetic backgrounds.

Another novel finding resulting from these analyses was the assessment of the vaccine-induced subdominant T cell responses, which we show comprised a significant percentage of the total T cell response, widely ranging between 12% - 74% (Figure 4D). This may be particularly important since subdominant responses can significantly contribute to protection. Thus, it may prove informative in the future to determine the specific contributions of the subdominant and immunodominant epitopic T cell responses to protection. Notably, these responses may have otherwise been overlooked using traditional matrix array peptide pools for epitope identification. As such, limited epitope detection in previous studies may have been directly related to lower

levels of vaccine-induced immunity, the use of less sensitive standard assays, and/or the use of peptide arrangements and/or algorithms favoring detection of immunodominant CD8+ epitopes.

Although immune correlates of protection against the filoviruses remain controversial, data generated by this highly immunogenic approach provides a unique opportunity with which to study T cell immunity as driven by a protective vaccine. DNA vaccination herein induced strong ZGP-specific T cells, a large part of which were characterized by T_h1-type multifunctional CTL expressing high levels of T-bet , also shown to correlate with T cell cytotoxicity in humans. It is clear that previous stand-alone DNA vaccine platforms capable of generating mainly humoral immune responses and cellular immunity skewed towards CD4+ T cells may likely benefit from in vivo EP delivery which has been recently demonstrated to induce potent CD8+ T cells in NHPs and the clinic. Thus, data herein are consistent with this approach as a stand-alone or prime-boost modality in NHP immunogenicity and efficacy studies. This approach offers an attractive vaccination strategy that can be quickly and inexpensively modified and/or produced for rapid response during *Filoviridae* bio-threat situations and outbreaks. In addition, this model approach provides an important tool for studying protective immune correlates against filoviral disease and could be applied to existing platforms to guide future strategies.

Example 2

A trivalent vaccine is provided which comprises three plasmids. The first plasmid comprises a nucleic acid sequence that encodes a *Zaire ebolavirus* consensus immunogen which is based upon ZEBOV CON, SEQ ID NO:1, modified to include an IgE signal peptide at the N terminus of the *Zaire ebolavirus* consensus immunogen. The second plasmid comprises a nucleic acid sequence that encodes a *Sudan ebolavirus* consensus immunogen which is based upon SUDV CON, SEQ ID NO:2, modified to include an IgE signal peptide at the N terminus of the *Sudan ebolavirus* consensus immunogen. The third plasmid comprises a nucleic acid sequence that encodes a *Marburg marburgvirus* Angola (MARV immunogen which is based upon MARV ANG, SEQ ID NO:3, modified to include an IgE signal peptide at the N terminus of the *Marburg marburgvirus* Angola immunogen.

Example 3

A five plasmid vaccine is provided. The first plasmid comprises a nucleic acid sequence that encodes a *Zaire ebolavirus* consensus immunogen which is ZEBOV CON, SEQ ID NO:1.

The second plasmid comprises a nucleic acid sequence that encodes a *Sudan ebolavirus* consensus immunogen which is SUDV CON, SEQ ID NO:2. The third plasmid comprises a nucleic acid sequence that encodes SEQ IDNO:4, a *Marburg marburgvirus* - Ravn cluster consensus (MARV-RAV CON) using *Marburg marburgvirus* Ravn, Durba (09DRC99) and Uganda (02Uga07Y). The fourth plasmid comprises a nucleic acid sequence that encodes SEQ IDNO:5, a *Marburg marburgvirus* - Ozolin cluster consensus (MARV-OZO CON) using Ozolin, Uganda (01Uga07), and Durba (05 and 07DRC99). The fifth plasmid comprises a nucleic acid sequence that encodes SEQ IDNO:6, a *Marburg marburgvirus* - Musoke cluster consensus (MARV-MUS CON) using (Musoke, Popp, and Leiden).

Example 4

A five plasmid vaccine is provided. The first plasmid comprises a nucleic acid sequence that encodes a *Zaire ebolavirus* consensus immunogen which is based upon ZEBOV CON, SEQ ID NO:1, modified to include an IgE signal peptide at the N terminus of the *Zaire ebolavirus* consensus immunogen. The second plasmid comprises a nucleic acid sequence that encodes a *Sudan ebolavirus* consensus immunogen which is based upon SUDV CON, SEQ ID NO:2, modified to include an IgE signal peptide at the N terminus of the *Sudan ebolavirus* consensus immunogen. The third plasmid comprises a nucleic acid sequence that encodes *Marburg marburgvirus* Rav consensus based upon SEQ IDNO:4, a *Marburg marburgvirus* - Ravn cluster consensus (MARV-RAV CON) using *Marburg marburgvirus* Ravn Durba (09DRC99) and Uganda (02Uga07Y) and modified to include an IgE signal peptide at the N terminus of the consensus *Marburg marburgvirus* - Rav immunogen. The fourth plasmid comprises a nucleic acid sequence that encodes *Marburg marburgvirus* Ozo consensus based upon SEQ IDNO:5, a *Marburg marburgvirus* - Ozolin cluster consensus (MARV-OZO CON) using Ozolin,Uganda (01Uga07), and Durba (05 and 07DRC99) and modified to include an IgE signal peptide at the N terminus of the consensus *Marburg marburgvirus* – Ozo immunogen. The fifth plasmid comprises a nucleic acid sequence that encodes *Marburg marburgvirus* Mus consensus based upon SEQ IDNO:6, a *Marburg marburgvirus* - Musoke cluster consensus (MARV-MUS CON) using (Musoke, Popp, and Leiden) and modified to include an IgE signal peptide at the N terminus of the consensus *Marburg marburgvirus* – Mus immunogen.

Example 5

A six plasmid vaccine is provided. The first plasmid comprises a nucleic acid sequence that encodes a *Zaire ebolavirus* consensus immunogen which is ZEBOV CON, SEQ ID NO:1. The second plasmid comprises a nucleic acid sequence that encodes a *Sudan ebolavirus* consensus immunogen which is SUDV CON, SEQ ID NO:2. The third plasmid comprises a nucleic acid sequence that encodes SEQ IDNO:4, a *Marburg marburgvirus* - Ravn cluster consensus (MARV-RAV CON) using *Marburg marburgvirus* - Ravn, Durba (09DRC99) and Uganda (02Uga07Y). The fourth plasmid comprises a nucleic acid sequence that encodes SEQ IDNO:5, a *Marburg marburgvirus* - Ozolin cluster consensus (MARV-OZO CON) using Ozolin, Uganda (01Uga07), and Durba (05 and 07DRC99). The fifth plasmid comprises a nucleic acid sequence that encodes SEQ IDNO:6, a *Marburg marburgvirus* - Musoke cluster consensus (MARV-MUS CON) using (Musoke, Popp, and Leiden). The sixth plasmid comprises a nucleic acid sequence that encodes SEQ IDNO:3, a *Marburg marburgvirus* Angola 2005 isolate glycoproteins immunogen.

Example 6

A five plasmid vaccine is provided. The first plasmid comprises a nucleic acid sequence that encodes a *Zaire ebolavirus* consensus immunogen which is based upon ZEBOV CON, SEQ ID NO:1, modified to include an IgE signal peptide at the N terminus of the *Zaire ebolavirus* consensus immunogen. The second plasmid comprises a nucleic acid sequence that encodes a *Sudan ebolavirus* consensus immunogen which is based upon SUDV CON, SEQ ID NO:2, modified to include an IgE signal peptide at the N terminus of the *Sudan ebolavirus* consensus immunogen. The third plasmid comprises a nucleic acid sequence that encodes *Marburg marburgvirus* Rav consensus based upon SEQ IDNO:4, a *Marburg marburgvirus* - Ravn cluster consensus (MARV-RAV CON) using *Marburg marburgvirus* Ravn Durba (09DRC99) and Uganda (02Uga07Y) and modified to include an IgE signal peptide at the N terminusof the consensus *Marburg marburgvirus* - Rav immunogen. The fourth plasmid comprises a nucleic acid sequence that encodes *Marburg marburgvirus* Ozo consensus based upon SEQ IDNO:5, a *Marburg marburgvirus* - Ozolin cluster consensus (MARV-OZO CON) using Ozolin,Uganda (01Uga07), and Durba (05 and 07DRC99) and modified to include an IgE signal peptide at the N terminusof the consensus *Marburg marburgvirus* – Ozo immunogen. The fifth plasmid

comprises a nucleic acid sequence that encodes *Marburg marburgvirus* Mus consensus based upon SEQ IDNO:6, a *Marburg marburgvirus* - Musoke cluster consensus (MARV-MUS CON) using (Musoke, Popp, and Leiden) and modified to include an IgE signal peptide at the N terminusof the consensus *Marburg marburgvirus* – Mus immunogen. The sixth plasmid comprises a nucleic acid sequence that encodes a *Marburg marburgvirus* Angola 2005 isolate glycoproteins immunogen which is based upon MARV ANG, SEQ ID NO:3, modified to include an IgE signal peptide at the N terminus of the *Marburg marburgvirus* Angola immunogen.

Example 7

Described herein is a DNA vaccine formulation expressing 3 synthetic Zaire Ebola virus (EBOV) glycoproteins (GP): 2 designed based on GP sequence alignments (1976-2014) and a 3rd construct matched to a 2014 outbreak strain. Plasmid IL-12 (pIL-12) was also included as an adjuvant to further enhance cellular immune responses. The multivalent GP DNA vaccine formulation was administered in macaques following a DNA-DNA prime-boost immunization regimen. Macaques (n=3 or 4/group) received the multivalent GP DNA formulation + pIL-12 by intramuscular delivery followed by electroporation. Differences in immunogenicity were assayed and protection between different doses, regimens (2, 3, 4, and 5 injections), and different spacing intervals between subsequent doses were monitored. Both antibody and T cell responses were observed in 83% of animals 2 weeks following the first injection and 100% of animals after the 2nd injection. The macaques were challenged with a lethal dose of the EBOV Guinea-Makona outbreak strain (1000pfu, 7-U virus) and monitored for 28 days following infection. 100% of animals receiving at least 3 injections at 4 week intervals survived lethal challenge. Animals were fully protected against signs of disease and did not exhibit elevated blood chemistry. Interestingly, 50% of animals receiving 2 injections survived lethal challenge. The surviving animals exhibited minimal signs of disease, suggesting that with further optimization complete protection with 2 injections is potentially achievable. In additional optimization studies in mice, single injections were found to be 100% protective and long-term immune responses 8 months post vaccination were induced.

METHODS

Developing an EBOV GP DNA vaccine formulation

Vaccines currently in clinical trials include rVSVΔG/ZEBOVGP, ChAd3 prime + MVA boost, and MVA pan-filovirus. While these vaccines are immunogenic, protective in non-human primates (NHPs), and provide single dose protection, they develop anti-vector immunity, have uncertain duration of memory response, give adverse reactions in human clinical trials and may not be suitable for all populations. Thus, an additional platform with a cleaner safety profile that can induce strong immune responses against heterologous Zaire Ebola viruses would be very beneficial (Figure 9).

Three EBOV DNA constructs were designed: a consensus sequence of ZEBOV (1976-1996) (ConEBOVGP#1), ZEBOV (2002-2008) (ConEBOVGP#2), and a matched ZEBOV sequence from the 2014 Guinea outbreak. (Guinea-GP). Five vaccines were developed, monovalent vaccines which comprise only a single DNA construct, a bivalent vaccine formulation which comprise either ConEBOVGP#1 with Guinea-GP and a trivalent formulation which comprises all three of ConEBOVGP#1, ConEBOVGP#2, and Guinea-GP (Figure 10).

RESULTS

A single immunization of DNA vaccine is immunogenic in mice

BALB/c mice received a single intramusclular (IM) immunization followed by electroporation (EP) of the vaccine formulation. On day 28, Total IgG antibody titer and ELISPOT-IFNγ were determined. Each vaccine formulation produced a robust IgG response. Polyfunctional CD4+ and CD8+ T cells secreting IFNγ, IL2, and TNFα were similar for all vaccines and formulations. (Figure 11).

A single immunization is fully protective in mice against lethal mouse-adapted Ebola virus challenge

BALB/c mice immunized with bivalent or trivalent vaccine formulations were lethal challenged with $1000LD_{50}$ of heterologous Ebolavirus. The challenge strain was mouse adapted-Ebola Mayinga 1976. Mice vaccinated with the control plasmid, pVax1, quickly lost weight and did not survive past day 7. However, both the mice vaccinated with the bivalent and mice

vaccinated with the trivalent vaccine formulations maintained their weight and had no deceased mice up to day 20 (Figure 12).

Individual GP DNA Vaccine constructs induce robust memory responses in mice.

BALB/c mice received a 3xIM 40μg immunization of a monovalent vaccine followed by electroporation on days 0, 28 and 84. IgG antibody titer, INFγ, CD4+CD44+ memory T cells, and CD8+CD44+ memory T cells were measured. Robust immune responses were detectable months after last injection (Figure 13).

GP DNA vaccine formulations are immunogenic in NHPs

Cynomolgus macaques were used as a NHP because they are a model for Ebola vaccine efficacy and lethal challenge. Macques were administered either a bivalent formulation with a Rhesus pIL12 adjuvant or a trivalent formulation with a Rhesus pIL12 adjuvant I.M. followed by EP. Different injection regimens in order to understand immunogenicity. Group 1 received 2 IM-EP injections of the bivalent formulation at a 4 week interval. Group 2 received 2 IM-EP injections of the trivalent formulation at a 4 week interval. Group 3 received 3 IM-EP injections of the trivalent formulation. Samples for immunogenicity studies were taken monthly and for one additional month following the last dose. (Figure 6). Each DNA vaccine formulation induced robust anti-Ebola GP antibody responses (GMT $>10^3$) & anti-GP T-cell responses. The immune responses were boosted following each injection (Figure 14).

GP DNA formulation vaccines protect against lethal Zaire Ebola virus (Makona) challenge

The Cynomolgus macaques from groups 1, 2 and 3 were challenged with a 1000 TCID50 Guinea-Makona 2014 C07 virus (7-U reference strain) dose 28 days post-final DNA immunization. Animals were monitored for 28 days post-challenge. While none of the control animals survived past day 10 post challenge, 4/4 group 3 animals survived 28 days post challenge, 4/8 group 2 animals survived 28 days post challenge and 3/4 group 1 animals survived 28 days post challenge (Figure 15). Surviving animals did not have any significant signs of disease. They also maintained normal CBC and enzyme levels. Bivalent and Trivalent DNA vaccines delivered by IM-EP elicit long-term antibody and T cell responses that were detectable >3months post final DNA injection (Figure 43).

Three doses of a Trivalent EBOV GP DNA vaccine is 100% protective against lethal EBOV challenge. Two doses of a Bivalent EBOV GP DNA vaccine affords 75% protection.

Overall, the data supports further study of DNA vaccines, delivered by IM-EP, for possible administration against Ebola and other infectious pathogens

EBOV-001 phase I clinical

An Open-Label study of INO-4212 (with or without INO-9012 was conducted. INO-4212 was administered IM or ID followed by electroporation in healthy volunteers. Safety and immunological assessments were monitored. Intradermal delivery and intramuscular delivery were compared. There were 69 total subjects. ELISA analysis was performed before immunization (baseline), and at weeks 2, 6, and 14. Seriopositive is defined as a positive IgG antibody response to Ebola Zaire glycoprotein.

INO-4201 is a DNA vaccine formulated with the consensus envelope glycoprotein of Zaire Ebolavirus (ConEBOVGP#1) generated by using the envelope glycoprotein sequences of the 1976, 1994, 1995, 1996, 2003, 2005, 2007 and 2008 outbreak strains, driven by a human CMV promoter (hCMV promoter) with the bovine growth hormone 3'end poly-adenylation signal (bGH polyA). pGX4201 was made by cloning the synthetic consensus envelope glycoprotein gene of Zaire Ebolavirus into pGX0001 at the BamHI and XhoI sites.

The ConEBOVGP#1 (ConGP1) sequence was constructed by generating a consensus envelope glycoprotein sequence of Zaire Ebolavirus using the envelope glycoprotein sequences of the 1976, 1994, 1995, 1996, 2003, 2005, 2007 and 2008 outbreak strains. Briefly, a consensus GP sequence was first generated based on six envelope sequences of the 1976, 1994, 1995, 1996, 2003 and 2005 outbreak strains. Then three non-consensus residues at the positions 377, 430 and 440 were weighted towards the 2003, 2005, 2007 and 2008 strains since they were the most recent and lethal outbreaks with published sequence data. The GenBank accession numbers for selected outbreak strain GP sequences are: Q05320, P87671, AAC57989, AEK25495, ABW34743, P87666, AER59718, AER59712, ABW34742, AAL25818. Once the consensus GP1 sequence was obtained, an upstream Kozak sequence was added to the N-terminal. Furthermore, in order to have a higher level of expression, the codon usage of this gene was adapted to the codon bias of Homo sapiens genes. In addition, RNA optimization was also

performed: regions of very high (>80%) or very low (<30%) GC content and the cis-acting sequence motifs such as internal TATA boxes, chi-sites and ribosomal entry sites were avoided. The synthesized ConGP1 was digested with BamHI and XhoI, and cloned into the expression vector.

INO-4202 is a DNA vaccine formulated with a DNA plasmid expressing the envelope glycoprotein of Zaire Ebolavirus isolated from the 2014 outbreak in Guinea (GuineaGP), driven by a human CMV promoter (hCMV promoter) with the bovine growth hormone 3'end poly-adenylation signal (bGH polyA).

INO-4212 is a bivalent vaccine of INO-4201 and INO-4202.

Figure 17 depicts the vaccine formulation schedule, route and dose for each cohort. After the first injection, 15% or less of the patients was seriopositive. After the second injection, 50-100% of the patients were seriopositive. After the third injection 79-100% of the patients were seriopositive (Figure 18). Two representative patients with a moderate IFNγ ELISpot, or a high IFNγ ELISpot showed specific T-cell responses (Figure 19).

While other Ebola vaccines platforms, including NIAID VRC/GSK and rVSV/ZEBOVGP, are currently in clinical trials the present bivalent and trivalent vaccines described herein have advantages not observed in the other vaccine platforms. For example, the bi- or tri-valent vaccines can be administered IM or ID, while the other vaccine platforms are only administered IM. Importantly, NIAID VRC/GSK and rVSV/ZEBOVGP show side effects including fever, fatigue, arthralgia, and lymphopenia while the bi- and tri-valent vaccines do not show any side effects. It should be noted however, that some of the side effects of rVSV/ZEBOVGP and ChAd3/MVAGP overlap with symptoms of Ebola. Further the bi- and tri-valent vaccines give antibody titers one to two orders of magnitude larger than rVSV/ZEBOVGP and ChAd3/MVAGP (Figure 20).

Subjects (n=15) were assigned to receive INO-4201 at a 2 mg DNA/dose given as two separate 1 mg (0.1 mL) ID (Mantoux) injections followed by EP with the CELLECTRA®-3P device. Subjects received a 3-dose series with immunizations at 0, 4 weeks, and 12 weeks (0-4-12 week schedule). Antibodies specific for EBOV glycoprotein (GP) were measured from the sera of vaccinated subjects with a binding ELISA. Reciprocal endpoint titers above Day 0 are

shown two weeks post each immunization (Figure 22). 100% of subjects vaccinated with INO-4201 seroconverted after 2 immunizations (Figure 22, Cohort 3 and Figure 24).

Example 8

Described herein is immune response data for the three EBOV DNA constructs described in Example 7: a consensus sequence of ZEBOV (1976-1996) (ConEBOVGP#1 or INO-4201), ZEBOV (2002-2008) (ConEBOVGP#2), and a matched ZEBOV sequence from the 2014 Guinea outbreak. (Guinea-GP). Five vaccines were developed, monovalent vaccines which comprise only a single DNA construct, a bivalent vaccine formulation which comprise either ConEBOVGP#1 with Guinea-GP and a trivalent formulation which comprises all three of ConEBOVGP#1, ConEBOVGP#2, and Guinea-GP. 69 Subjects from cohorts 1-5 were included in analysis of immune response by ELISA and 75 subjects from cohorts 1-5 were included in analysis of immune response by ELISpot (Figures 21, 25).

ELISA Titers by Cohort and Timepoint

Titers of anti-EBOVR were determined for each cohort at weeks 2, 6 and 14. By week 6, each cohort saw an increase in antibody titer above Day 0 (Figures 22-23). There was little to no reactivity for the first dose in each cohort. Dose 2 begins to drive seroconversion, with Cohorts 3 and 5 seeing the largest frequency. Dose three drives >90% serconversion in 4/5 cohorts. Cohorts 3 and 5 show 100% seroconversion at this time point (Figure 24)

Subject responses by peptide pool

Cohort responses were analyzed by peptide pool (Figures 26-33). To analyze ELISpot outliers, day 0 values for each pool and total EBOV responses were used to create an outlier threshold (Mean day 0 values +(3x STDEV of day 0 values)). This threshold should encompass 99% of a normally distributed population. Any subject that displayed baseline values greater than the outlier threshold was removed and a responder criteria was generated with the remaining subjects.

ICS Analysis

47 subjects from all cohorts were included in the analysis, however cohort 5 is underrepresented (Figure 35). ICS analysis performed at baseline and week 14. A single EBOV

peptide pool composed of Pools 1-4 is used for stimulation. Analysis of T cell activity in the form of IFNg or TNFa production from both CD4 and CD8 compartments suggests significant elevation of TNFa in both CD4 and CD8 compartments as well as elevation of TNFa and/or IFNg in Cohort 3 only (Wilcoxon matched paired analysis, two-tailed)

Immunology Summary

100% of Cohort 3 (ID) patients seroconverted after 2 doses. 92% of Cohort 5 (IM+IL12) patients seroconverted after 2 doses and 100% after 3 doses. Other cohorts showed 67% at best after 2 doses and ranged as high as 93% after 3 doses.

When analyzing all patients: the best response frequency were Cohorts 2 and 4 with 53% and 57% respectively. Cohort 3 showed 40% responders. Addition of IL-12 in Cohort 5 did not seem to influence response rates (47%). When analyzing patients with 8 outliers removed the response frequency were Cohorts 2 and 4 with 84.6% and 76.9% respectively. Cohort 3 showed 64.3% responders. Addition of IL-12 in Cohort 5 did not seem to influence response rates (53.3%).

Both CD4 and CD8 T cells showed high expression of TNFa and TNFa and/or IFNg in Cohort 3 (statistically significant to baseline, Wilcoxon matched pairs test, 2 tailed).

Immunization with INO-4201 was well tolerated in healthy volunteers with no Grade 3 or Grade 4 SAEs noted. INO-4201 induced robust Ebola GP-specific antibody (GMT 46,968) and resulted in 100% seroconversion, as gauged by binding ELISA, after only two doses of INO-4201. Administration of INO-4201 generated EBOV GP specific T cell responses as assessed by Interferon gamma (IFNγ) ELISpot (295.3 SFU per 10^6 PBMCs) and significant increases in in the production of IFNγ or TNFα in both the CD8+ T and CD4+ T cell compartments. Intradermal administration of INO-4201 using the Cellectra device is both well tolerated and immunogenic as assessed by both humoral and cellular EBOV GP-specific immunoassays. These results indicate that INO-4201 is a strong candidate for further clinical development of a prophylactic Ebola vaccine.

Example 9 - Lasting humoral and cellular immune responses in cynomolgus macaques following administration of a Zaire Ebola virus (EBOV) GP DNA vaccine delivered by intramuscular electroporation

Presented herein are novel Ebola virus disease (EVD) DNA vaccines that have a clean safety profile and are serology independent, allowing for possible repeat vector administration. Three novel synthetic Zaire Ebola virus (EBOV) GP DNA vaccines were designed and Bivalent, or Trivalent formulations were developed. Both EBOV-GP DNA vaccines were highly protective (75-100%) against lethal EBOV Makona C07 challenge in cynomolgus macaques. Animals (n=4-5/group) with different regimens were followed to monitor long-term immunogenicity following DNA immunization. All NHPs rapidly seroconverted. NHPs have durable total IgG antibody titers and T cells responses to EBOV GP antigen, including polyfunctional CD4 and CD8 T cells expressing IFNγ, IL2, and TNFα and responses in memory subset populations (Figures 47-59). Together, the data strong support EBOV-GP DNA vaccine delivery for protection and the generation of robust memory immune responses.

The EBOV-GP Dan vaccines elicit long-term immune responses and have a strong recall response following a 1 year boost (Figures 49-50). The recall response was remarkably high in the group receiving a single IM injection (Figure 50).

Example 10

Presented herein are the peptide sequences and the nucleic acid sequences for the peptides.

TABLE 1

Plasmid Vaccine pMARV
GP sequence MARV ANG

Peptide Number	Sequence	SEQ ID NO:	Position	H-2	ELISPOT AVE	±SEM	FACS T cell restr.
3	IQGVKTLPILEIASN	7	13-27	d	62	34	4+
5	ASNIQPQNVDSVCSG	8	25-39	b	743	186	8+
12	SKRWAFRAGVPPKNV	9	67-81	b	694	204	4+
27	GKVFTEGNIAAMIVN	10	157-171	d	602	75	4+
28	GNIAAMIVNKTVHKM	11	163-177	b/d	126	28	8+
	GNIAAMIVNKTVHKM	12		d	30	10	4+

29	IVNKTVHKMIFSRQG	13	169-183	d	92	17	4+
30	HKMIFSRQGQGYRHM	14	175-189	d	31	10	4+
31	RQGQGYRHMNLTSTN	15	181-195	b	674	112	8+
32	RHMNLTSTNKYWTSS	16	187-201	b	44	16	8+
65	LPTENPTTAKSTNST	17	385-399	b/d	398/16	107/2	4+
71	PNSTAQHLVYFRRKR	18	421-435	d	29	6	4+
72	HLVYFRRKRNILWRE	19	427-441	d	145	18	4+
89	GLSWIPFFGPGIEGL	20	529-543	b	26	8	4+
92	GLIKNQNNLVCRLRR	21	547-561	d	29	10	4+
93	NNLVCRLRRLANQTA	22	553-567	d	34	13	4+
97	TTEERTFSLINRHAI	23	577-591	b	46	18	8+
99	HAIDFLLARWGGTCK	24	589-603	d	63	12	4+
101	TCKVLGPDCCIGIED	25	601-615	b	97	37	4+

"Epitope-containing peptides were identified by IFNγ ELISPOT (≥ 10 SFC/10^6 splenocytes AND ≥ 80% response rate) and then confirmed by FACS (≥ 3-5x10^4 CD3+ cells were acquired). Responses for each were further characterized by FACS (expression of CD4 and/or CD8 by CD3+/CD44+/IFNγ+ cells). Predicted CD8+ epitopes are underlined (best consensus % rank by IEDB) and previously-described epitopes are referenced. Immunodominant epitopes are displayed (*).

TABLE 2
Plasmid Vaccine pEBOS
GP sequence SUDV CON

Peptide Number	Sequence	SEQ ID NO:	Position	H-2	ELISPOT AVE	±SEM	FACS T cell restr.
4	FFVWVIILFQKAFSM	26	19-33	b	310	139	8+
15	RWGFRSGVPPKVVSY	27	85-99	b	108	59	4+
19	YNLEIKKPDGSECLP	28	109-123	b	55	25	4+
24	HKAQGTGPCPGDYAF	29	139-153	d	13	3	8+
27	GAFFLYDRLASTVIY	30	157-171	d	29	9	8+
30	NFAEGVIAFLILAKP	31	175-189	d	31	6	4+
36	SYYATSYLEYEIENF	32	211-225	b	60	16	4+
41	FVLLDRPHTPQFLFQ	33	241-255	d	338	55	8+
78	NITTAVKTVLPQEST	34	463-477	b/d	28/105	12/18	4+
82	TGILGSLGLRKRSRR	35	487-501	d	82	14	4+
83	LGLRKRSRRQVNTRA	36	493-507	d	69	12	4+
89	IAWIPYFGPGAEGIY	37	529-543	b	123	40	8+/4+
97	TELRTYTILNRKAID	38	577-591	d	12	5	4+
101	CRILGPDCCIEPHDW	39	601-615	b	80	41	4+
105	QIIHDFIDNPLPNQD	40	625-639	b	28	23	4+
110	GIGITGIIIAIIALL	41	655-669	b	27	19	8+

"Epitope-containing peptides were identified by IFNγ ELISPOT (≥ 10 SFC/10^6 splenocytes AND ≥ 80% response rate) and then confirmed by FACS (≥ 3-5x10^4 CD3+ cells were acquired). Responses for each were further characterized by FACS (expression of CD4 and/or CD8 by CD3+/CD44+/IFNγ+ cells). Predicted CD8+ epitopes are underlined (best consensus % rank by IEDB) and previously-described epitopes are referenced. Immunodominant epitopes are displayed (*).

TABLE 3
Plasmid Vaccine pEBOZ
GP sequence ZEBOV CON

Peptide Number	Sequence	SEQ ID NO:	Position	ELISPOT H-2	AVE	±SEM	FACS T cell restr.
6	FSIPLGVIHNSTLQV	42	31-45	d	78	31	8+
15	RWGFRSGVPPKVVNY	43	85-99	b	44	12	4+
19	YNLEIKKPDGSECLP	44	109-123	b	29	12	4+
24	HKVSGTGPCAGDFAF	45	139-153	d	484	85	8+
27	GAFFLYDRLASTVIY	46	157-171	d	72	18	8+
30	TFAEGVVAFLILPQA	47	175-189	d	581	85	4+
32	PQAKKDFFSSHPLRE	48	187-201	b	18	6	4+
33	FFSSHPLREPVNATE	49	193-207	b	21	8	4+
40	EVDNLTYVQLESRFT	50	235-249	d	32	17	4+
41	YVQLESRFTPQFLLQ	51	241-255	d	97	23	4+
48	TTIGEWAFWETKKNL	52	283-297	d	219	70	4+
49	AFWETKKNLTRKIRS	53	289-303	d	32	15	4+
50	KNLTRKIRSEELSFT	54	295-309	d	105	37	4+
60	SQGREAAVSHLTTLA	55	355-369	b	16	7	4+
65	DNSTHNTPVYKLDIS	56	385-399	d	29	18	4+
66	TPVYKLDISEATQVE	57	391-405	d	371	118	4+
71	PPATTAAGPPKAENT	58	421-435	b	21	8	4+
84	TRREAIVNAQPKCNP	59	499-513	b	12	5	8+
89	LAWIPYFGPAAEGIY	60	529-543	b	93	8	8+/4+
97	TELRTFSILNRKAID	61	577-591	b/d	14/82	4/42	8+
101	CHILGPDCCIEPHDW	62	601-615	b	96	62	4+

"Epitope-containing peptides were identified by IFNγ ELISPOT (≥ 10 SFC/10^6 splenocytes AND ≥ 80% response rate) and then confirmed by FACS (≥ 3-5x10^4 CD3+ cells were acquired). Responses for each were further characterized by FACS (expression of CD4 and/or CD8 by CD3+/CD44+/IFNγ+ cells). Predicted CD8+ epitopes are underlined (best consensus % rank by IEDB) and previously-described epitopes are referenced. Immunodominant epitopes are displayed (*).

TABLE 4
Plasmid Vaccine pMARV
GP sequence MARV ANG

Best con % rank (IEDB)

Peptide Number	Sequence	SEQ ID NO:	CD8+ (≤0.5)					CD4+ (<25)			Previously defined (80% Blast; Allele
			D^b	K^b	D^d	K^d	L^d	I-A^b	I-A^d	I-E^d	
3	IQGVKTLPILEIASN	7							12.1		
5	ASNIQPQNVDSVCSG	8	0.4								
12	SKRWAFRAGVPPKNV	9						0.8			
27	GKVFTEGNIAAMIVN	10						12.9			
28	GNIAAMIVNKTVHKM	11	0.2						3.9		H-2^d class I
	GNIAAMIVNKTVHKM	12	0.2						3.9		
29	IVNKTVHKMIFSRQG	13							17.2		
30	HKMIFSRQGQGYRHM	14									
31	RQGQGYRHMNLTSTN	15		0.1					23.9		
32	RHMNLTSTNKYWTSS	16									
65	LPTENPTTAKSTNST	17						24.0			
71	PNSTAQHLVYFRRKR	18								7.5	
72	HLVYFRRKRNILWRE	19				0.3				8.3	
89	GLSWIPFFGPGIEGL	20						7.0			
92	GLIKNQNNLVCRLRR	21									
93	NNLVCRLRRLANQTA	22							13.3		
97	TTEERTFSLINRHAI	23	0.1			0.4					
99	HAIDFLLARWGGTCK	24								21.8	
101	TCKVLGPDCCIGIED	25			0.4						

"Epitope-containing peptides were identified by IFNγ ELISPOT (≥ 10 SFC/10^6 splenocytes AND ≥ 80% response rate) and then confirmed by FACS (≥ 3-5x10^4 CD3+ cells were acquired). Responses for each were further characterized by FACS (expression of CD4 and/or CD8 by CD3+/CD44+/IFNγ+ cells). Predicted CD8+ epitopes are underlined (best consensus % rank by IEDB) and previously-described epitopes are referenced. Immunodominant epitopes are displayed (*).

TABLE 5
Plasmid Vaccine pEBOS
GP sequence SUDV CON

Best con % rank (IEDB)

Peptide Number	Sequence	SEQ ID NO:	CD8+ (≤0.5) D^b	K^b	D^d	K^d	L^d	CD4+ (<25) I-A^b	I-A^d	I-E^d	Previously defined (80% Blast; Allele
4	FFVWVIILFQKAFSM	26		0.4							
15	RWGFRSGVPPKVVSY	27						1.2			
19	YNLEIKKPDGSECLP	28									
24	HKAQGTGPCPGDYAF	29					0.3				
27	GAFFLYDRLASTVIY	30		0.3				21.1		23.4	H-2^b class I
30	NFAEGVIAFLILAKP	31			0.1						
36	SYYATSYLEYEIENF	32	0.4		0.3	0.1					
41	FVLLDRPHTPQFLFQ	33					0.1				
78	NITTAVKTVLPQEST	34							7.2		
82	TGILGSLGLRKRSRR	35								17.2	
83	LGLRKRSRRQVNTRA	36									
89	IAWIPYFGPGAEGIY	37			0.1			3.0			
97	TELRTYTILNRKAID	38	0.1						18.5	21.2	
101	CRILGPDCCIEPHDW	39									
105	QIIHDFIDNPLPNQD	40	0.3								
110	GIGITGIIIAIIALL	41									

"Epitope-containing peptides were identified by IFNγ ELISPOT (≥ 10 SFC/10^6 splenocytes AND ≥ 80% response rate) and then confirmed by FACS (≥ 3-5x10^4 CD3+ cells were acquired). Responses for each were further characterized by FACS (expression of CD4 and/or CD8 by CD3+/CD44+/IFNγ+ cells). Predicted CD8+ epitopes are underlined (best consensus % rank by IEDB) and previously-described epitopes are referenced. Immunodominant epitopes are displayed (*).

TABLE 6
Plasmid Vaccine pEBOZ
GP sequence ZEBOV CON

Best con % rank (IEDB)

			CD8+ (≤0.5)					CD4+ (<25)			Previously defined (80% Blast; Allele
Peptide Number	Sequence	SEQ ID NO:	D^b	K^b	D^d	K^d	L^d	I-A^b	I-A^d	I-E^d	
6	FSIPLGVIHNSTLQV	42					0.2				H-2^d class I
15	RWGFRSGVPPKVVNY	43						1.2			H-2^d class I
19	YNLEIKKPDGSECLP	44									
24	HKVSGTGPCAGDFAF	45					0.1	14.9			
27	GAFFLYDRLASTVIY	46		0.3				21.1		23.4	
30	TFAEGVVAFLILPQA	47			0.2				21.6		
32	PQAKKDFFSSHPLRE	48	0.1	0.4				16.4			
33	FFSSHPLREPVNATE	49						14.7			
40	EVDNLTYVQLESRFT	50				0.4			19.6		H-2^b class I
41	YVQLESRFTPQFLLQ	51									H-2^k class I
48	TTIGEWAFWETKKNL	52								12.9	
49	AFWETKKNLTRKIRS	53								22.9	
50	KNLTRKIRSEELSFT	54							22.7		
60	SQGREAAVSHLTTLA	55	0.3					23.1	3.9		
65	DNSTHNTPVYKLDIS	56									
66	TPVYKLDISEATQVE	57						22.6	5.5		
71	PPATTAAGPPKAENT	58						2.1			
84	TRREAIVNAQPKCNP	59	0.3					14.6	7.9		
89	LAWIPYFGPAAEGIY	60			0.1			0.8			
97	TELRTFSILNRKAID	61	0.1							22.2	
101	CHILGPDCCIEPHDW	62									

"Epitope-containing peptides were identified by IFNγ ELISPOT (≥ 10 SFC/10^6 splenocytes AND ≥ 80% response rate) and then confirmed by FACS (≥ 3-5x10^4 CD3+ cells were acquired). Responses for each were further characterized by FACS (expression of CD4 and/or CD8 by CD3+/CD44+/IFNγ+ cells). Predicted CD8+ epitopes are underlined (best consensus % rank by IEDB) and previously-described epitopes are referenced. Immunodominant epitopes are displayed (*).

CLAIMS

1. An isolated nucleic acid molecule comprising one or more nucleic acid sequences selected from the group consisting of a nucleic acid encoding a first consensus Zaire ebolavirus envelope glycoprotein immunogen (ZEBOVCON), a nucleic acid encoding a second consensus Zaire ebolavirus envelope glycoprotein immunogen (ZEBOVCON2), and a nucleic acid encoding a ZEBOV Guinea 2014 Outbreak envelope glycoprotein immunogen (ZEBOVGUI), wherein

the ZEBOVCON comprises an amino acid sequence that is at least 95% homologous to SEQ ID NO:1, or a fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:1;

the ZEBOVGUI comprises an amino acid sequence that is at least 95% homologous to SEQ ID NO:67, or a fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:67; and

the ZEBOVCON2 comprises an amino acid sequence that is at least 95% homologous to SEQ ID NO:68, or a fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:68.

2. The isolated nucleic acid molecule of claim 1, wherein the ZEBOVCON comprises an amino acid sequence that is at least 99% homologous to SEQ ID NO:1, or a fragment of an amino acid sequence that is at least 99% homologous to SEQ ID NO:1.

3. The isolated nucleic acid molecule of claim 1, wherein the ZEBOVCON comprises an amino acid sequence of SEQ ID NO:1, or a fragment of SEQ ID NO:1.

4. The isolated nucleic acid molecule of claim 1, wherein the ZEBOVGUI comprises an amino acid sequence that is at least 99% homologous to SEQ ID NO: 67, or a fragment of an amino acid sequence that is at least 99% homologous to SEQ ID NO: 67.

5. The isolated nucleic acid molecule of claim 1, wherein the ZEBOVGUI comprises an amino acid sequence of SEQ ID NO: 67, or a fragment of SEQ ID NO: 67.

6. The isolated nucleic acid molecule of claim 1, wherein the ZEBOVCON2 comprises an amino acid sequence that is at least 99% homologous to SEQ ID NO: 68, or a fragment of an amino acid sequence that is at least 99% homologous to SEQ ID NO: 68.

7. The isolated nucleic acid molecule of claim 1, wherein the ZEBOVCON2 comprises an amino acid sequence of SEQ ID NO: 68, or a fragment of SEQ ID NO: 68.

8. The isolated nucleic acid molecule of claim 1, wherein the fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:1 comprises at least 600 amino acids, at least 630 amino acids, or at least 660 amino acids.

9. The isolated nucleic acid molecule of claim 1, wherein the fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:67 comprises at least 600 amino acids, at least 630 amino acids, or at least 660 amino acids.

10. The isolated nucleic acid molecule of claim 1, wherein the fragment of an amino acid sequence that is at least 95% homologous to SEQ ID NO:68 comprises at least 600 amino acids, at least 630 amino acids, or at least 660 amino acids.

11. The isolated nucleic acid molecule of claim 1, ZEBOVCON is linked to an IgE signal peptide.

12. The isolated nucleic acid molecule of claim 1, ZEBOVGUI is linked to an IgE signal peptide.

13. The isolated nucleic acid molecule of claim 1, ZEBOVCON2 is linked to an IgE signal peptide.

14. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid encoding ZEBOVCON comprises a nucleic acid sequence at least 95% homologous SEQ ID NO:69, or a fragment thereof.

15. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid encoding ZEBOVGUI comprises a nucleic acid sequence at least 95% homologous SEQ ID NO:72, or a fragment thereof.

16. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid encoding ZEBOVCON2 comprises a nucleic acid sequence at least 95% homologous SEQ ID NO:70, or a fragment thereof.

17. A composition comprising a nucleic acid molecule of claim 1.

18. The composition of claim 18, wherein the composition comprises two nucleic acid molecules.

19. The composition of claim 18, wherein the composition comprises three nucleic acid molecules.

20. The composition of any of claims 1 wherein the nucleic acid molecule is a plasmid.

21. The composition of any of claims 1-20 formulated for delivery to an individual using electroporation.

22. The composition of any of claims 1-20 further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

23. A method of inducing an immune response against a filovirus comprising administering the composition of any of claims 1-20 to an individual in an amount effective to induce an immune response in said individual.

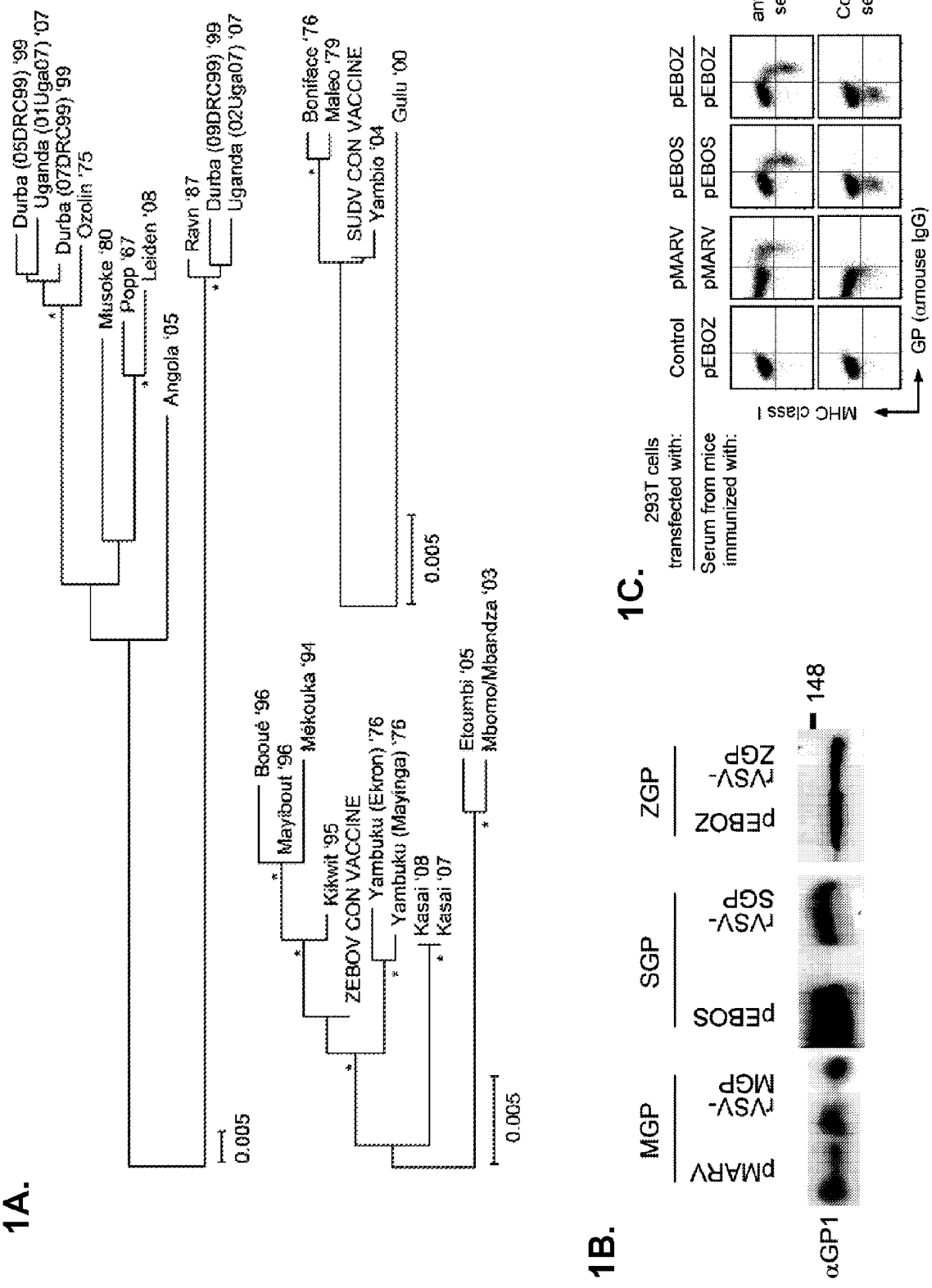
24. A method of inducing an immune response against an Ebolavirus, the method comprising administering the composition of any of claims 1-20 to an individual in an amount effective to induce an immune response in said individual.

25. A method of treating an individual who has been diagnosed with Ebolavirus comprising administering a therapeutically effective amount of the composition of any of claims 1-20 to an individual.

26. A method of treating an individual who has been diagnosed with Ebolavirus, the method comprising administering a therapeutically effective amount of the composition of any of claims 1-20 to an individual.

27. A method of preventing Ebolavirus infection in an individual comprising administering a prophylactically effective amount of the composition of any of claims 1-20 to an individual.

28. A method of preventing Ebolavirus infection in an individual, the method comprising administering a prophylactically effective amount of the composition of any of claims 1-20 to an individual.



Figures 1A-1C

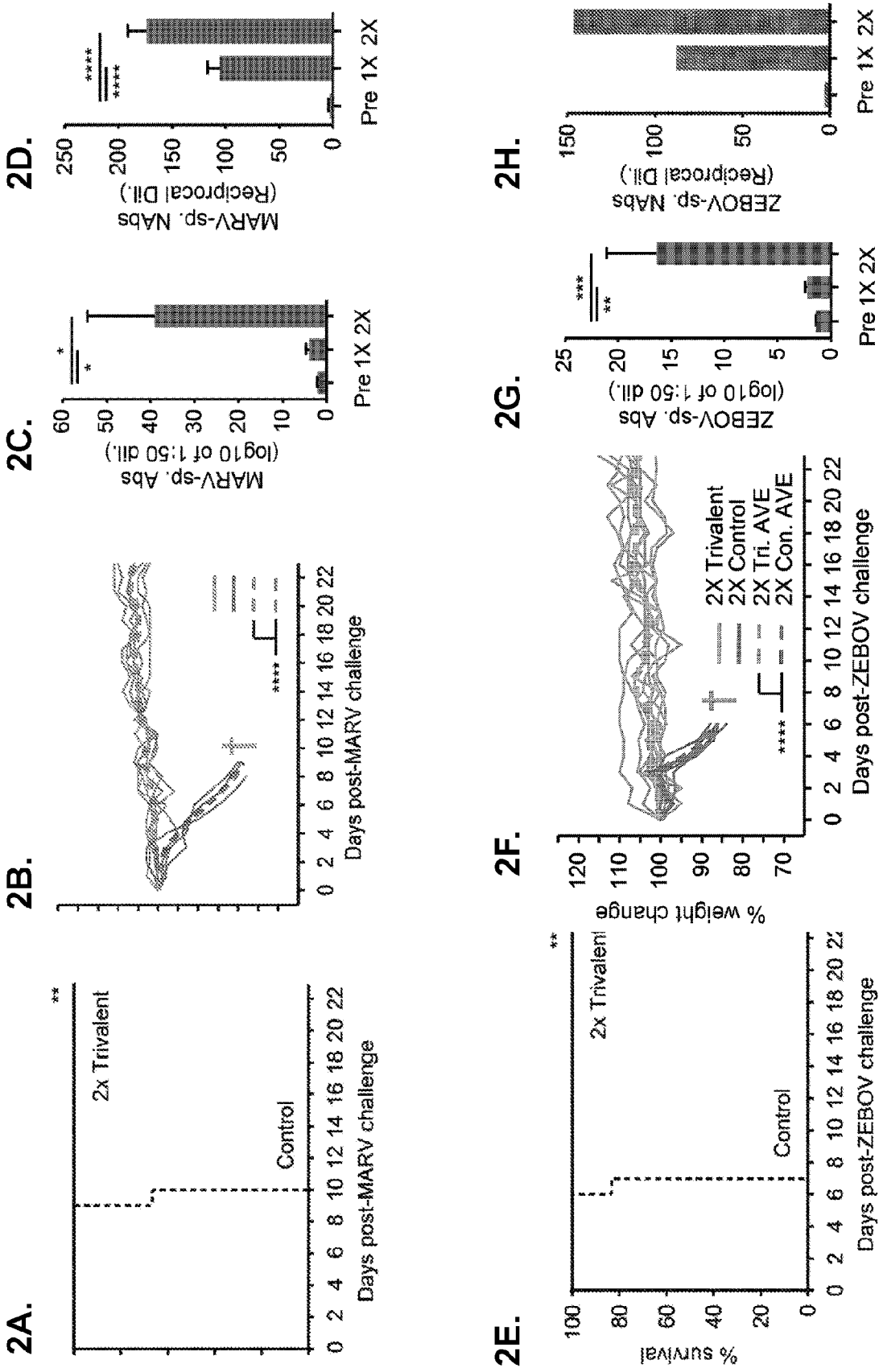

2A.
2x Trivalent
Control
**
Days post-MARV challenge
2B.
2X Trivalent
2X Control
2X Tri. AVE
2X Con. AVE
****
Days post-MARV challenge
2C.
MARV-sp. Abs
(log10 of 1:50 dil.)
Pre 1X 2X
2D.
MARV-sp. NAbs
(Reciprocal Dil.)
Pre 1X 2X
2E.
% survival
2x Trivalent
Control
Days post-ZEBOV challenge
2F.
% weight change
Days post-ZEBOV challenge
2G.
ZEBOV-sp. Abs
(log10 of 1:50 dil.)
Pre 1X 2X
2H.
ZEBOV-sp. NAbs
(Reciprocal Dil.)
Pre 1X 2X

Figure 2A-2H

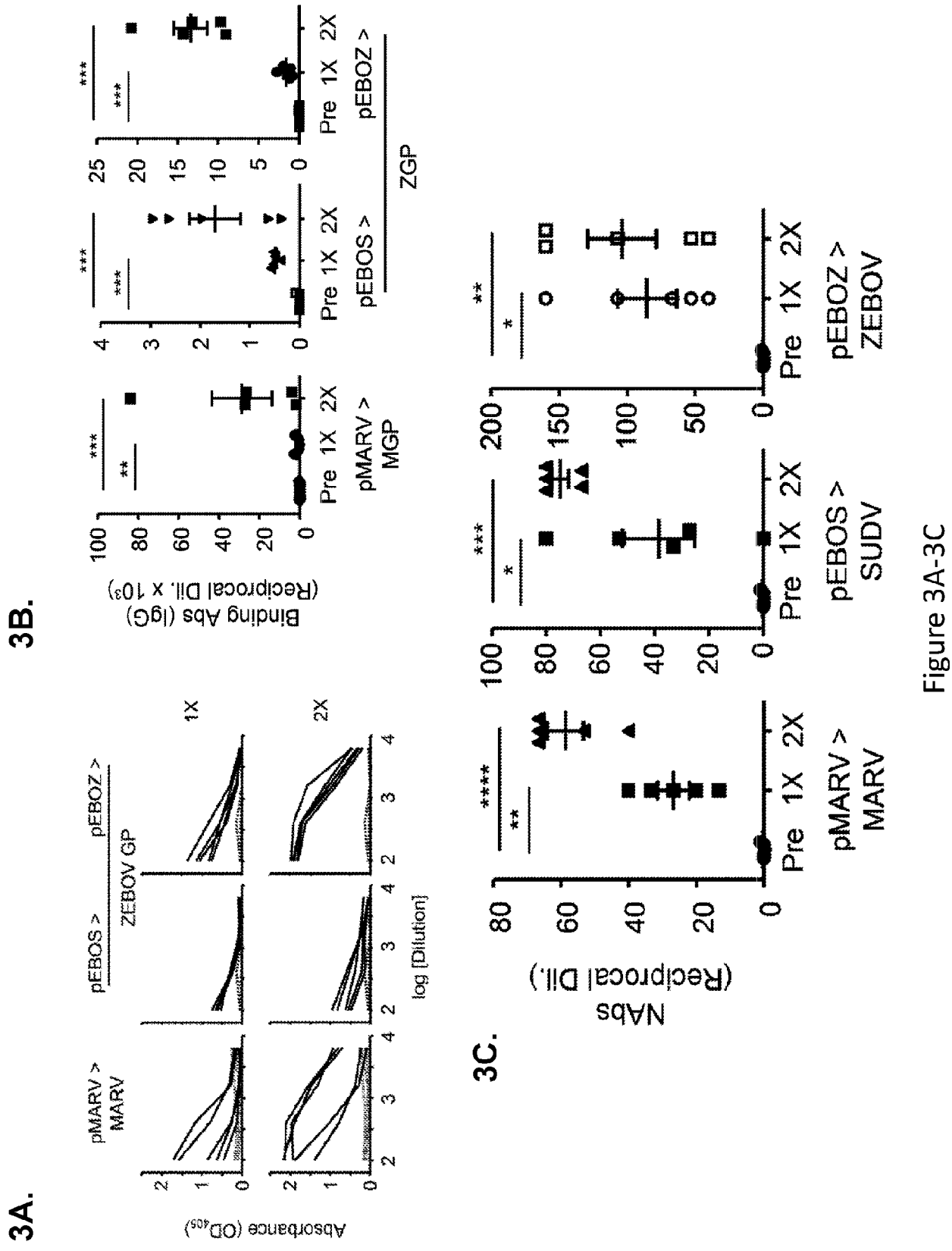

3A.
pMARV > MARV
pEBOS > ZEBOV GP
pEBOZ >
1X
2X
Absorbance (OD405)
log [Dilution]
3B.
Binding Abs (IgG) (Reciprocal Dil. x 10³)
pMARV > MGP
pEBOS > ZGP
pEBOZ >
Pre 1X 2X
3C.
NAbs (Reciprocal Dil.)
pMARV > MARV
pEBOS > SUDV
pEBOZ > ZEBOV

Figure 3A-3C

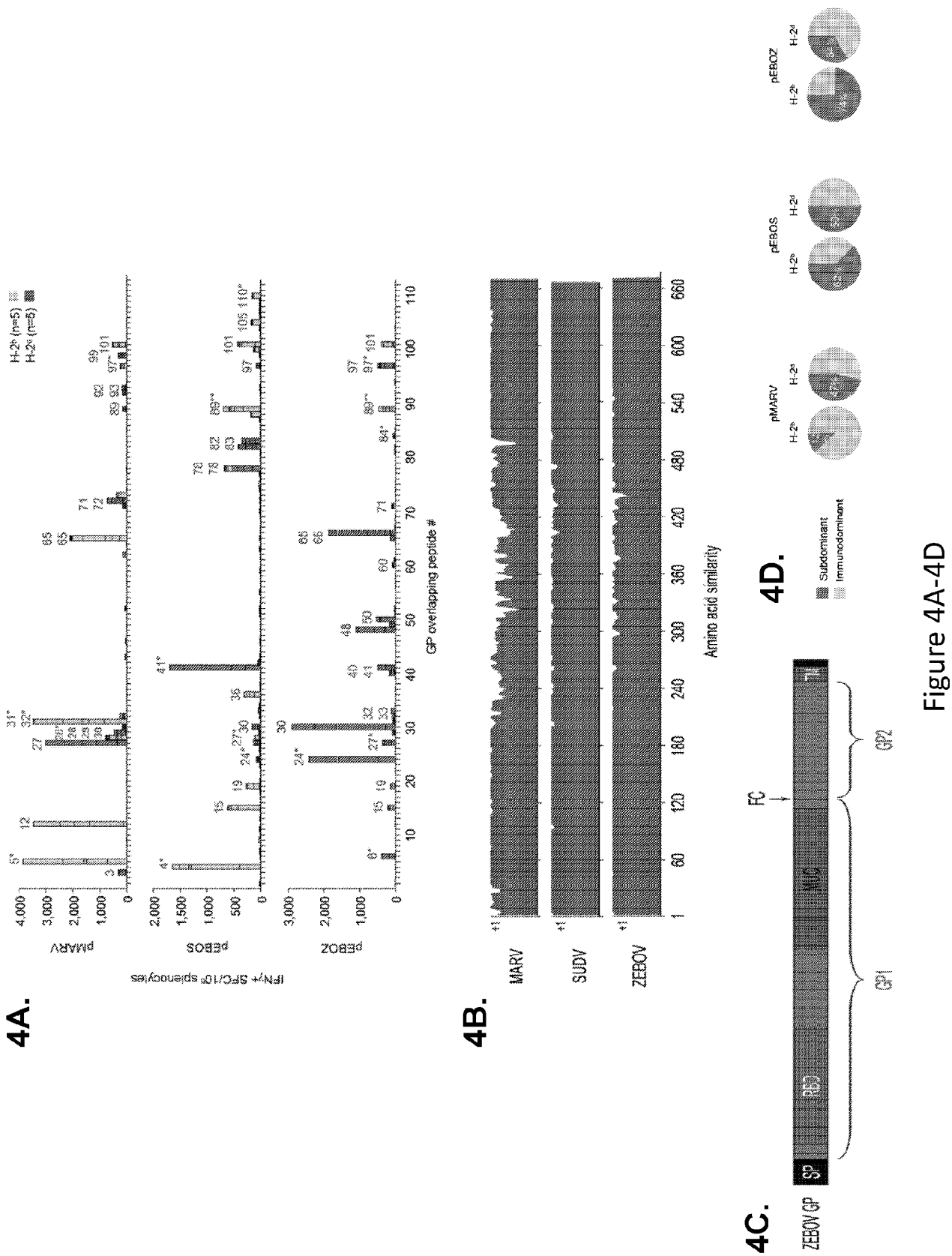

4A.
H-2b (n=5)
H-2d (n=5)
pMARV
pEBOS
pEBOZ
IFNγ+ SFC/10e6 splenocytes
GP overlapping peptide #
4B.
MARV
SUDV
ZEBOV
Amino acid similarity
4C.
ZEBOV GP
SP
RBD
MLD
FC
GP1
GP2
4D.
pMARV
pEBOS
pEBOZ
H-2b
H-2d
Subdominant
Immunodominant

Figure 4A-4D

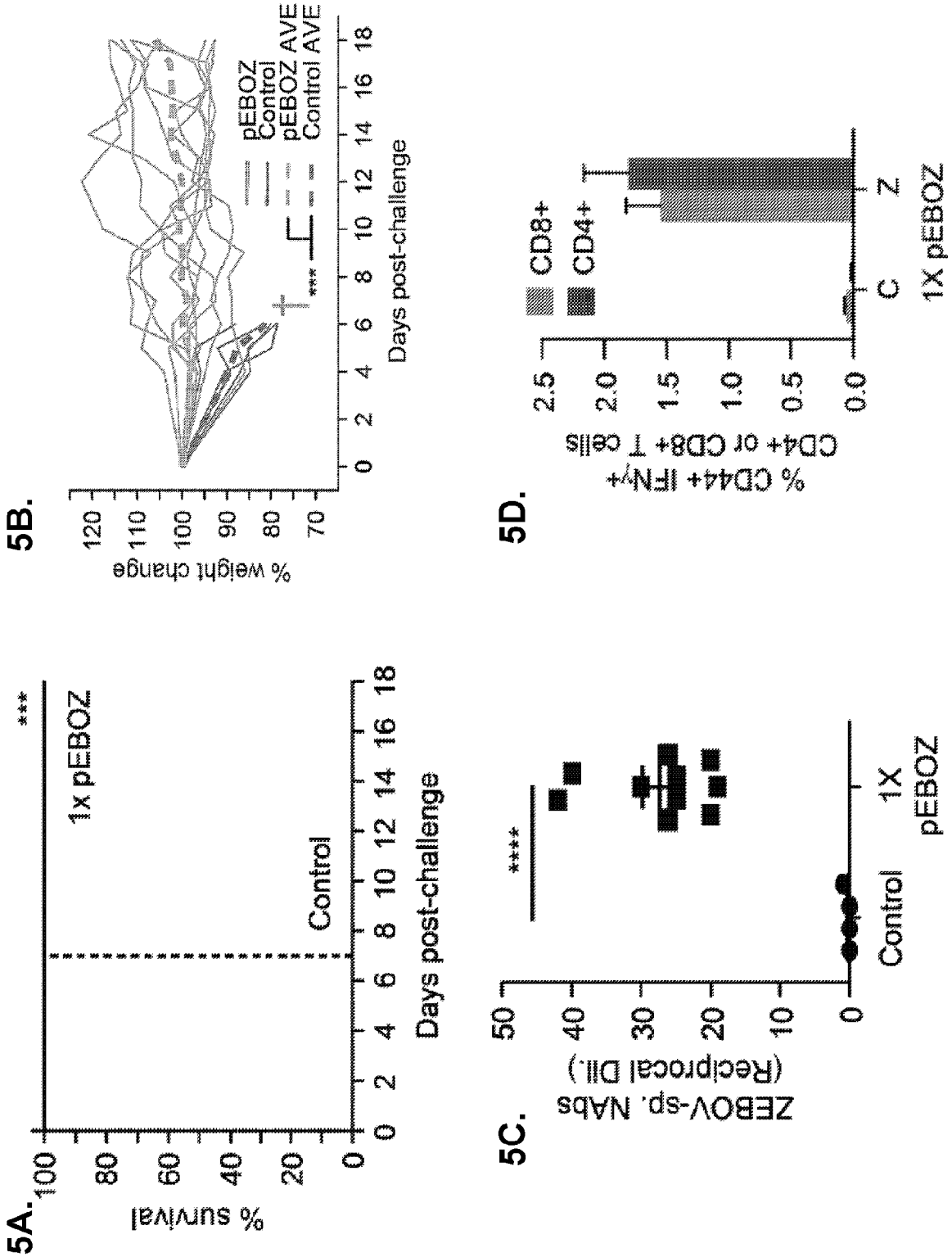

5A.
% survival
1x pEBOZ
Control
***
Days post-challenge
5B.
% weight change
pEBOZ
Control
pEBOZ AVE
Control AVE
***
Days post-challenge
5C.
ZEBOV-sp. NAbs
(Reciprocal DIl.)
****
Control
1X pEBOZ
5D.
% CD44+ IFNγ+
CD4+ or CD8+ T cells
CD8+
CD4+
C
Z
1X pEBOZ

Figure 5A-5D

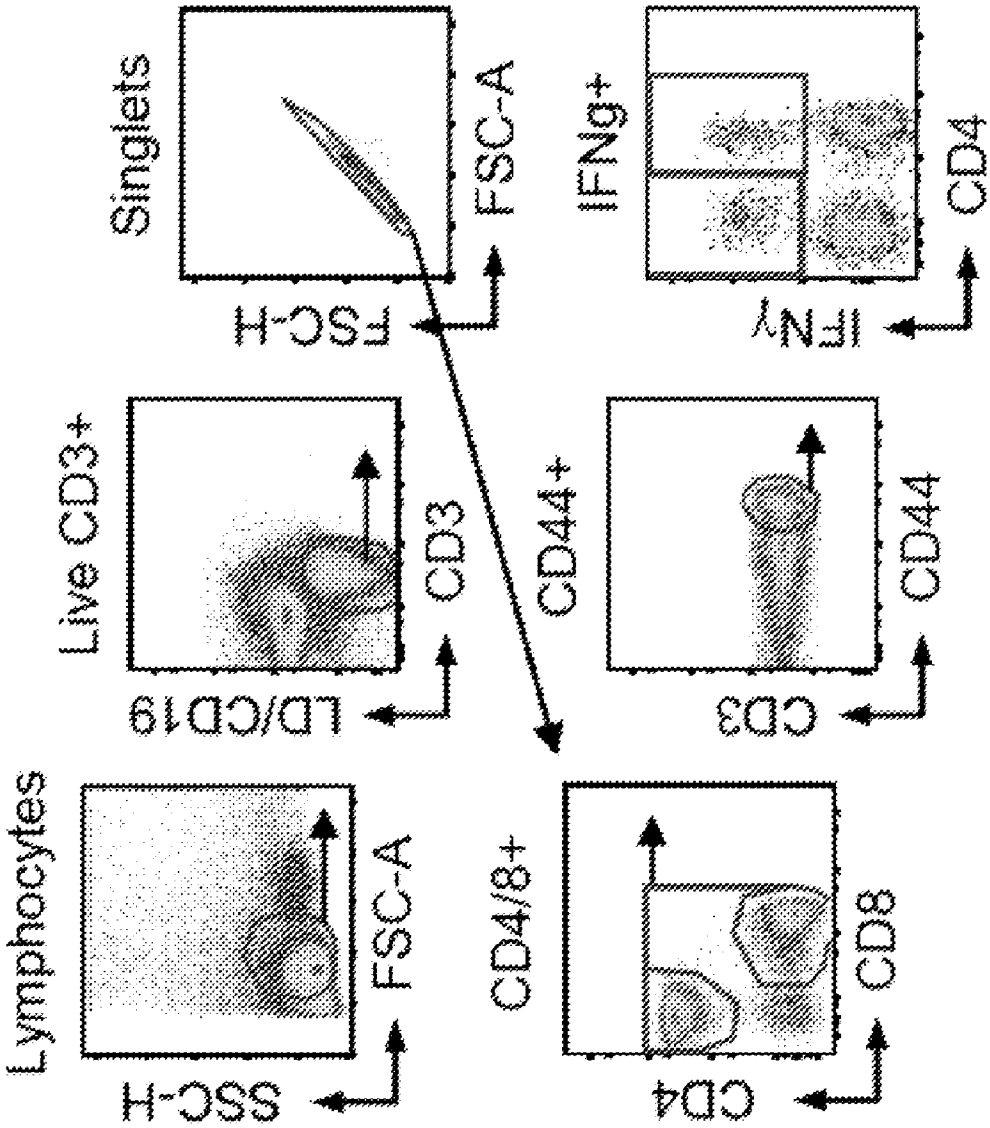
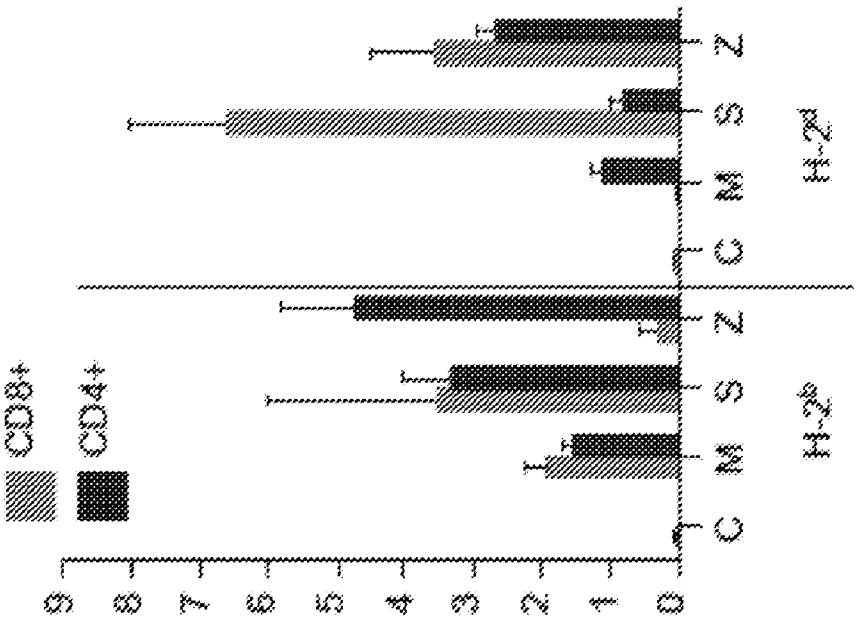

Singlets
FSC-H
FSC-A
IFNg+
IFNγ
CD4
Live CD3+
LD/CD19
CD3
CD44+
CD3
CD44
Lymphocytes
SSC-H
FSC-A
CD4/8+
CD4
CD8

Figure 6


CD8+
CD4+
C
M
S
Z
H-2d
H-2k
0
1
2
3
4
5
6
7
8
9

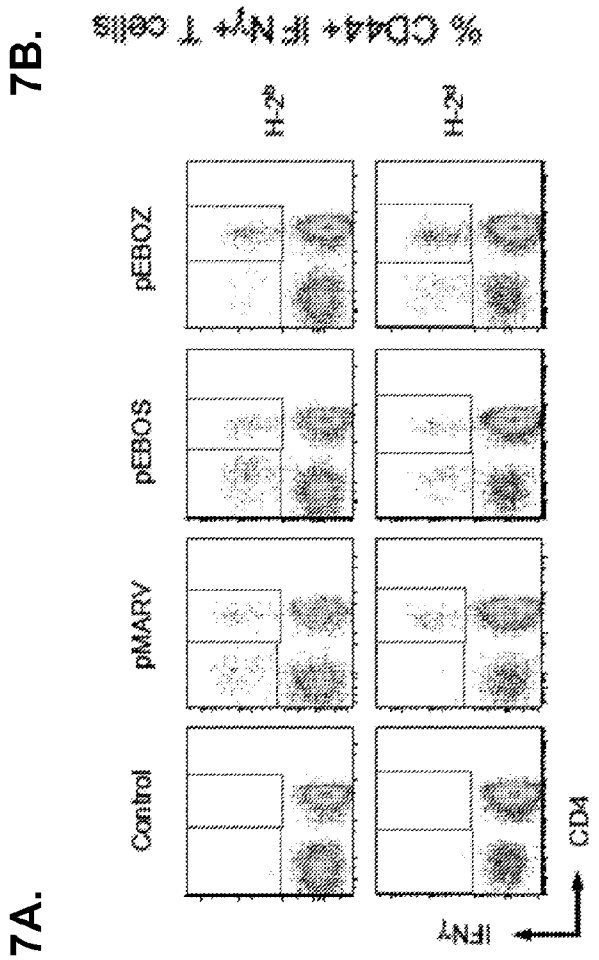

7B.
% CD44+ IFNγ+ T cells
H-2d
H-2k
pEBOZ
pEBOS
pMARV
Control
IFNγ
CD4
7A.

Figure 7A-7B

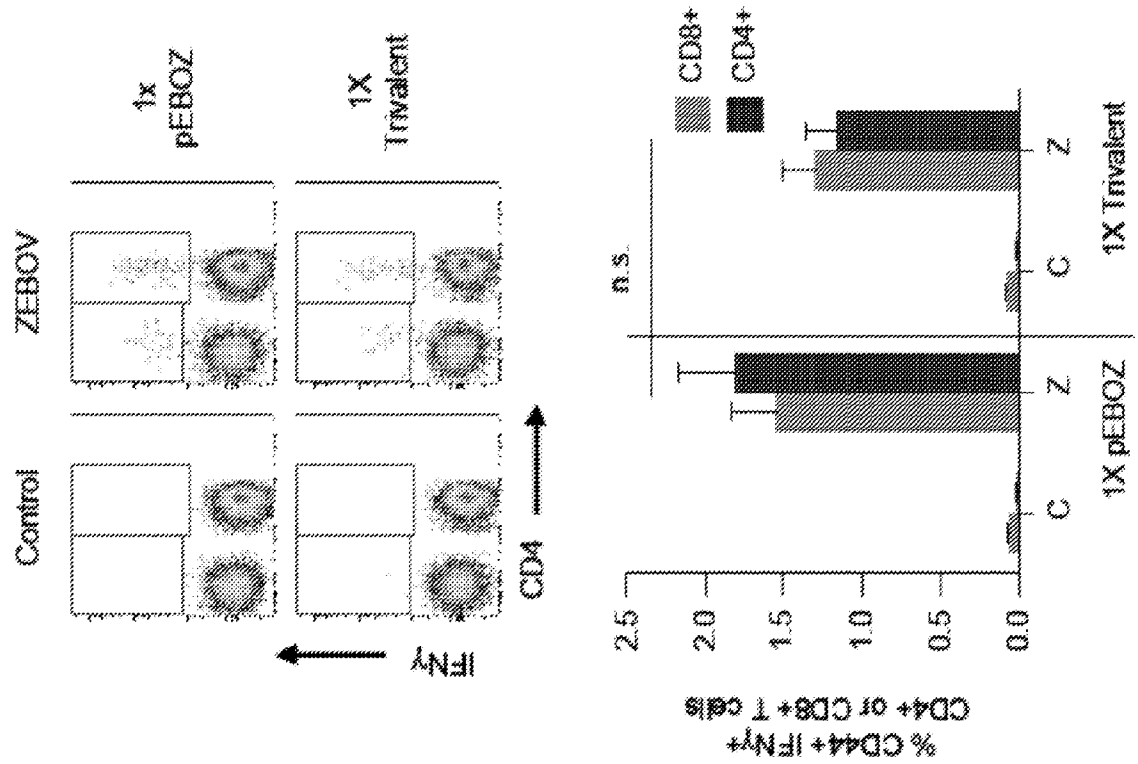
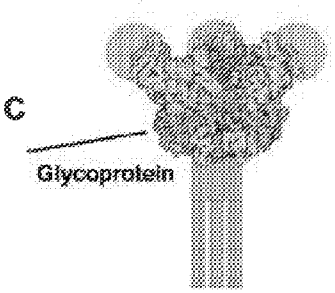

8A.
Control
ZEBOV
1x pEBOZ
1X Trivalent
IFNγ
CD4
8B.
CD8+
CD4+
n.s.
C
Z
1X pEBOZ
1X Trivalent
% CD44+ IFNγ+ CD4+ or CD8+ T cells
2.5
2.0
1.5
1.0
0.5
0.0

Figure 8A-8B

Experimental vaccines against Ebola

The Ebola viral glycoprotein (GP) is the major antigenic target for vaccines: Antibody & T cell target



Vaccines currently in clinical trials

- rVSVΔG/ZEBOVGP
- ChAd3 prime + MVA boost
- MVA pan-filovirus

} **Advantages:** immunogenic, protective in NHPs, single dose protection

Drawbacks:

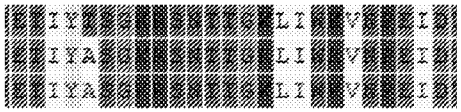
- development of anti-vector immunity
- duration of memory response uncertain
- adverse reactions in human clinical trials
- may not be suitable for all populations

An additional platform with a cleaner safety profile that can induce strong immune responses against heterologous Zaire Ebola viruses would be very beneficial

Figure 9

Developing an EBOV GP DNA vaccine formulation

DNA Vaccine Development



Constructs
2 Consensus:
ConEBOVGP#1
ConEBOVGP#2

1 matched:
Guinea-GP

- **Single vaccines**
- **Bivalent formulation**
  - **ConEBOVGP#1 + Guinea-GP**
- **Trivalent Formulation**
  - **All three constructs**

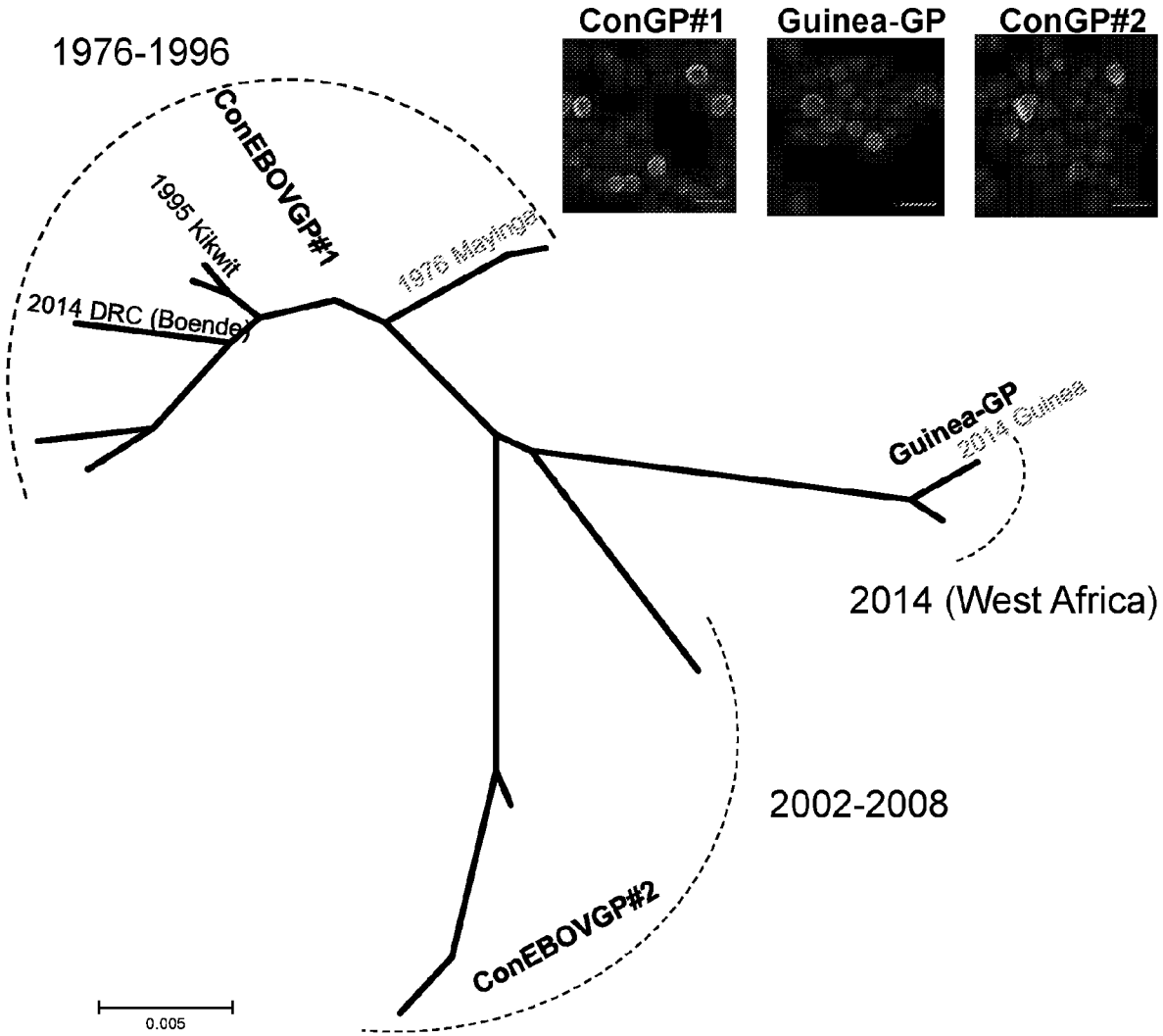
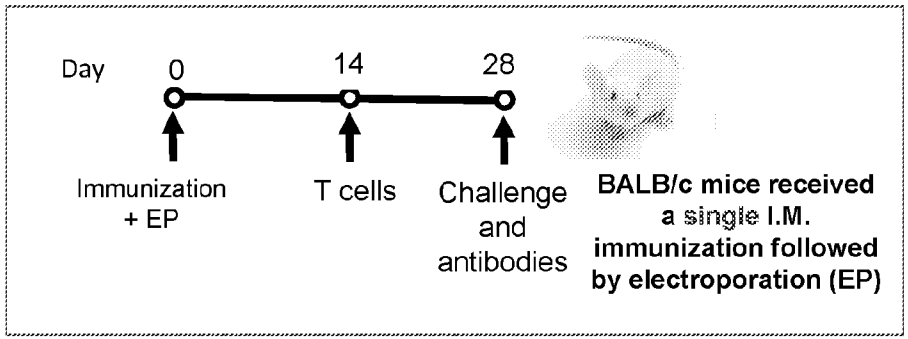
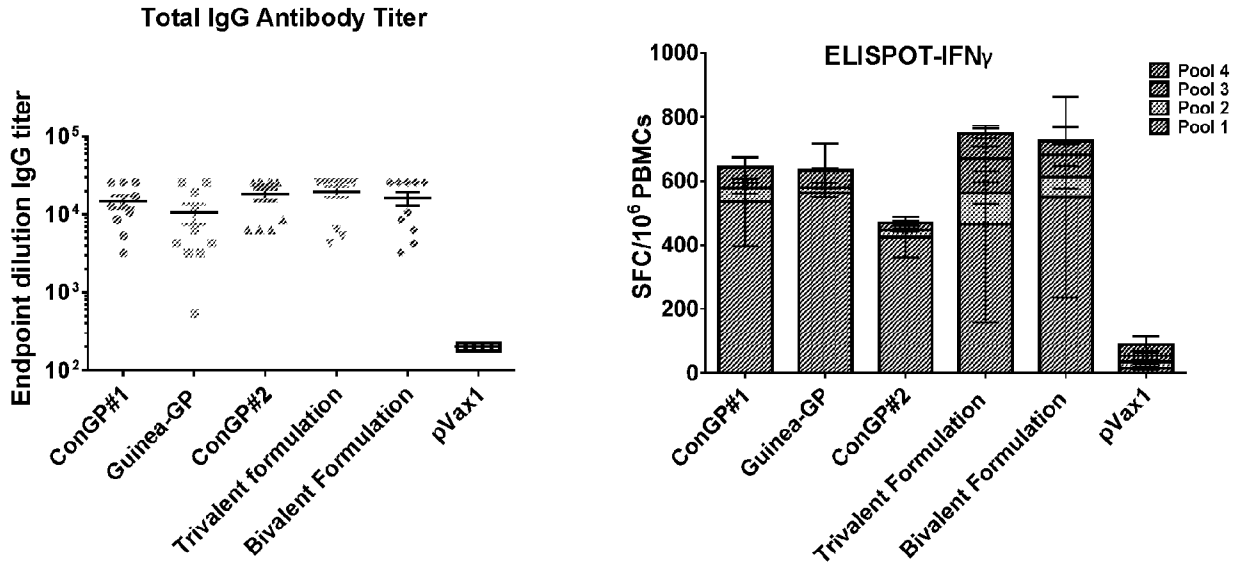


Figure 10

A single immunization of DNA vaccine is immunogenic in mice





Polyfunctional CD4+ and CD8+ T cells secreting IFNγ, IL2, and TNFα were similar for all vaccines and formulations.

Figure 11

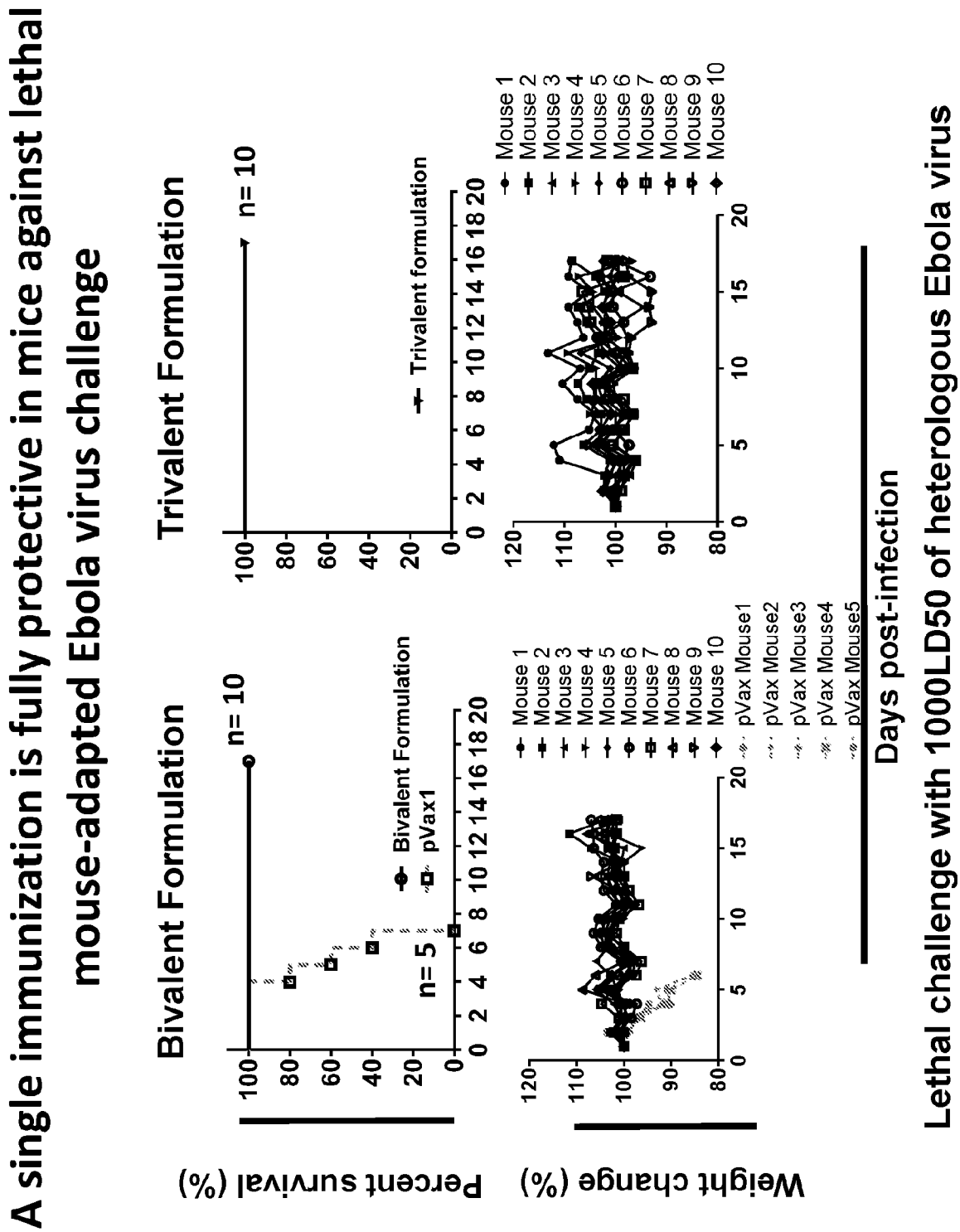
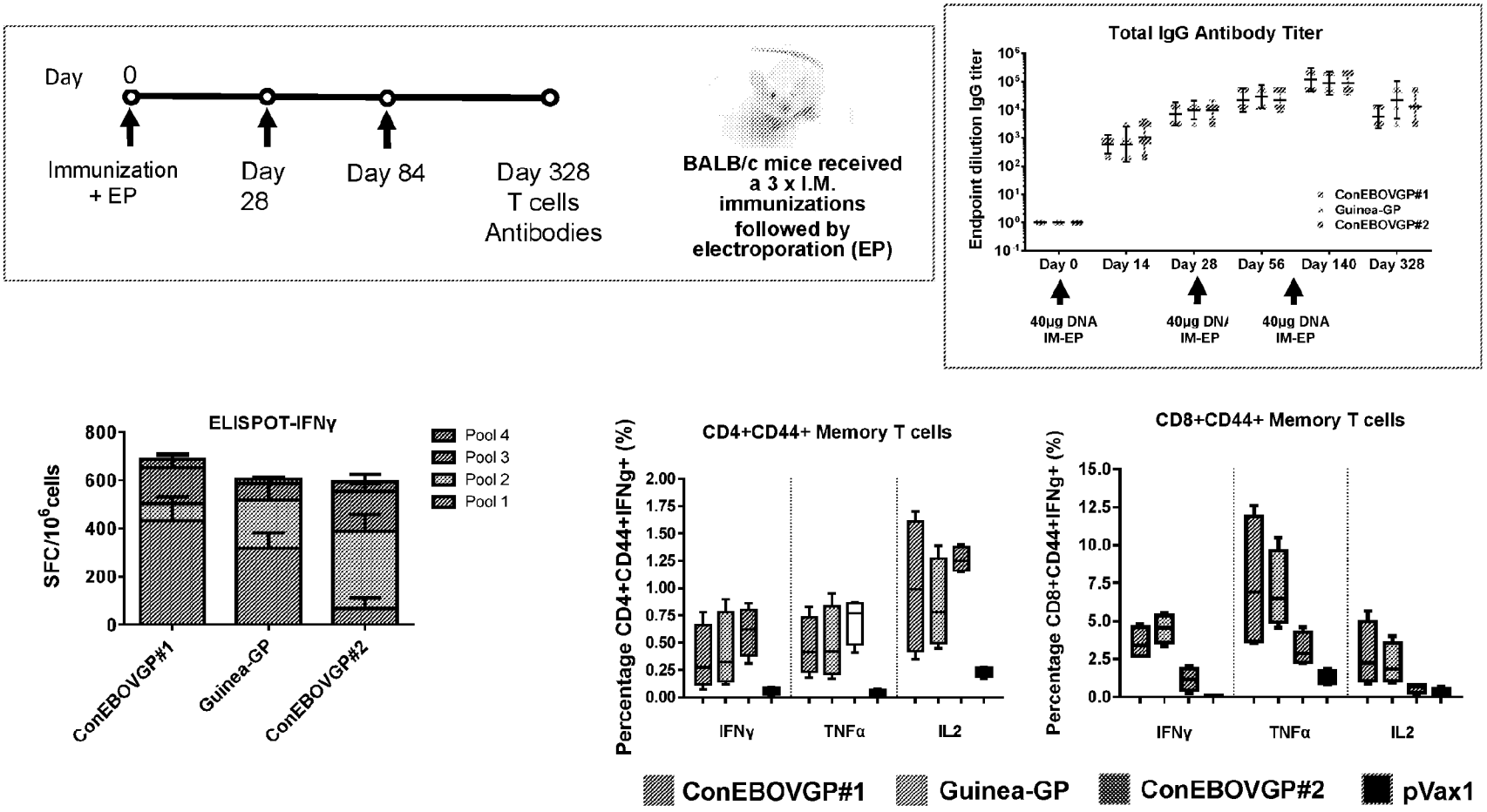

A single immunization is fully protective in mice against lethal mouse-adapted Ebola virus challenge
Bivalent Formulation
Trivalent Formulation
n= 10
n= 5
Bivalent Formulation
pVax1
Trivalent formulation
Percent survival (%)
Weight change (%)
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
pVax Mouse1
pVax Mouse2
pVax Mouse3
pVax Mouse4
pVax Mouse5
Days post-infection
Lethal challenge with 1000LD50 of heterologous Ebola virus
Challenge strain = mouse adapted-Ebola Mayinga 1976

Figure 12

Individual GP DNA vaccine constructs induce robust memory responses in mice



Robust immune responses are detectable months after last injection

Figure 13

Efficacy of GP DNA vaccines in non-human primates

Administered I.M. followed by EP

Bivalent Formulation	Trivalent Formulation
1. ConEBOVGP#1 2. Outbreak-matched GP + Adjuvant: Opt. Rhesus plL12	1. ConEBOVGP#1 2. Outbreak-matched GP 3. ConEBOVGP#2 + Adjuvant: Opt. Rhesus plL12

Cynomolgus macaques are a model for Ebola vaccine efficacy and lethal challenge

Evaluated different injection regimens in order to understand immunogenicity

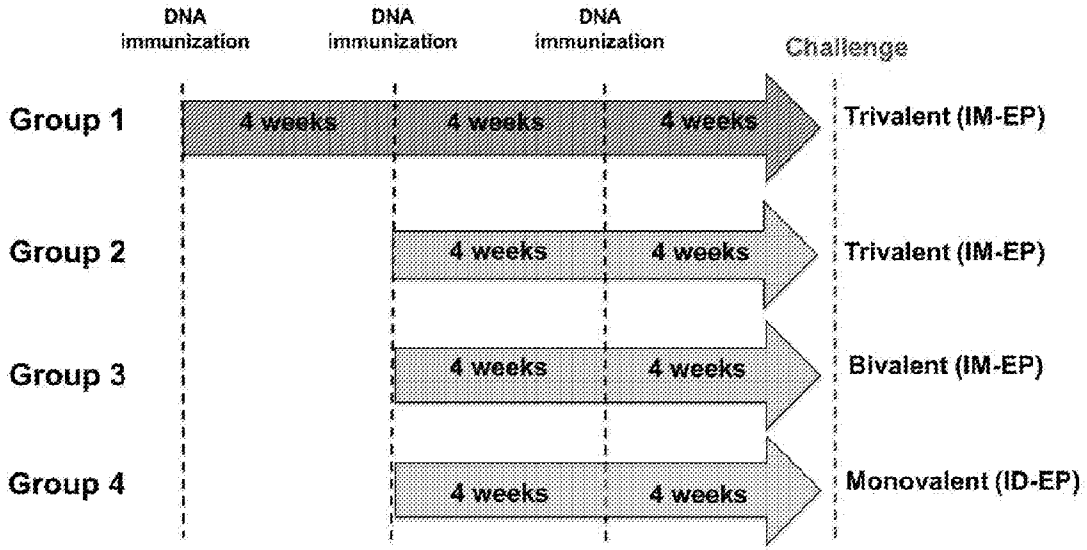
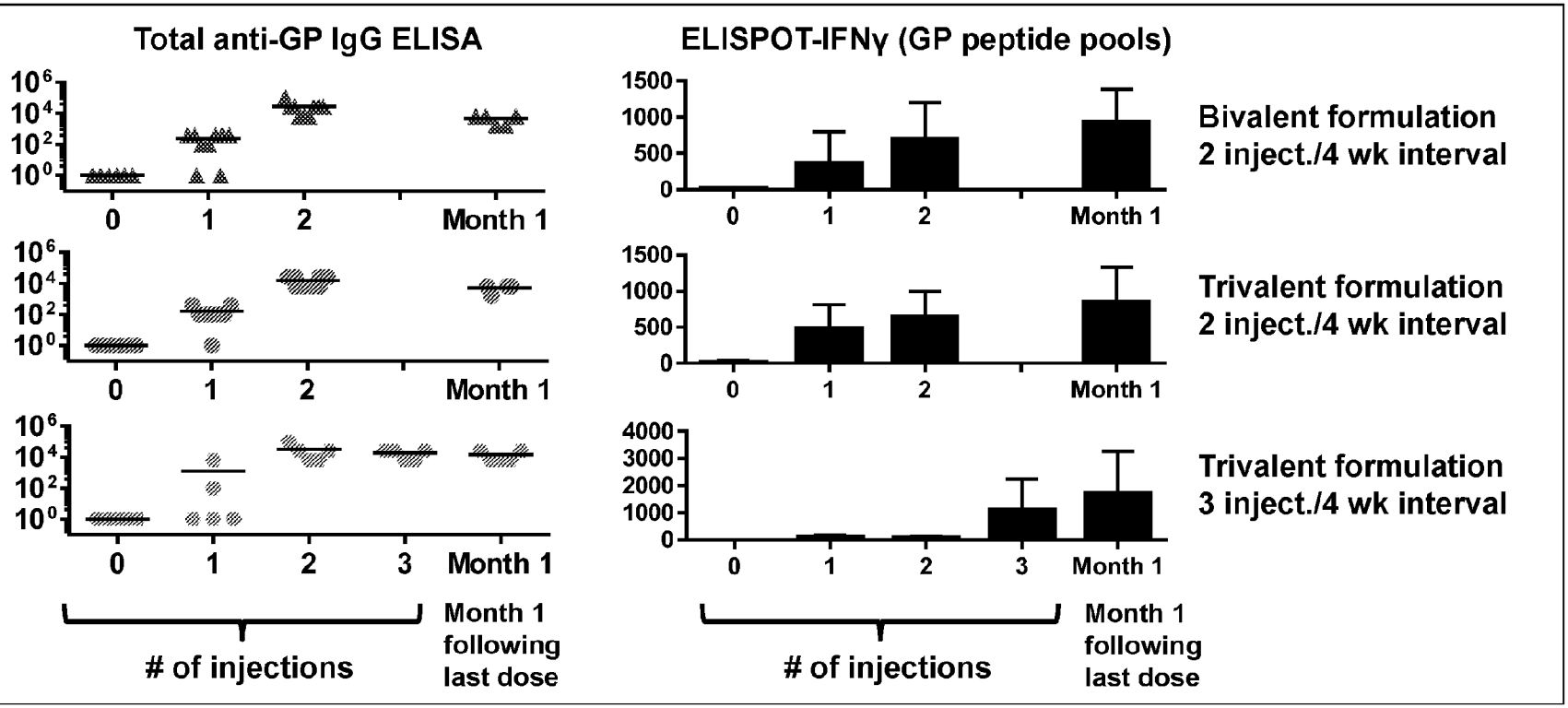


Figure 14

GP DNA vaccine formulations are immunogenic in NHPs



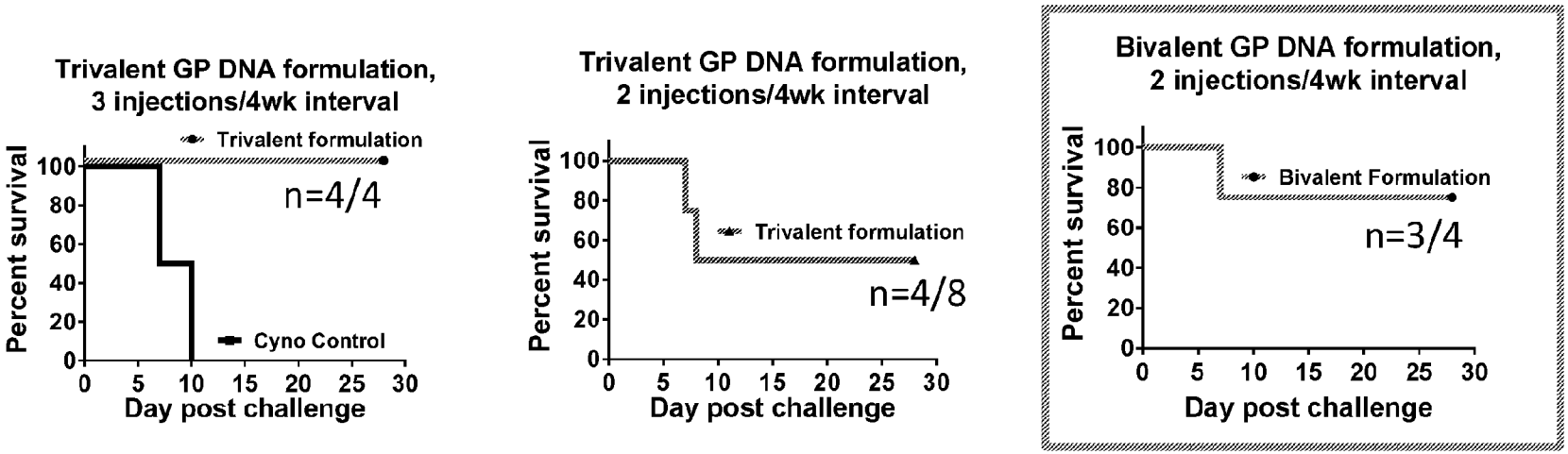
DNA formulations vaccines induce robust anti-Ebola GP antibody responses (GMT >10^3) & anti-GP T-cell responses.

Immune responses are boosted following each injection.

Figure 15

GP DNA formulation vaccines protect against lethal Zaire Ebola virus (Makona) challenge

Challenge dose = 1000 TCID50 Guinea-Makona 2014 C07 virus (7-U reference strain), 28 days post-final DNA immunization



Animals were monitored for 28 days post-challenge.

Surviving animals did not have any significant signs of disease. They also maintained normal CBC and enzyme levels

Figure 16

EBOV-001 phase I clinical (NCT02464670)

Open-Label Study of INO-4212 With or Without INO-9012, Administered IM or ID Followed by Electroporation in Healthy Volunteers

Primary outcome: safety assessment
Secondary outcome: immunological assessment
Other: intradermal (ID) delivery vs intramuscular (IM) delivery

Cohort	Vaccine	Schedule	Route	Dose (mg)
1	ConEBOVGP#1	0-4-12 weeks	IM	2
2	Guinea-GP	0-4-12 weeks	IM	2
3	ConEBOVGP#1	0-4-12 weeks	ID	2
4	ConEBOVGP#1 + Guinea-GP	0-4-12 weeks	IM	4
5	ConEBOVGP#1 + Guinea-GP + plasmid IL12	0-4-12 weeks	IM	4+1

Figure 17

EBOV-001 seroconversion

69 subjects total

ELISA analysis:
- baseline
- week 2
- week 6
- week 14

Seropositive = + IgG antibody response to Ebola Zaire glycoprotein

Week 14: IgG GMT = 10^{3}-10^{5}

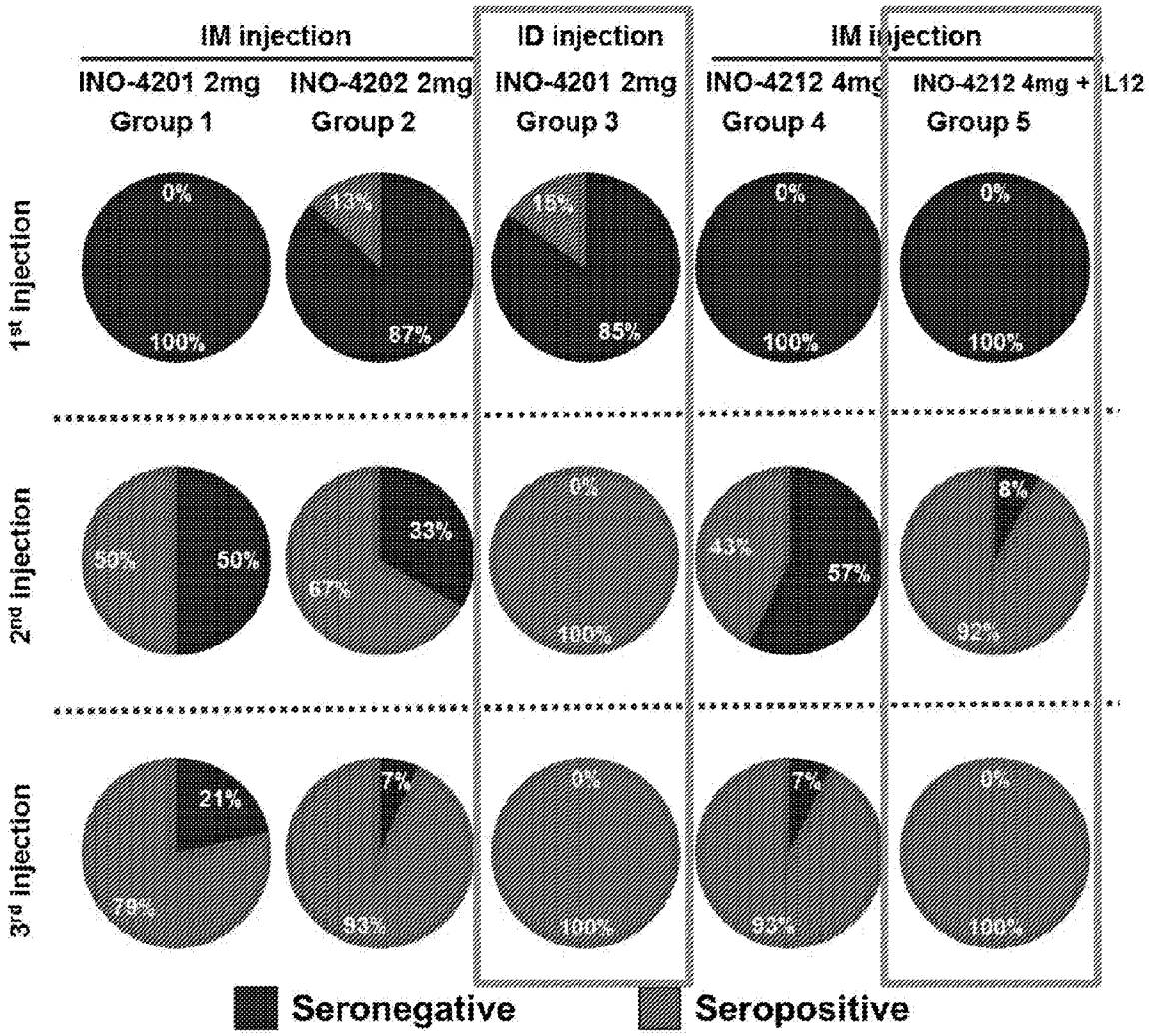
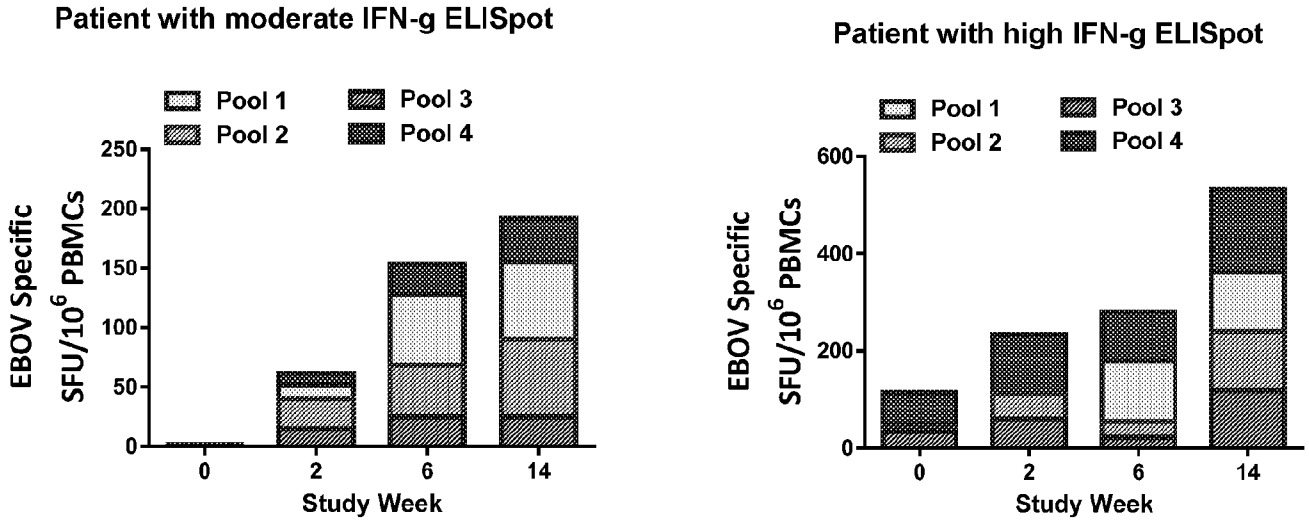


Figure 18

EBOV 001: Induction of Ebola GP Specific T-Cell Responses (ELISpot): Representative Patients



- ELISpot analysis: baseline, week 2, week 6, week 14.
- 4 peptide pools used for stimulation were GP pool 1, GP pool 2, GP pool 3, GP pool 4.

Figure 19

Comparison with other Ebola vaccine platforms currently in clinical trials

	ChAd3/MVA-GP	rVSV/ZEBOVGP	GP DNA Vaccine
	NIAID VRC/GSK	PHAC/Newlink/Merck	Inovio
Transgene	1976 GP	1995 GP	Consensus GP 2014 GP
Route of administration	I.M.	I.M.	I.M. or I.D.
Regimen	ChAd3 prime (60-90% sero), MVA boost	Single injection	2 injection (100% sero. ID) 3 injection (100% sero. IM)
Side Effects	Fever, Fatigue, Arthralgia Lymphopenia	Fever, Fatigue, Arthralgia	None
Injection Site Pain	Yes	Yes	Yes
Antibody titers	$>10^3$	$>10^3$	10^3-10^5
T cell responses	Yes	N/A	Yes
Cold-chain	-20C	-20C	4C
Other notes	Need MVA boost to generate long-term memory	Ring-vaccine trial, long-term memory not confirmed	Can be re-administered Memory study ongoing

***Rampling et al 2015, Legerwood et al 2014, Agnandji et al 2015, Henao-Restrepo et al 2015, Tapia et al 2015*

Some of the side effects of rVSV/ZEBOVGP and ChAd3/MVAGP overlap with symptoms of Ebola

Figure 20

EBOV001 ELISA

- **69 subjects from all cohorts (1-5) are included in analysis.**
- ELISA analysis from baseline, week 2, week 6, week 14.
- Protein used was Ebola Zaire

Cohort descriptions

Cohort	Vaccine	Plasmids included in vaccine	Schedule	Route	Dose (mg)
1	INO-4201	pGX4201	0-4-12 weeks	IM	2
2	INO-4202	pGX4202	0-4-12 weeks	IM	2
3	INO-4201	pGX4201	0-4-12 weeks	ID	2
4	INO-4212	pGX4201 and pGX4202	0-4-12 weeks	IM	4
5	INO-4212 +INO-9012	pGX4201, pGX4202, and pGX6001	0-4-12 weeks	IM	4+1

		Ethnicity		Race				West African Origin	
		Hispanic	Not Hispanic	Black	White	Asian	BI-RACIAL		
Number subjects	74	8	66	34	37	2	1	1	Sierra Leone
Male	25	4	21	9	15	1	0		Subject 4201-007, Cohort 1
Female	49	4	45	25	22	1	1		

Figure 21

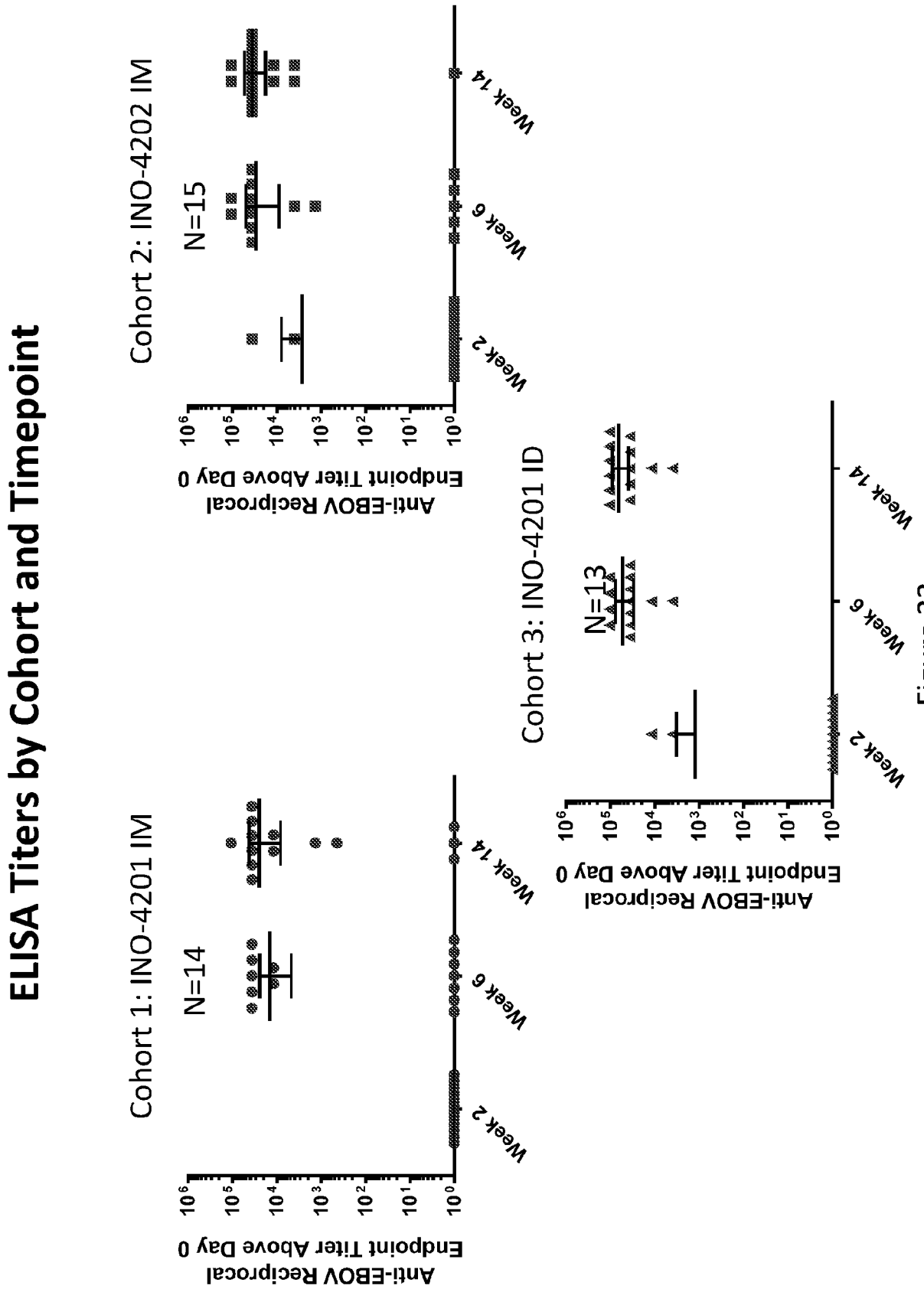
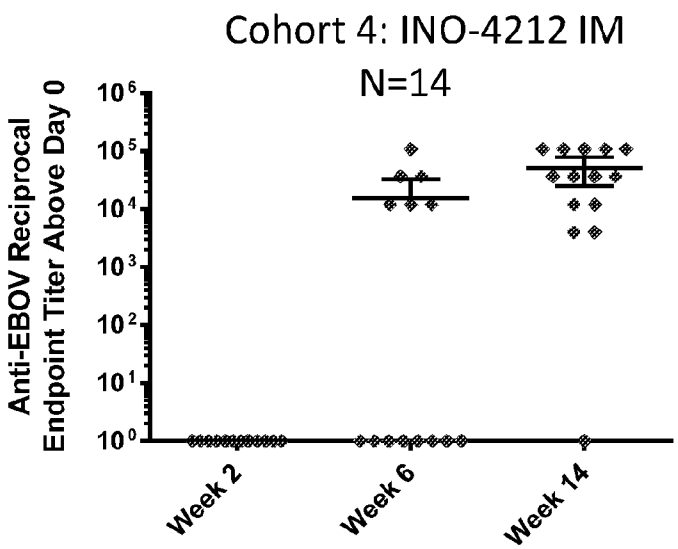
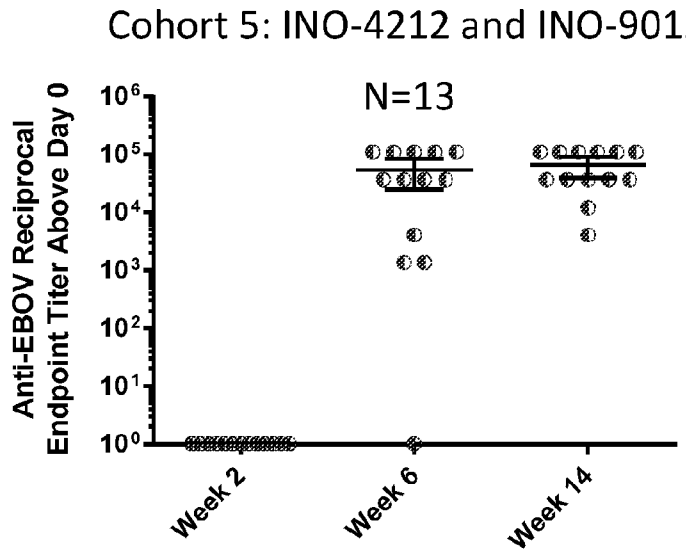

ELISA Titers by Cohort and Timepoint
Cohort 1: INO-4201 IM
N=14
Cohort 2: INO-4202 IM
N=15
Cohort 3: INO-4201 ID
N=13
Anti-EBOV Reciprocal Endpoint Titer Above Day 0
Week 2
Week 6
Week 14

Figure 22

ELISA Titers by Cohort and Timepoint





	Reciprocal EBOV Geometric Mean Titers				
Cohort	**1**	**2**	**3**	**4**	**5**
Week 2 GMT (range)	**1** (1-1)	**3.50** (1-36,450)	**3.91** (1-12,150)	**1** (1-1)	**1** (1-1)
Week 6 GMT (range)	**163.88** (1-36,450)	**882.49** (1-109,350)	**39,664.20** (4050-109,350)	**77.06** (1-109,350)	**12,609.40** (1-109,350)
Week 14 GMT (range)	**2,049.04** (1-109,350)	**13,500.60** (1-109,350)	**46,968.00**(4050-109,350)	**15,914.10** (1-109,350)	**46,968.00** (1-109,350)

Figure 23

ELISA Summary

- Summary:
  - Little to no reactivity after the first dose
  - Dose 2 begins to drive seroconversion, with Cohorts 3 and 5 seeing he largest frequency
  - Dose three drives >90% serconversion in 4/5 cohorts. Cohorts 3 and 5 show 100% seroconversion at this timepoint

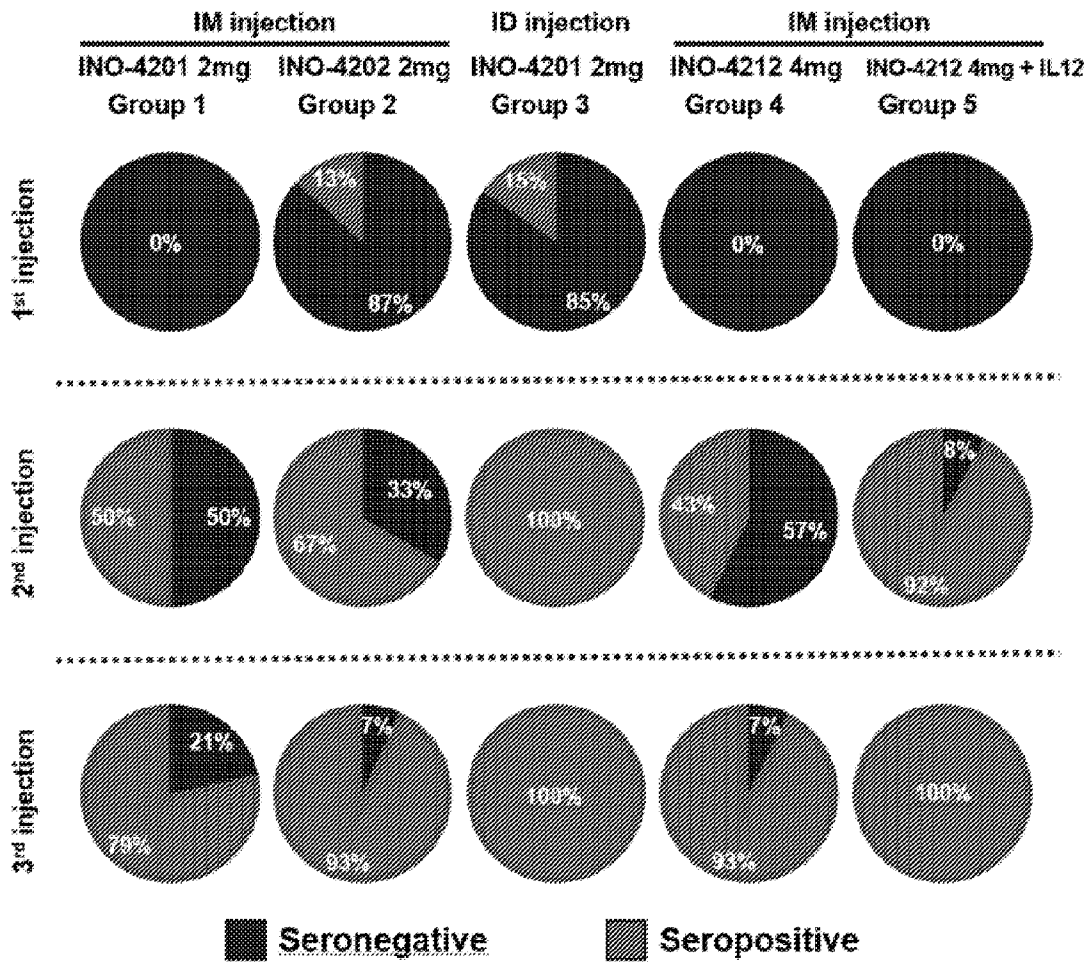


Figure 24

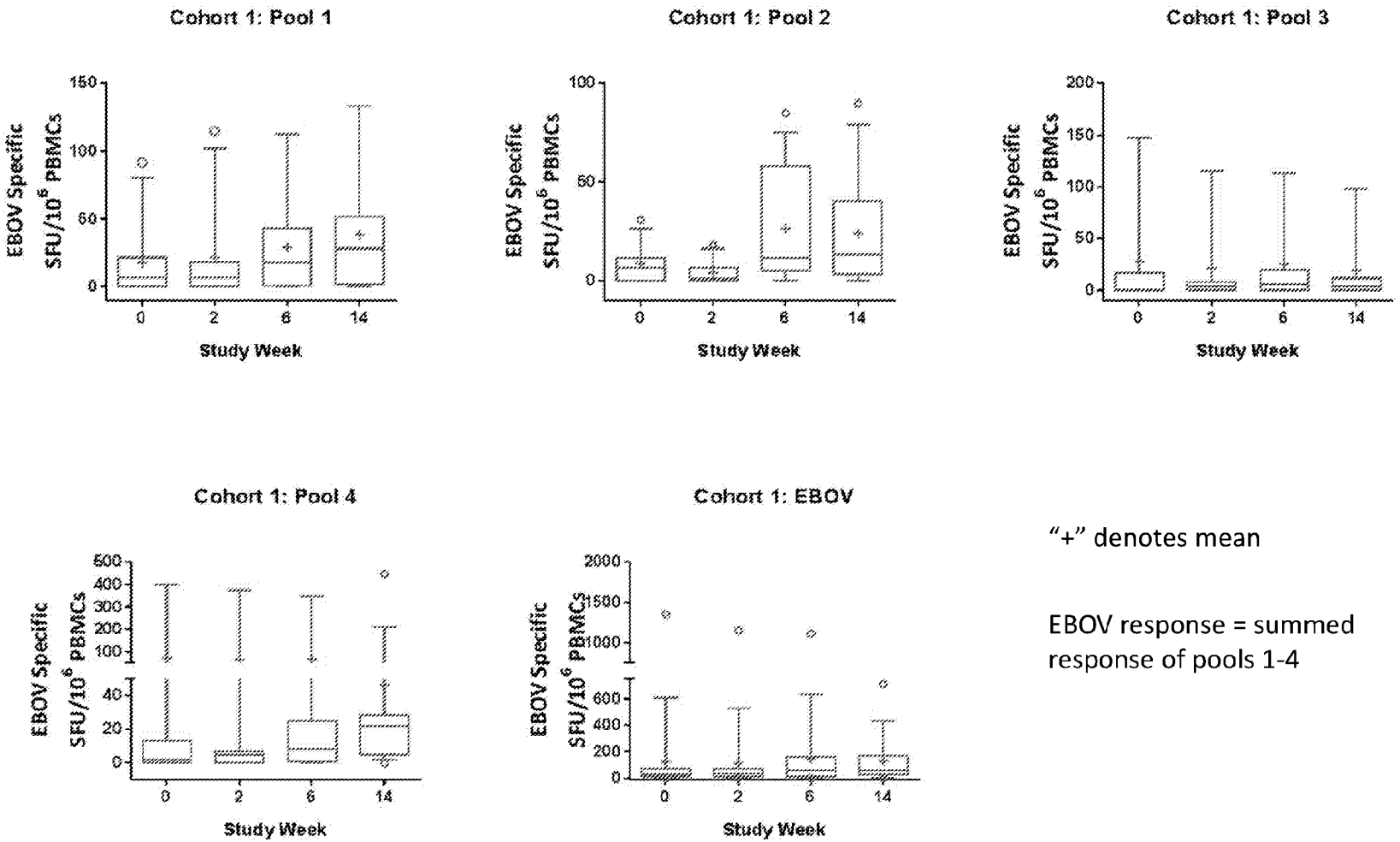
EBOV001 ELISpot

- **75 subjects from all cohorts (1-5) are included in analysis.**
- ELISpot analysis from baseline, week 2, week 6, week 14.
- 4 peptide pools (Zaire) used for stimulation:
  - Pool 1 (pp1-28)
  - Pool 2 (pp29-56)
  - Pool 3 (pp57-84)
  - Pool 4 (pp85-112)

Cohort	Vaccine	Plasmids included in vaccine	Schedule	Route	Dose (mg)
1	INO-4201	pGX4201	0-4-12 weeks	IM	2
2	INO-4202	pGX4202	0-4-12 weeks	IM	2
3	INO-4201	pGX4201	0-4-12 weeks	ID	2
4	INO-4212	pGX4201 and pGX4202	0-4-12 weeks	IM	4
5	INO-4212 +INO-9012	pGX4201, pGX4202, and pGX6001	0-4-12 weeks	IM	4+1

Figure 25

Cohort 1 : Subject responses by peptide pool (all subjects, n = 15)



"+" denotes mean

EBOV response = summed response of pools 1-4

Figure 26

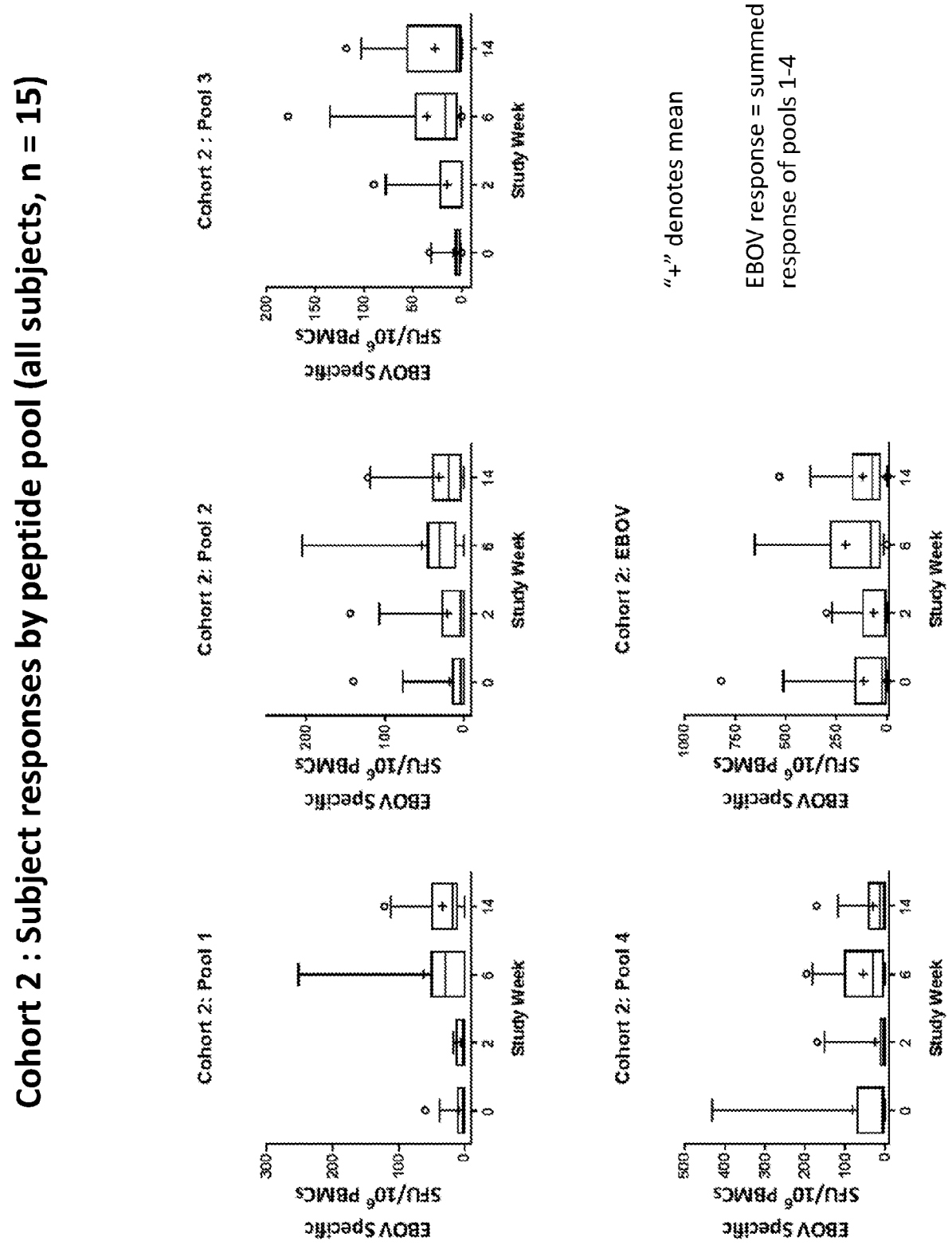

Cohort 2 : Subject responses by peptide pool (all subjects, n = 15)
Cohort 2: Pool 1
Cohort 2: Pool 2
Cohort 2 : Pool 3
Cohort 2: Pool 4
Cohort 2: EBOV
EBOV Specific SFU/10^6 PBMCs
Study Week
"+" denotes mean
EBOV response = summed response of pools 1-4

Figure 27

Cohort 3 : Subject responses by peptide pool (all subjects, n = 15)

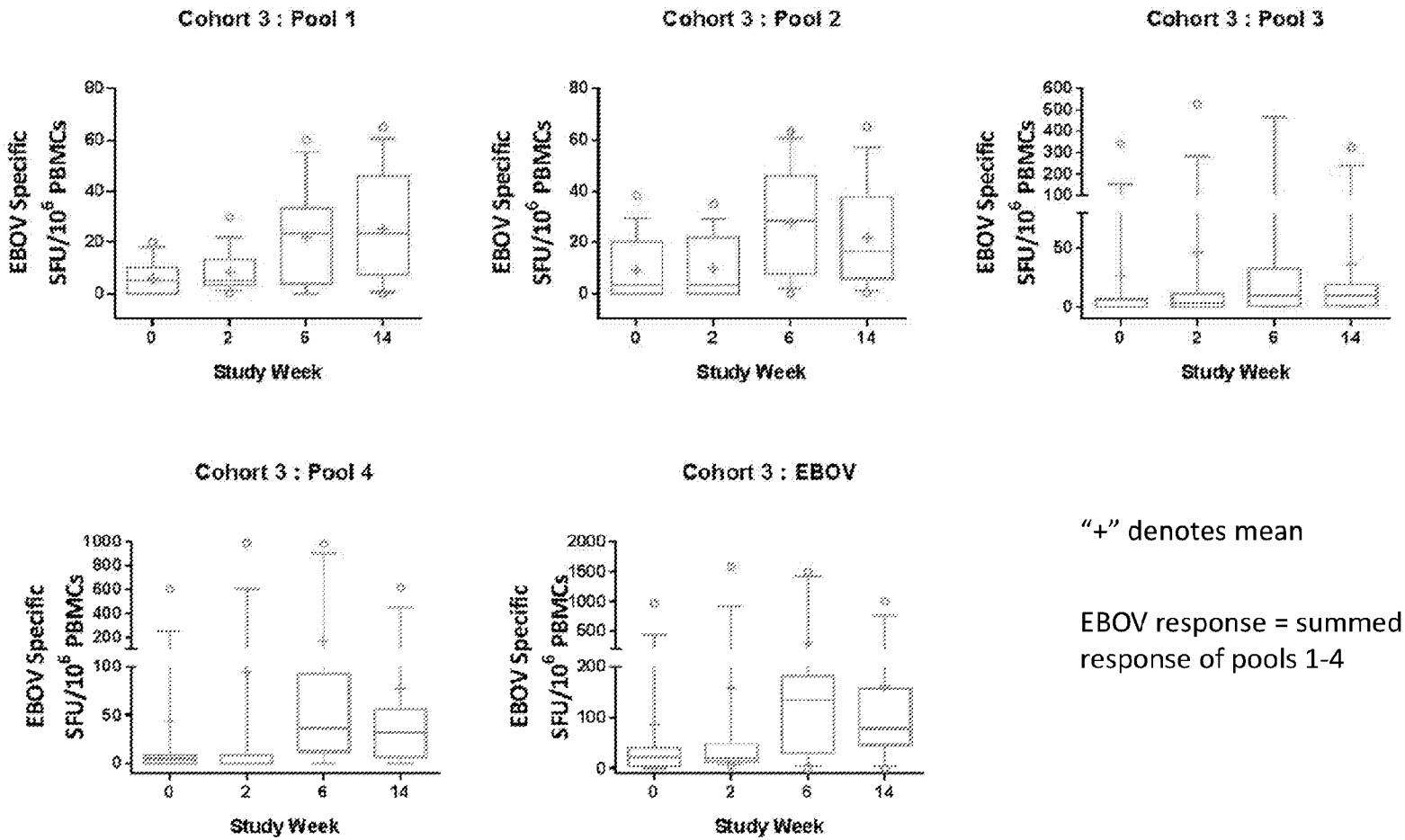


Figure 28

Cohort 4 : Subject responses by peptide pool (all subjects, n = 14)

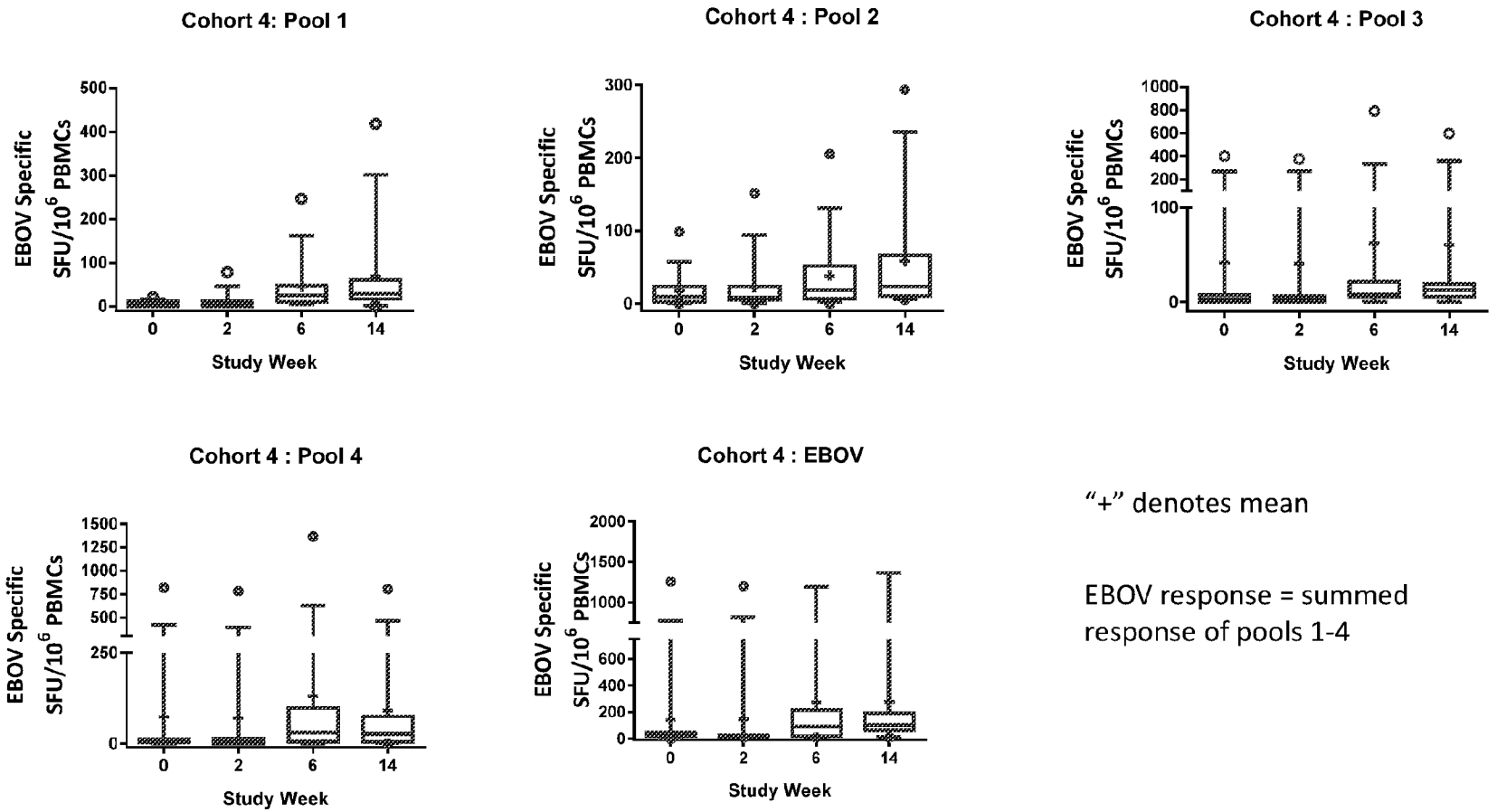


Figure 29

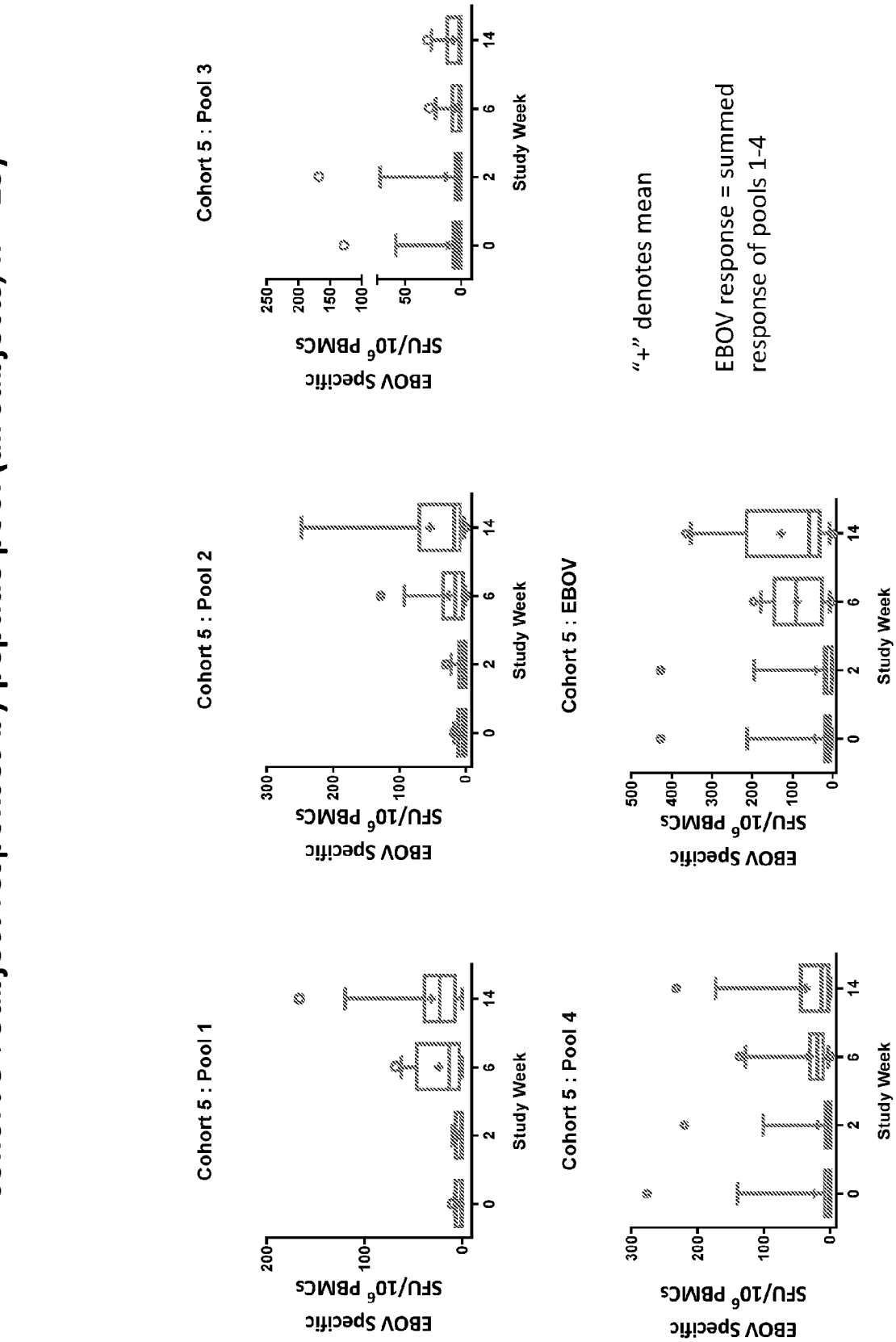

Cohort 5 : Subject responses by peptide pool (all subjects, n = 15)
Cohort 5 : Pool 1
Cohort 5 : Pool 2
Cohort 5 : Pool 3
Cohort 5 : Pool 4
Cohort 5 : EBOV
EBOV Specific SFU/10^6 PBMCs
Study Week
"+" denotes mean
EBOV response = summed response of pools 1-4

Figure 30

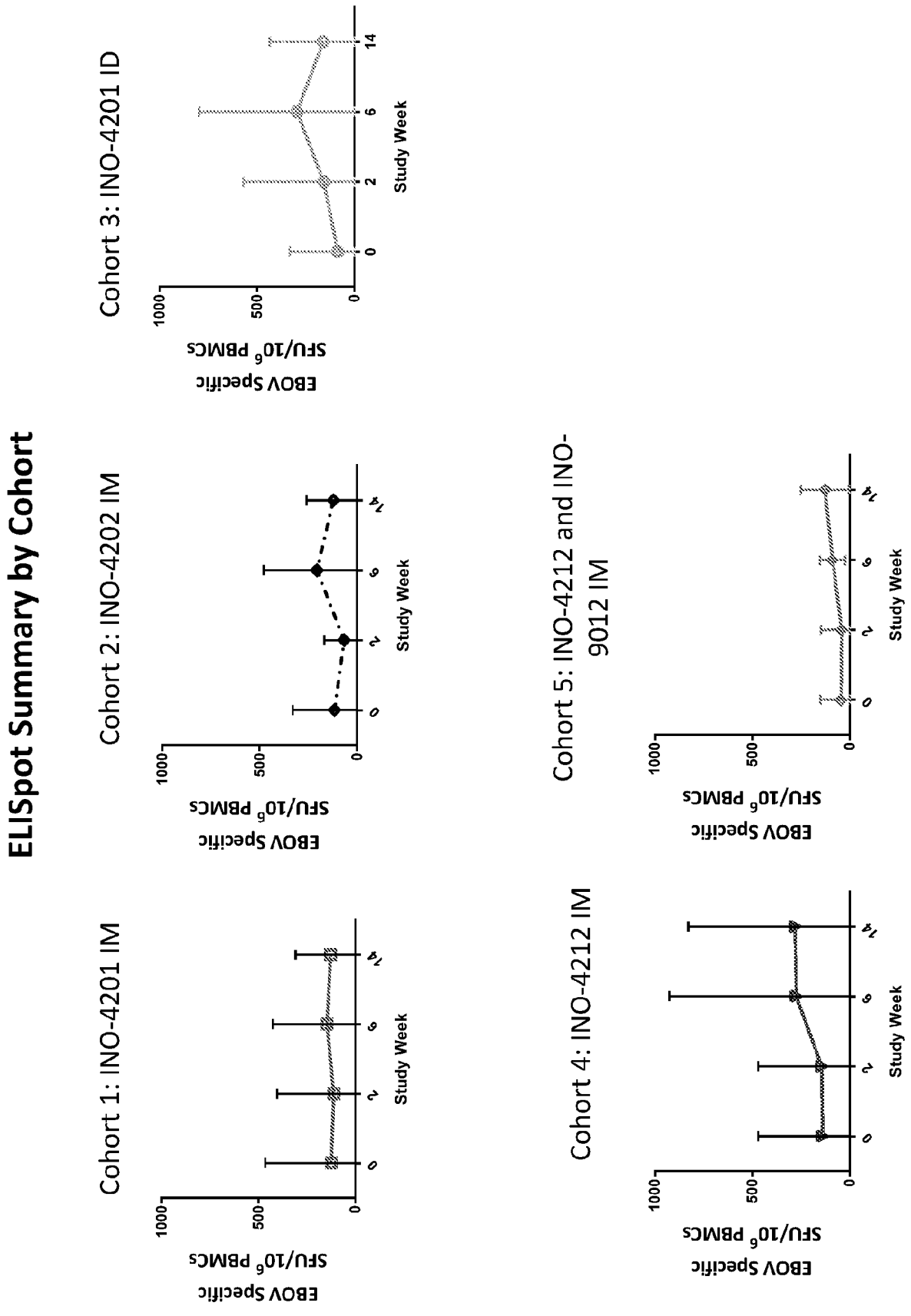

ELISpot Summary by Cohort
Cohort 1: INO-4201 IM
Cohort 2: INO-4202 IM
Cohort 3: INO-4201 ID
Cohort 4: INO-4212 IM
Cohort 5: INO-4212 and INO-9012 IM
EBOV Specific SFU/10^6 PBMCs
Study Week
0
500
1000
0
2
6
14

Figure 31

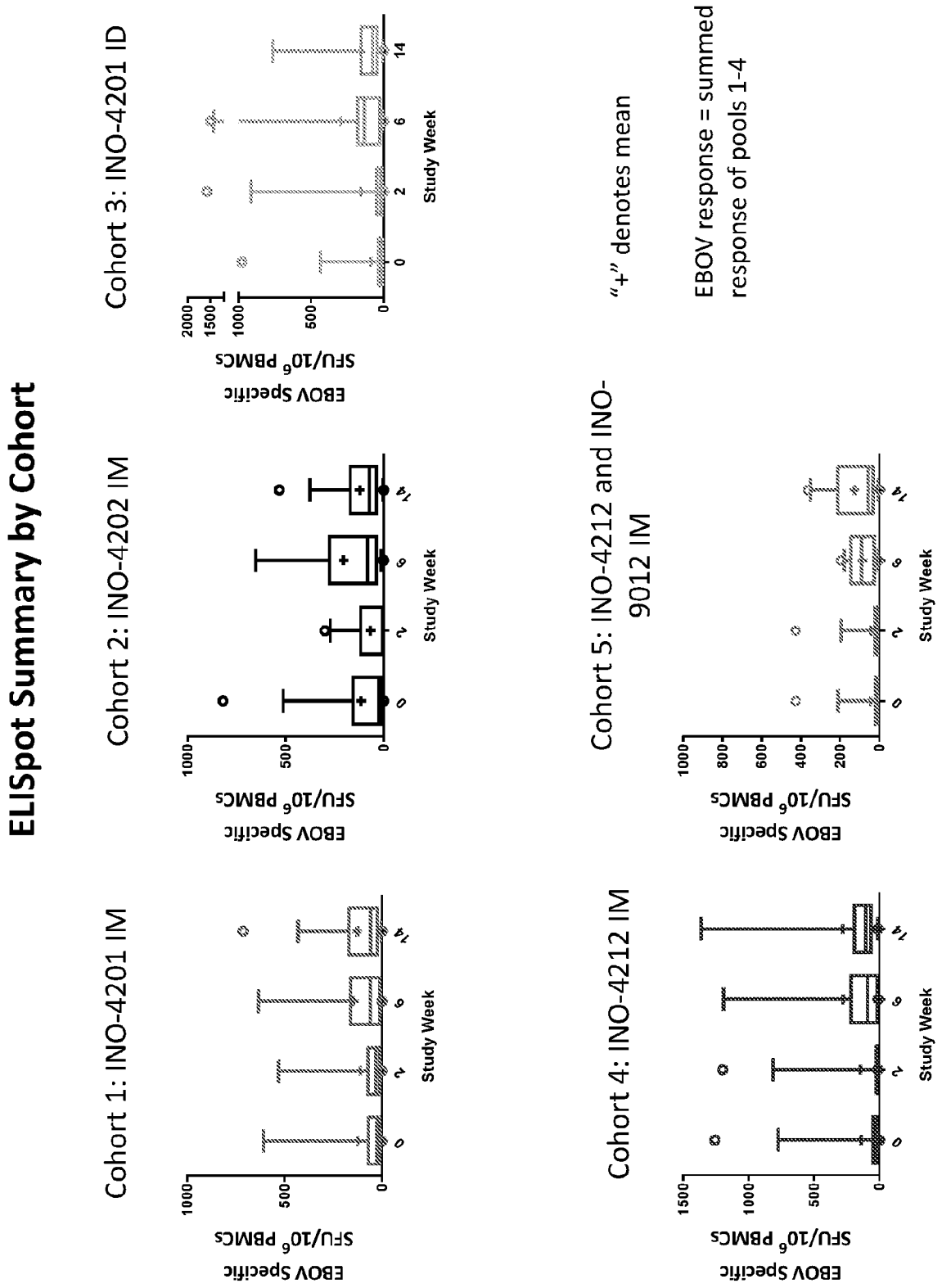

ELISpot Summary by Cohort
Cohort 1: INO-4201 IM
Cohort 2: INO-4202 IM
Cohort 3: INO-4201 ID
Cohort 4: INO-4212 IM
Cohort 5: INO-4212 and INO-9012 IM
EBOV Specific SFU/10^6 PBMCs
Study Week
"+" denotes mean
EBOV response = summed response of pools 1-4

Figure 32

Responder Analysis

Vaccine Responders – All Subjects

All Subjects			
	Responder	Total	% Responders
EBOV	4	75	5.3%
GP Pool1	31	75	41.3%
GP Pool 2	22	75	29.3%
GP Pool 3	5	75	6.7%
GP Pool 4	3	75	4.0%

Response by pool					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	0/15	1/15	2/15	1/15	0/15
GP pool 1	6/15	8/15	4/15	7/15	6/15
GP pool 2	5/15	4/15	3/15	5/15	5/15
GP pool 3	0/15	1/15	2/15	2/15	0/15
GP pool 4	0/15	0/15	2/15	1/15	0/15

Response by Cohort					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	0/15	1/15	2/15	1/15	0/15
Any Pool	7/15	8/15	6/15	8/15	7/15

All Subjects			
	Responder	Total	% Responders
EBOV	4	75	5.3%
Any Pool	36	75	48.0%

% Response by pool					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	0.0%	6.7%	13.3%	6.7%	0.0%
GP pool 1	40.0%	53.3%	26.7%	46.7%	40.0%
GP pool 2	33.3%	26.7%	20.0%	33.3%	33.3%
GP pool 3	0.0%	6.7%	13.3%	13.3%	0.0%
GP pool 4	0.0%	0.0%	13.3%	6.7%	0.0%

% Response by Cohort					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	0.0%	6.7%	13.3%	6.7%	0.0%
Any Pool	46.7%	53.3%	40.0%	53.3%	46.7%

Figure 33

Responder Analysis

Vaccine Responders – Baseline Outliers (n=8) Removed

All Subjects			
	Responder	Total	% Responders
EBOV	19	67	28.4%
GP Pool1	44	67	65.7%
GP Pool 2	33	67	49.3%
GP Pool 3	6	67	9.0%
GP Pool 4	4	67	6.0%

Response by pool					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	2/12	5/13	3/14	5/13	4/15
GP pool 1	7/12	11/13	8/14	10/13	8/15
GP pool 2	6/12	9/13	7/14	5/13	6/15
GP pool 3	0/12	4/13	1/14	0/13	1/15
GP pool 4	0/12	2/13	1/14	0/13	1/15

Response by Cohort					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	2/12	5/13	3/14	5/13	4/15
Any Pool	8/12	11/13	9/14	10/13	8/15

All Subjects			
	Responder	Total	% Responders
EBOV	19	67	28.4%
Any Pool	46	67	68.7%

% Response by pool					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	16.7%	38.5%	21.4%	38.5%	26.7%
GP pool 1	58.3%	84.6%	57.1%	76.9%	53.3%
GP pool 2	50.0%	69.2%	50.0%	38.5%	40.0%
GP pool 3	0.0%	30.8%	7.1%	0.0%	6.7%
GP pool 4	0.0%	15.4%	7.1%	0.0%	6.7%

% Response by Cohort					
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5
EBOV	16.7%	38.5%	21.4%	38.5%	26.7%
Any Pool	66.7%	84.6%	64.3%	76.9%	53.3%

Figure 34

EBOV001 ICS

- **47 subjects from all cohorts (1-5) are included in analysis. Cohort 5 is currently underrepresented.**
- ICS analysis performed at baseline and week 14
- A single EBOV peptide pool composed of Pools 1-4 is used for stimulation

Cohort descriptions

Cohort	Vaccine	Plasmids included in vaccine	Schedule	Route	Dose (mg)
1	INO-4201	pGX4201	0-4-12 weeks	IM	2
2	INO-4202	pGX4202	0-4-12 weeks	IM	2
3	INO-4201	pGX4201	0-4-12 weeks	ID	2
4	INO-4212	pGX4201 and pGX4202	0-4-12 weeks	IM	4
5	INO-4212 +INO-9012	pGX4201, pGX4202, and pGX6001	0-4-12 weeks	IM	4+1

Figure 35

EBOV001 ICS

Wilcoxon Paired analysis based on cohort

	Cohort 1 (2-tailed)	Cohort 2 (2-tailed)	Cohort 3 (2-tailed)	Cohort 4 (2-tailed)	Cohort 5 (2-tailed)
CD4 IFNg and TNFa	.770	.252	.695	.438	1.000
CD4 IFNg	.688	.105	.713	.063	1.000
CD4 IFNg and/or TNFa	.945	.121	.0039	.547	1.000
CD4 TNFa	.945	.301	.0078	.406	1.000
CD8 IFNg and TNFa	.770	.922	.027	.164	1.000
CD8 IFNg	.844	.133	.221	.125	1.000
CD8 IFNg and/or TNFa	.945	.695	.0010	.844	1.000
CD8 TNFa	.910	.734	.0010	.742	1.000

Evaluated subjects

Cohort	n=
1	12
2	12
3	11
4	9
5	3

Yellow = significant

Red = unevaluable due to N

Figure 36

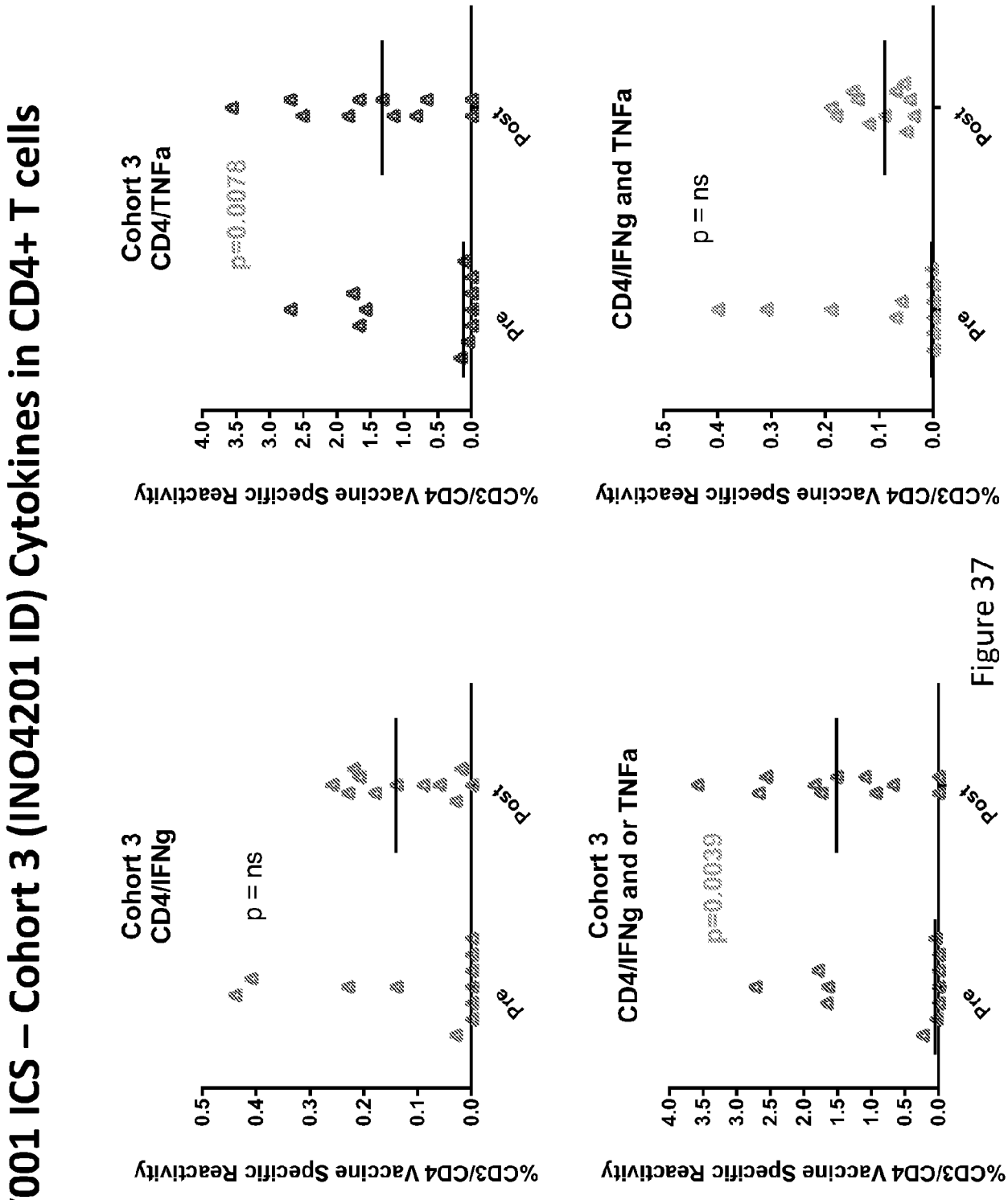
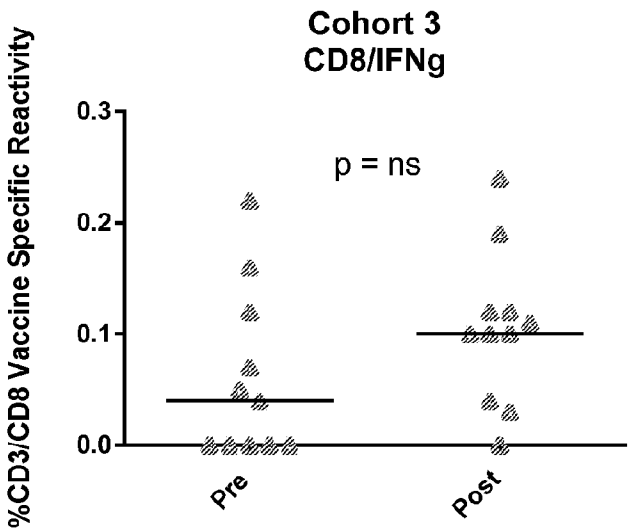
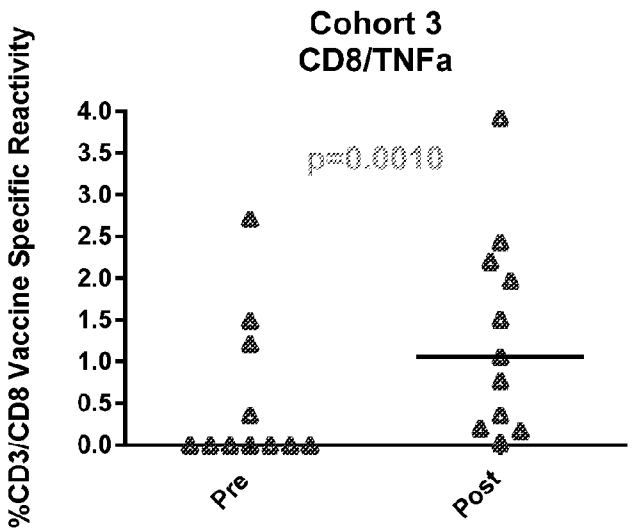
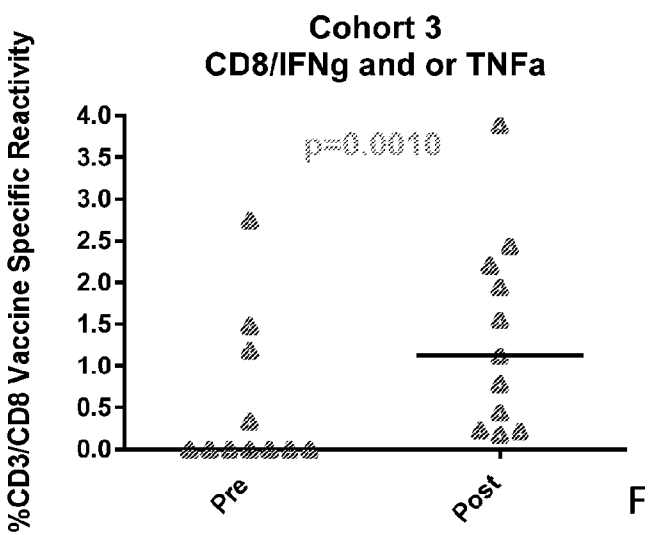

EBOV001 ICS – Cohort 3 (INO4201 ID) Cytokines in CD4+ T cells
Cohort 3
CD4/IFNg
p = ns
%CD3/CD4 Vaccine Specific Reactivity
0.0
0.1
0.2
0.3
0.4
0.5
Pre
Post
Cohort 3
CD4/TNFa
p=0.0078
%CD3/CD4 Vaccine Specific Reactivity
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Pre
Post
Cohort 3
CD4/IFNg and or TNFa
p=0.0039
%CD3/CD4 Vaccine Specific Reactivity
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Pre
Post
CD4/IFNg and TNFa
p = ns
%CD3/CD4 Vaccine Specific Reactivity
0.0
0.1
0.2
0.3
0.4
0.5
Pre
Post

Figure 37

EBOV001 ICS – Cohort 3 Cytokines in CD8+ T cells







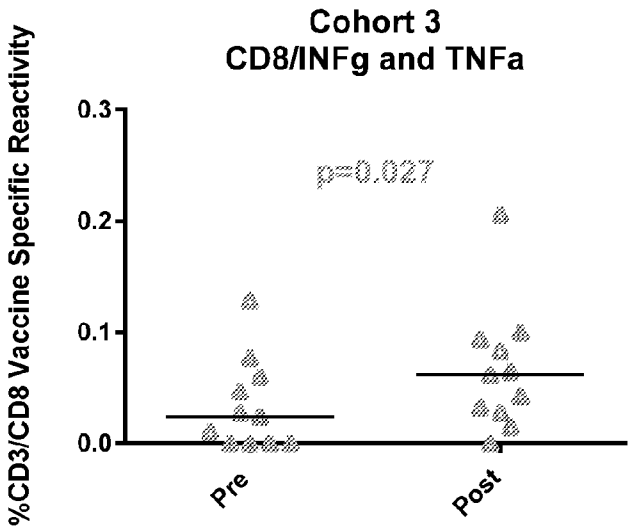


Figure 38

Vaccine Responders: All (n=75) subjects by cohort

- Vaccine responder is defined as meeting the threshold and having more SFUs post day 0
- Threshold = Mean + 2 x STDEV of total population's response on Day 0 for each pool

	4201-003	4201-004	4201-007	4201-008	4202-013	4202-025	4203-001	4203-006	4203-011	4203-016	4203-019	4203-020	4203-029	4203-031	4204-004
Cohort	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GP1	Non	Response	Response	Non	Response	Response	Non	Non	Response	Non	Non	Response	Non	Non	Non
GP2	Non	Response	Response	Non	Response	Non	Non	Non	Non	Response	Non	Response	Non	Non	Non
GP3	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non
GP4	Non	Non	Non	Non	N	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non
Any pool	Non	Response	Response	Non	Response	Response	Non	Non	Response	Response	Non	Response	Non	Non	Non

	4201-001	4201-005	4201-010	4202-006	4202-014	4202-016	4202-020	4203-005	4203-007	4203-013	4203-018	4203-021	4203-026	4203-032	4203-035
Cohort	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GP1	Non	Response	Response	Response	Response	Non	Response	Non	Response	Response	Non	Non	Response	Non	Non
GP2	Non	Response	Response	Response	Non	Non	Non	Non	Non	Non	Non	Non	Res	Non	Non
GP3	Non	Non	Response	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non
GP4	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non
Any pool	Non	Response	Response	Response	Response	Non	Response	Non	Response	Response	Non	Non	Response	Non	Non

	4201-002	4201-006	4201-012	4202-007	4202-015	4202-017	4202-026	4203-003	4203-009	4203-015	4203-023	4203-024	4203-028	4203-030	4203-037
Cohort	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
GP1	Non	Non	Non	Response	Response	Non	Response	Non	Non	Non	Non	Response	Non	Non	Non
GP2	Non	Non	Non	Response	Response	Response	Non	Non	Non	Non	Non	Non	Non	Non	Non
GP3	Non	Response	Non	Non	Non	Non	Res	Non	Non	Non	Non	Non	Non	Non	Non
GP4	Non	Response	Non	Non	Non	Non	Res	Non	Non	Non	Non	Non	Non	Non	Non
Any pool	Non	Response	Non	Response	Response	Response	Response	Non	Non	Non	Non	Response	Non	Non	Non

Figure 39

Vaccine Responders: All (n=75) subjects by cohort

- Vaccine responders is defined as meeting the threshold and having more SFUs post day 0
- Threshold = Mean + 2 x STDEV of total population's response on Day 0 for each pool

	4201-009	4201-011	4201-014	4201-017	4202-011	4202-027	4202-028	4202-030	4202-031	4203-014	4203-017	4203-040	4203-042	4203-043
Cohort	4	4	4	4	4	4	4	4	4	4	4	4	4	4
GP1	Response	Non	Non	Response	Response	Response	Response	Response	Non	Response	Non	Non	Non	Non
GP2	Response	Non	Non	Res	Non	Response	Response	Non	Non	Non	Non	Response	Non	Non
GP3	Non	Non	Non	Res	Non	Non	Res	Non	Non	Non	Non	Non	Non	Non
GP4	Non	Non	Non	Non	Non	Non	Res	Non	Non	Non	Non	Non	Non	Non
Any pool	Response	Non	Non	Response	Response	Response	Response	Response	Non	Response	Non	Response	Non	Non

	4201-018	4201-019	4201-020	4201-021	4201-022	4202-023	4202-024	4202-033	4203-034	4203-044	4203-045	4203-046	4203-047	4203-049	4203-050
Cohort	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GP1	Non	Response	Non	Response	Non	Response	Non	Non	Response	Non	Non	Non	Response	Non	Response
GP2	Response	Response	Non	Response	Non	Response	Non	Non	Response	Non	Non	Non	Non	Non	Non
GP3	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non
GP4	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non	Non
Any pool	Response	Response	Non	Response	Non	Response	Non	Non	Response	Non	Non	Non	Response	Non	Response

Figure 40

Median responses by cohort and pool (n=75)

	Median ELISpot Response					
Cohort	1	2	3	4	5	All Subjects
EBOV-Baseline	26.7	25.0	20.8	27.9	13.3	21.7
EBOV-Wk2	33.3	8.3	20.0	21.7	15.0	19.2
EBOV-Wk6	60.0	83.3	133.3	102.5	90.8	83.3
EBOV-Wk14	58.3	75.4	77.5	105.8	58.3	77.5
GP pool 1 -Baseline	6.7	3.3	5.0	6.3	1.7	4.2
GP pool 1 -Wk2	6.7	3.3	5.0	3.3	1.7	5.0
GP pool 1 -Wk6	17.5	30.0	23.3	25.8	13.3	23.3
GP pool 1 -Wk14	28.3	18.3	23.3	29.2	23.3	25.0
GP pool 2-Baseline	6.7	3.3	3.3	10.8	3.3	6.3
GP pool 2-Wk2	0.8	3.3	3.3	9.2	3.3	5.0
GP pool 2-Wk6	11.7	30.0	28.3	21.7	15.8	18.3
GP pool 2-Wk14	13.3	18.3	16.7	23.3	16.7	19.2
GP pool 3-Baseline	0.0	5.0	0.0	3.3	3.3	3.3
GP pool 3-Wk2	3.3	0.0	3.3	3.3	1.7	2.5
GP pool 3-Wk6	5.0	16.7	10.0	8.3	1.7	8.3
GP pool 3-Wk14	3.3	5.0	10.0	12.5	1.7	5.8
GP pool 4-Baseline	1.7	6.7	5.0	5.8	1.7	3.3
GP pool 4-Wk2	5.0	3.3	8.3	5.0	5.0	5.0
GP pool 4-Wk6	8.3	29.2	36.7	30.0	19.2	21.7
GP pool 4-Wk14	21.7	14.2	31.7	26.7	13.3	21.7

EBOV response = summed response of pools 1-4

Figure 41

Mean responses by cohort and pool (n=75)

	Mean ELISpot Response					
Cohort	1	2	3	4	5	All Subjects
EBOV-Baseline	124.6	116.3	85.6	148.9	44.6	103.4
EBOV-Wk2	112.5	66.9	158.7	154.1	41.7	107.2
EBOV-Wk6	147.1	206.0	295.3	291.3	88.3	203.5
EBOV-Wk14	128.3	121.8	160.6	280.1	128.1	163.9
GP pool 1 -Baseline	17.9	8.9	5.8	7.2	2.7	8.5
GP pool 1 -Wk2	21.9	6.0	8.4	11.4	3.1	10.3
GP pool 1 -Wk6	29.4	62.4	22.1	47.1	24.0	37.5
GP pool 1 -Wk14	38.7	33.8	25.3	69.6	31.7	40.3
GP pool 2-Baseline	8.8	17.4	9.2	18.5	5.8	11.8
GP pool 2-Wk2	4.2	20.7	9.8	23.7	6.6	12.6
GP pool 2-Wk6	26.4	52.7	27.8	40.2	26.2	34.9
GP pool 2-Wk14	24.0	31.0	21.9	58.7	53.4	37.8
GP pool 3-Baseline	27.8	8.2	26.6	44.8	12.0	23.6
GP pool 3-Wk2	21.1	15.0	46.6	43.6	13.4	28.1
GP pool 3-Wk6	25.0	35.5	80.5	66.0	5.2	41.6
GP pool 3-Wk14	19.1	27.2	36.1	60.6	6.7	30.0
GP pool 4-Baseline	69.9	81.8	44.1	78.4	24.1	59.4
GP pool 4-Wk2	65.3	25.2	93.9	75.4	18.6	56.2
GP pool 4-Wk6	66.2	55.4	164.9	137.9	32.9	89.6
GP pool 4-Wk14	46.6	29.9	77.2	91.2	36.3	55.8

EBOV response = summed response of pools 1-4

Figure 42

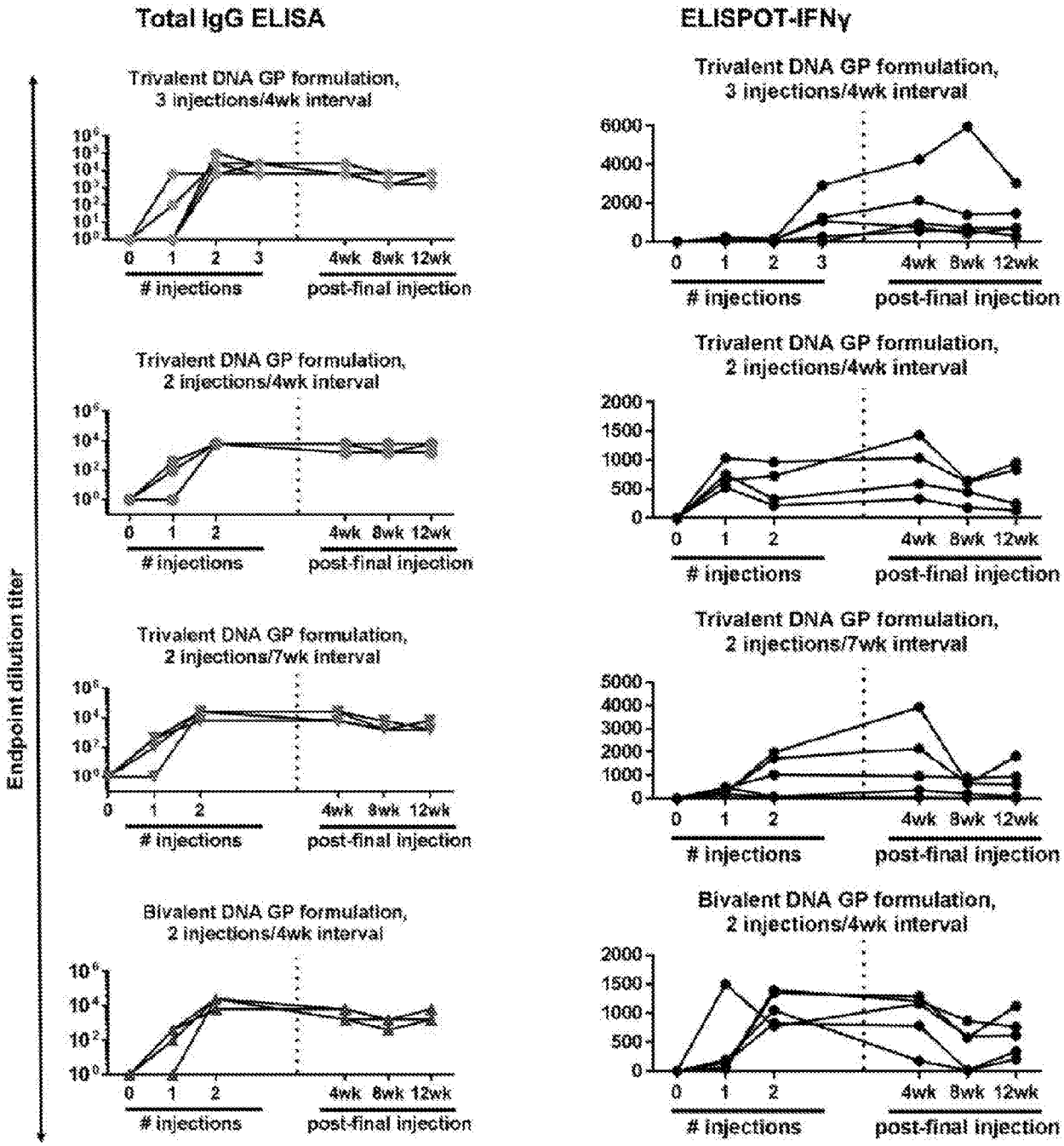
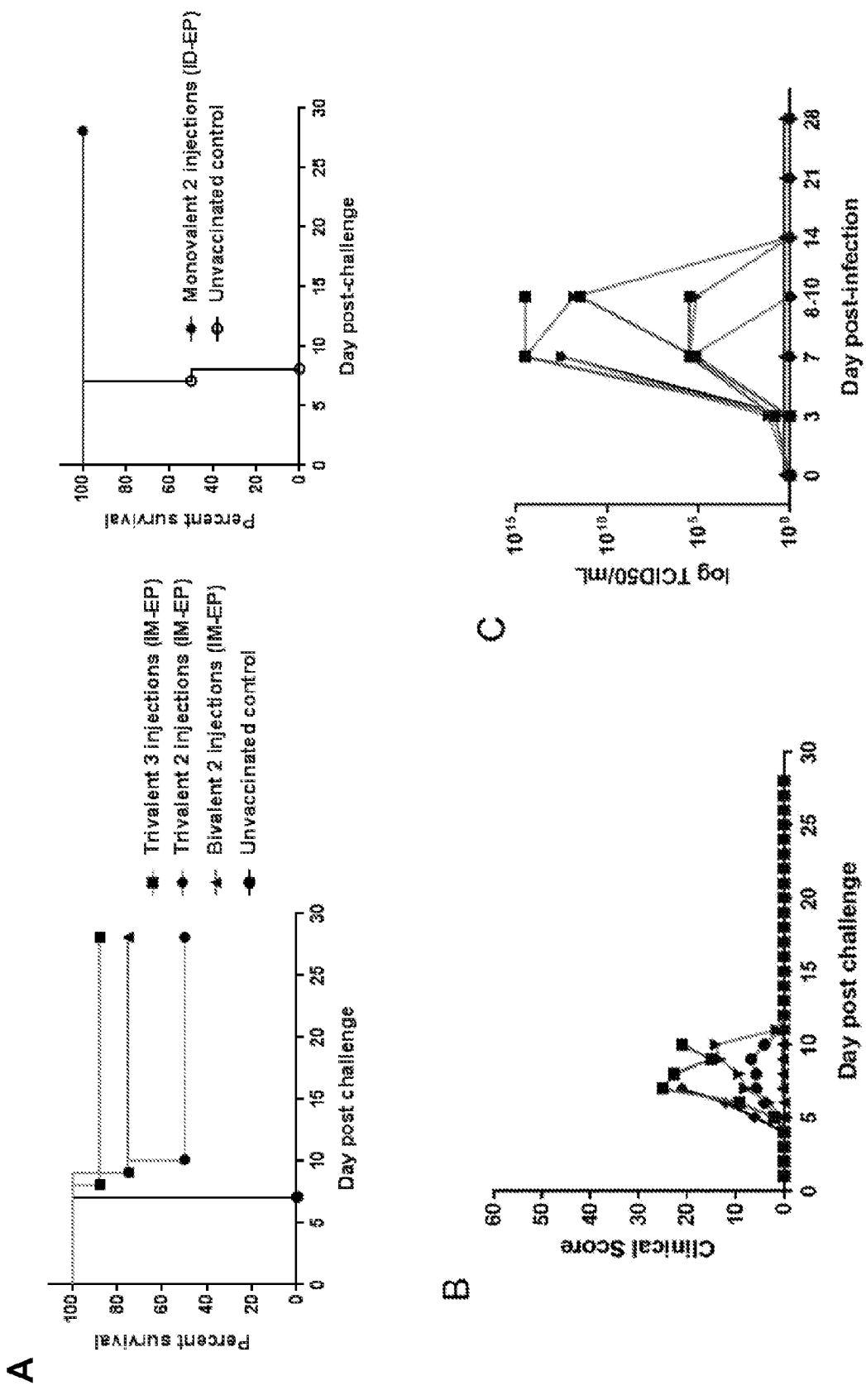
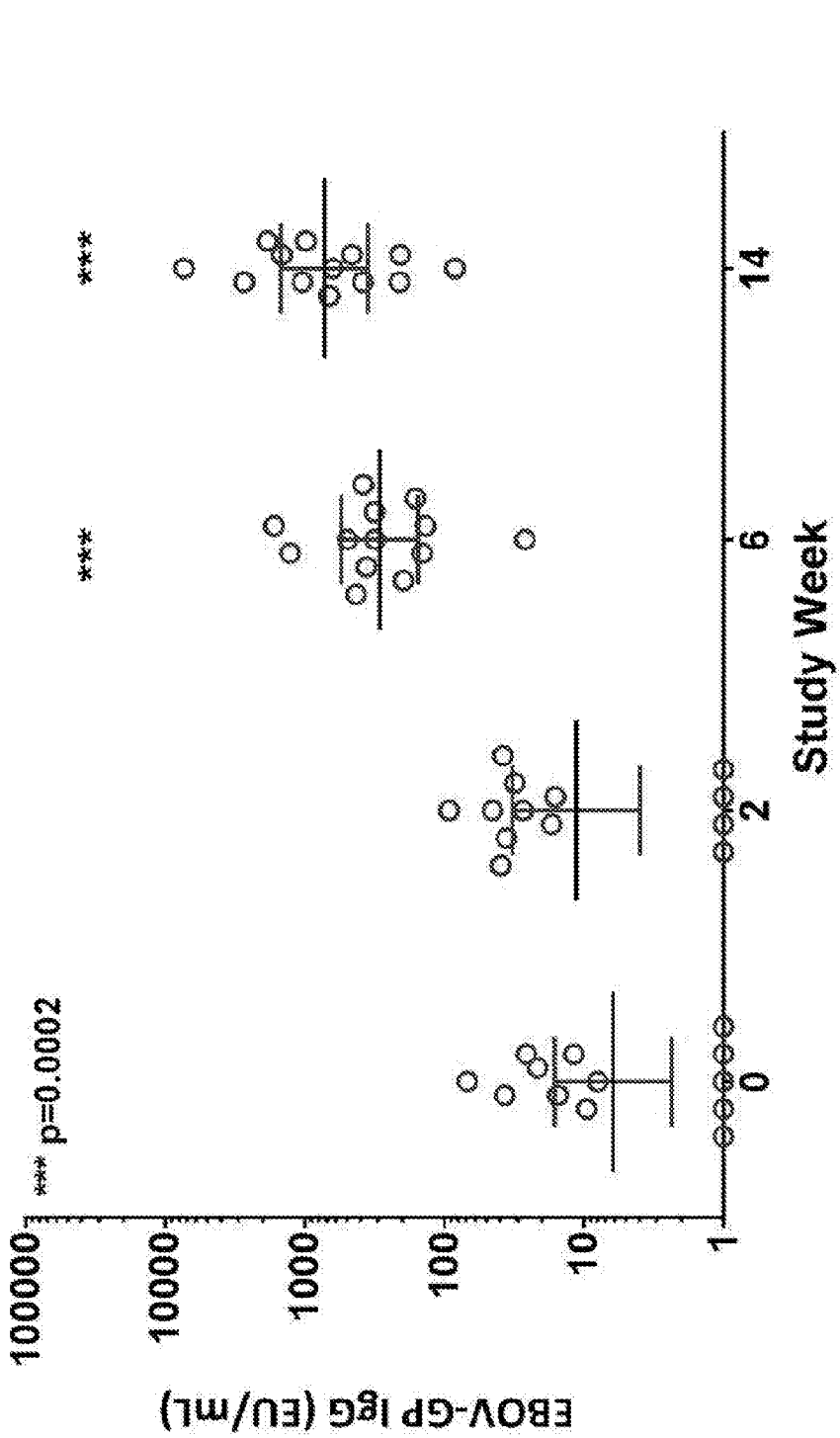

Total IgG ELISA
ELISPOT-IFNγ
Endpoint dilution titer
Trivalent DNA GP formulation,
3 injections/4wk interval
Trivalent DNA GP formulation,
2 injections/4wk interval
Trivalent DNA GP formulation,
2 injections/7wk interval
Bivalent DNA GP formulation,
2 injections/4wk interval
injections
post-final injection
0 1 2 3
4wk 8wk 12wk
6000
4000
2000
5000
3000
1500
1000
500

Figure 43



Figures 44A – 44C



EBOV-GP IgG (EU/mL)	
Week 2 EU/mL (range)	11.4 (1-68.7)
Week 6 EU/mL (range)	290.8 (1-92.5)
Week 14 EU/mL (range)	726.6 (84.3-7379.4)

Figure 45

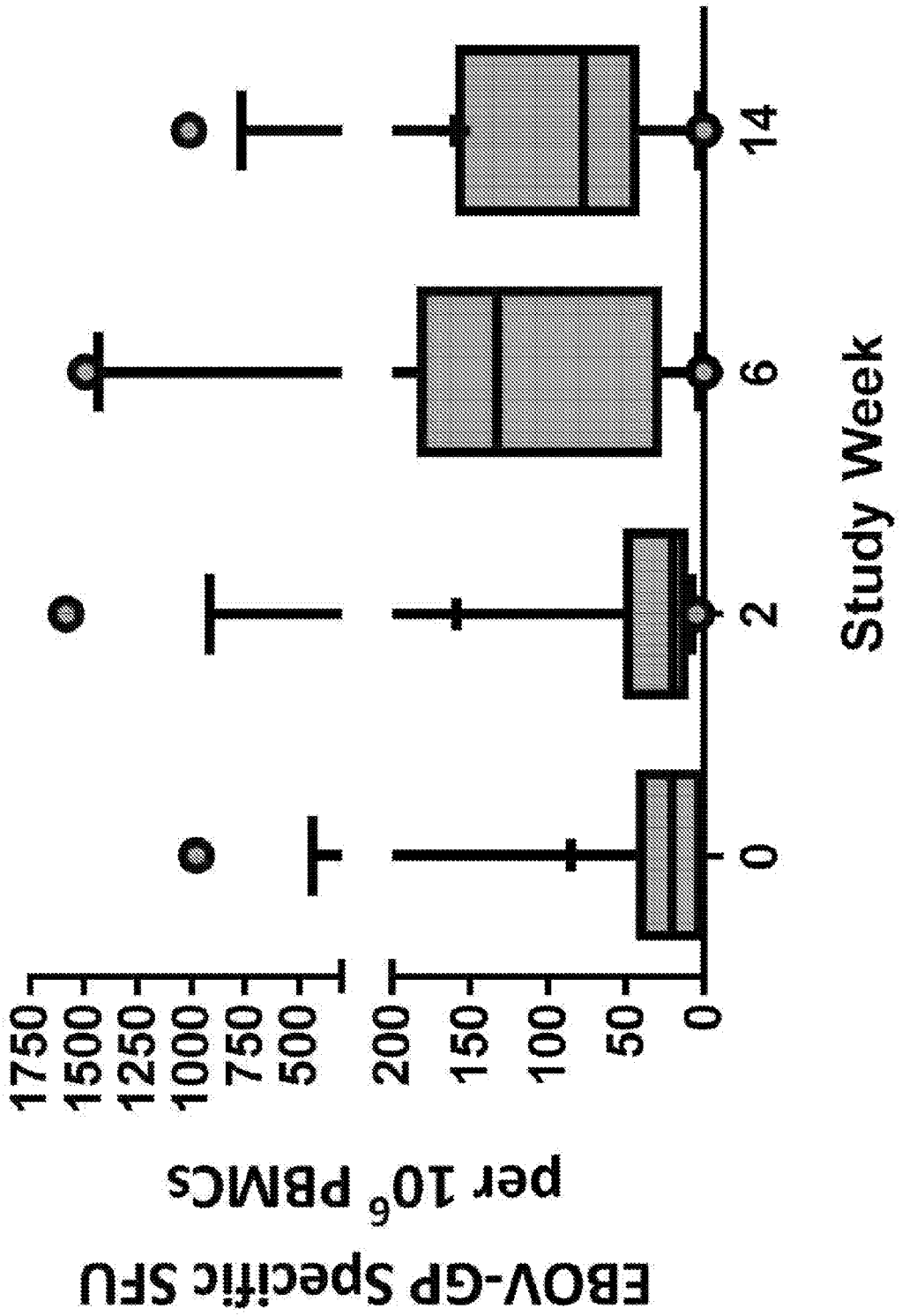
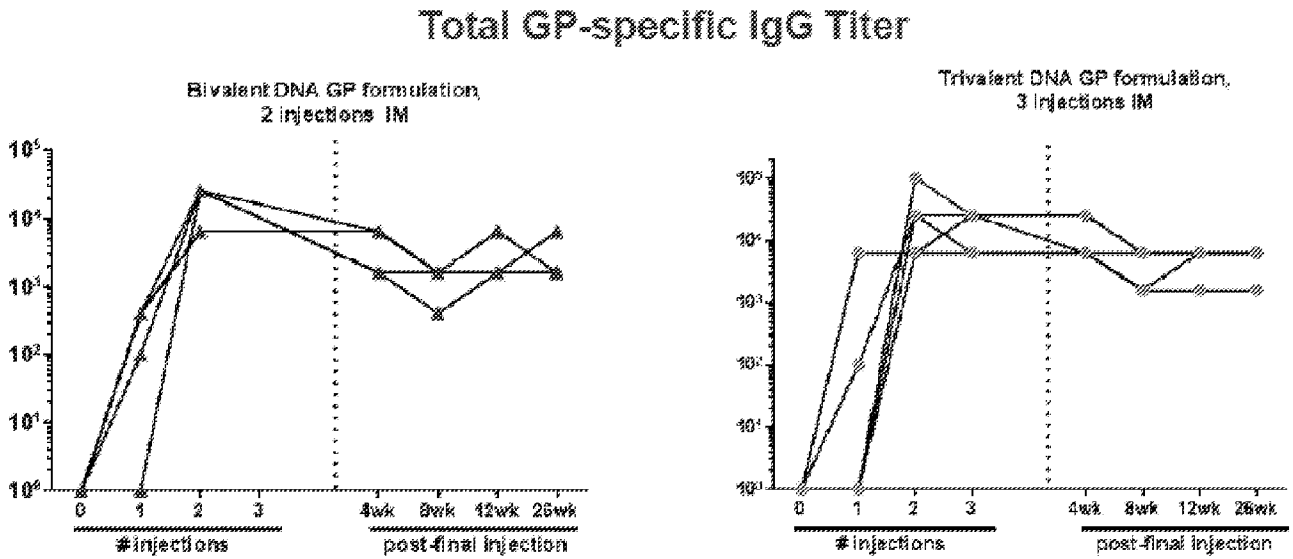

EBOV-GP Specific SFU
per 10^6 PBMCs
1750
1500
1250
1000
750
500
200
150
100
50
0
0
2
6
14
Study Week

Figure 46

Long-term immunogenicity with Ebola GP DNA Vaccine formulations in NHPs

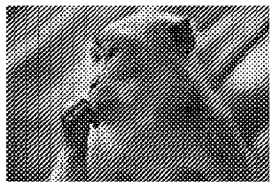


Strong antibody responses >6months post-vaccination

Figure 47

EBOV GP DNA Vaccine Memory Study: IM Delivery

Group	Vaccine	Constructs	Schedule	n	Dose
1	Trivalent	pGX4201 + pGX4202 + pGX4203 + opt IL-12 (pGX6006)	0-4-8 (3 injections)	5	3 mg + 3 mg + 3 mg + 0.2 mg
2	Trivalent	pGX4201 (old con) + pGX4202 (Guinea-GP) + pGX4203 (new con) + opt IL-12 (pGX6006)	0-4 (2 injections)	5	3 mg + 3 mg + 3 mg + 0.2 mg
3	Bivalent	pGX4201 + pGX4202 + opt IL-12 (pGX6006)	0-4 (2 injections)	5	4.5 mg + 4.5 mg + 0.2 mg
4	Trivalent	pGX4201 + pGX4202 + pGX4203 + opt IL-12 (pGX6006)	0 (1 injection)	5	3 mg + 3 mg + 3 mg + 0.2 mg



- immunized animals Jan-March 2016
- followed immune responses over 12 months following final dose
- gave a 1-year boost February 2017

Figure 48

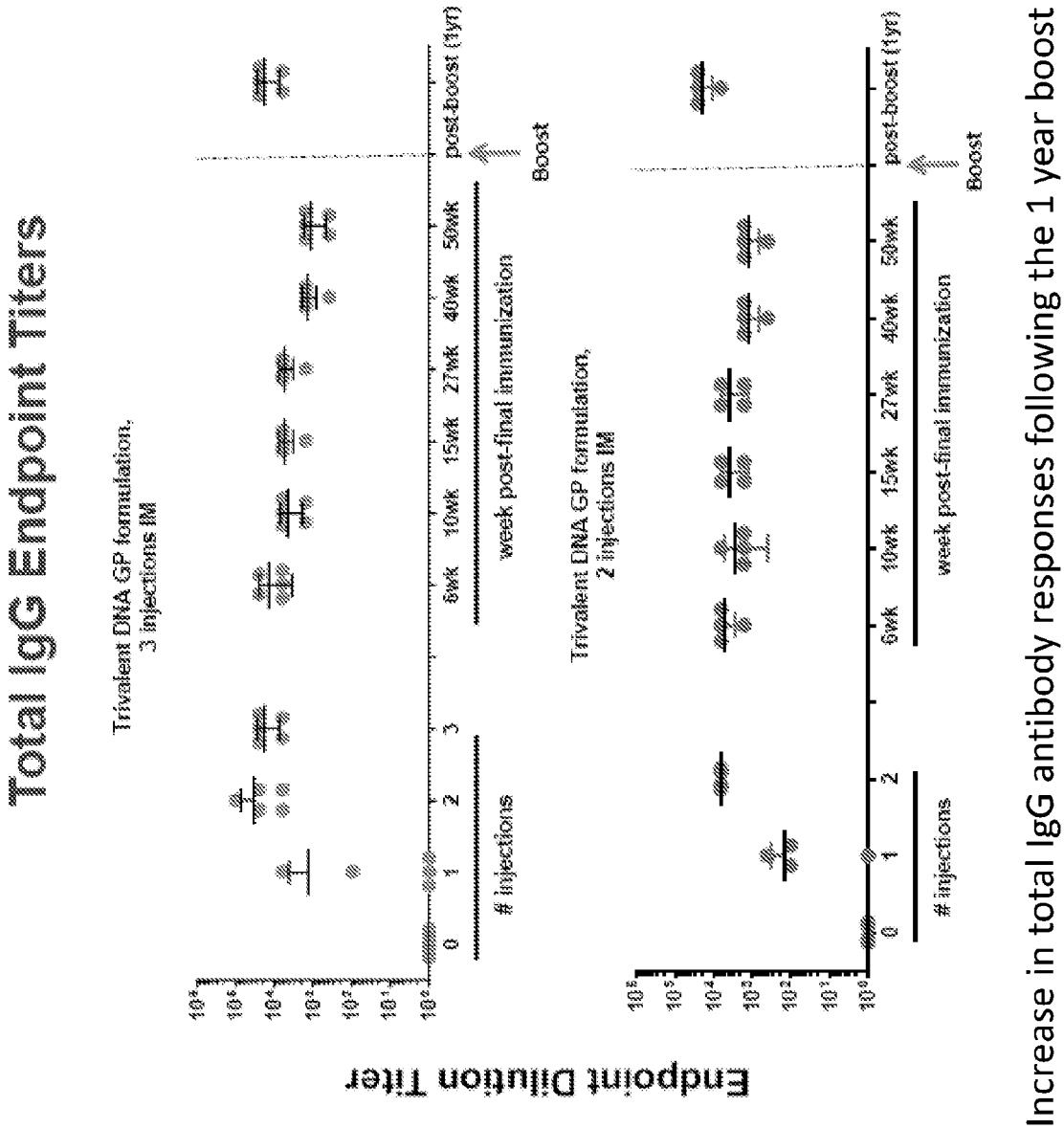
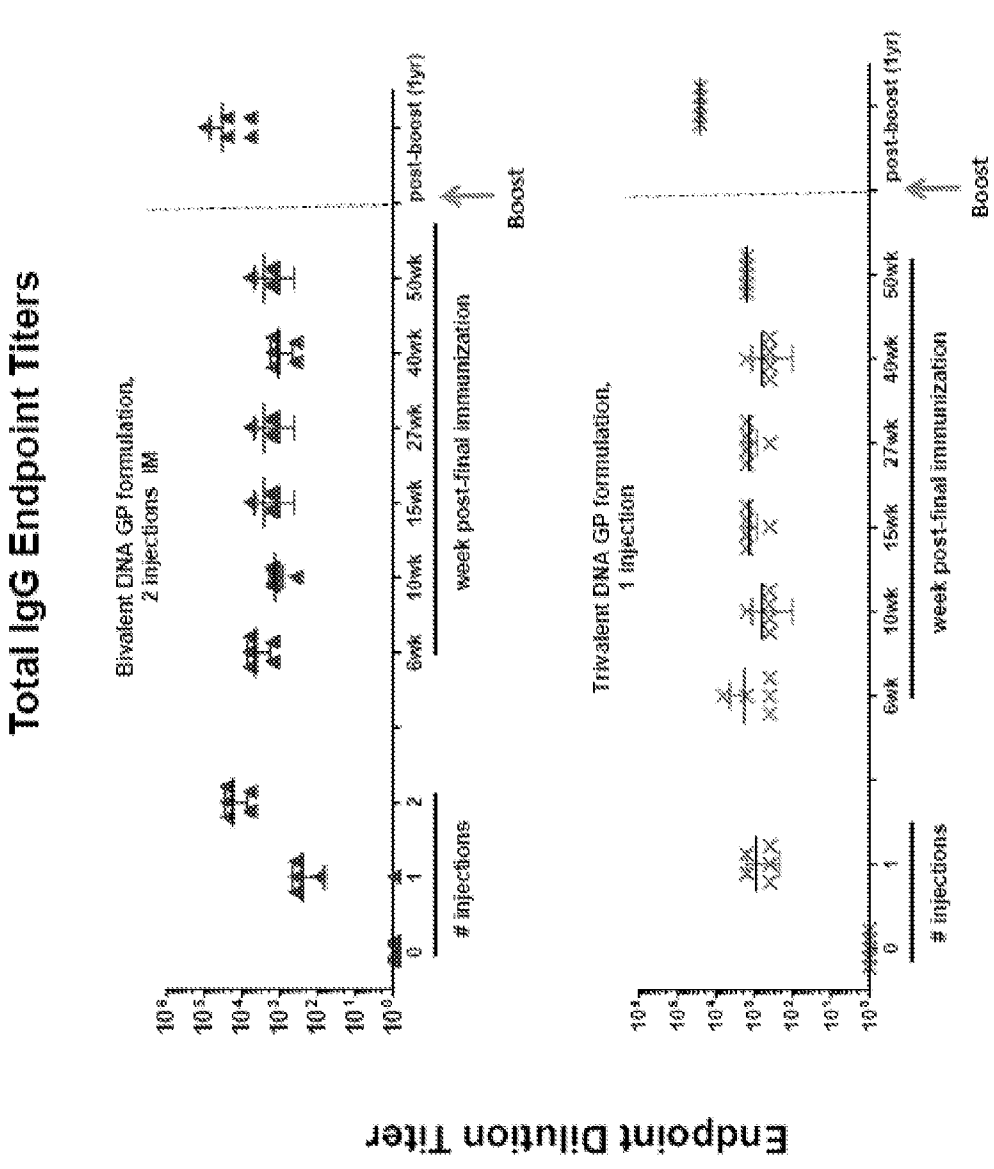


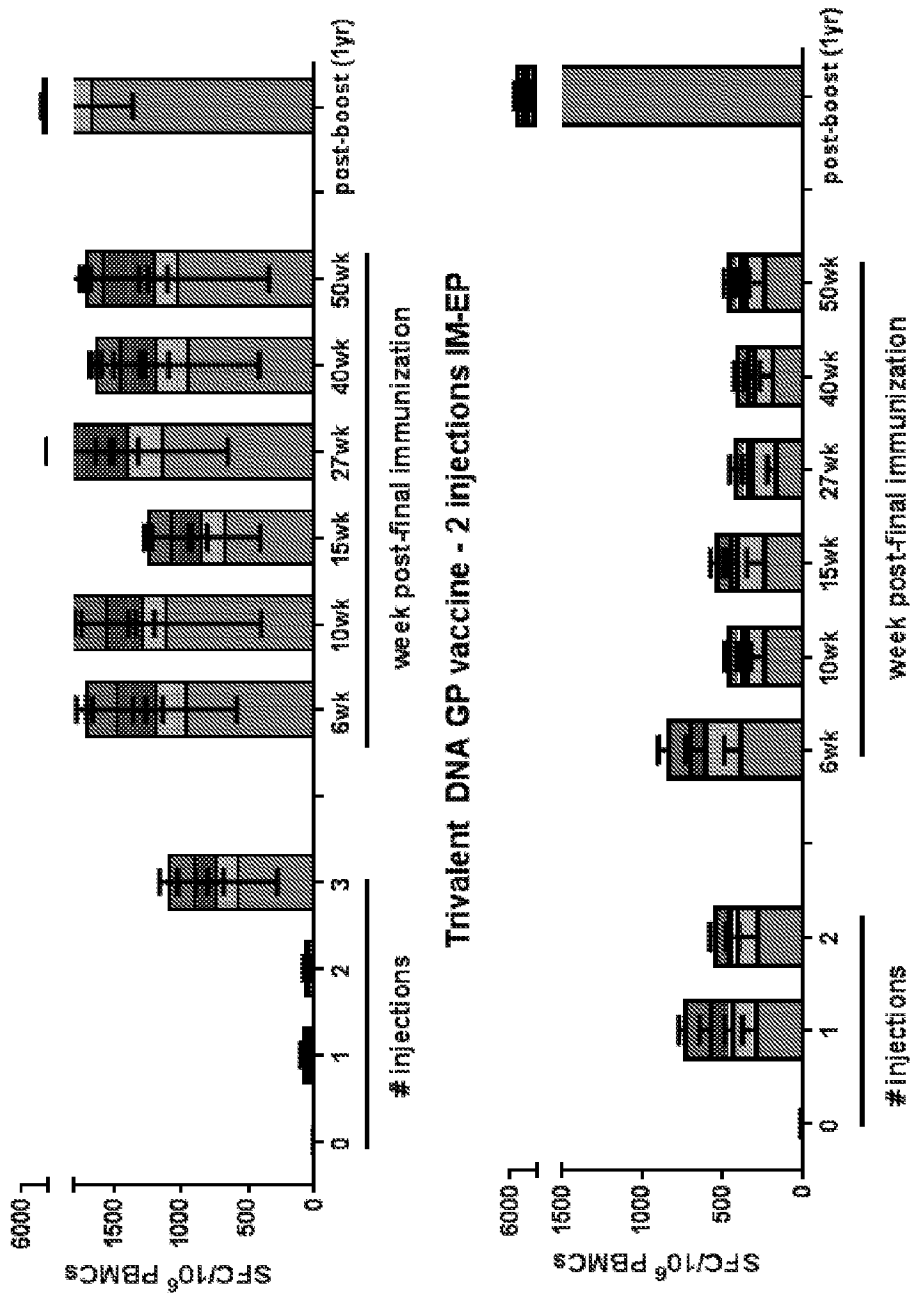
Figure 49

Increase in total IgG antibody responses following the 1 year boost



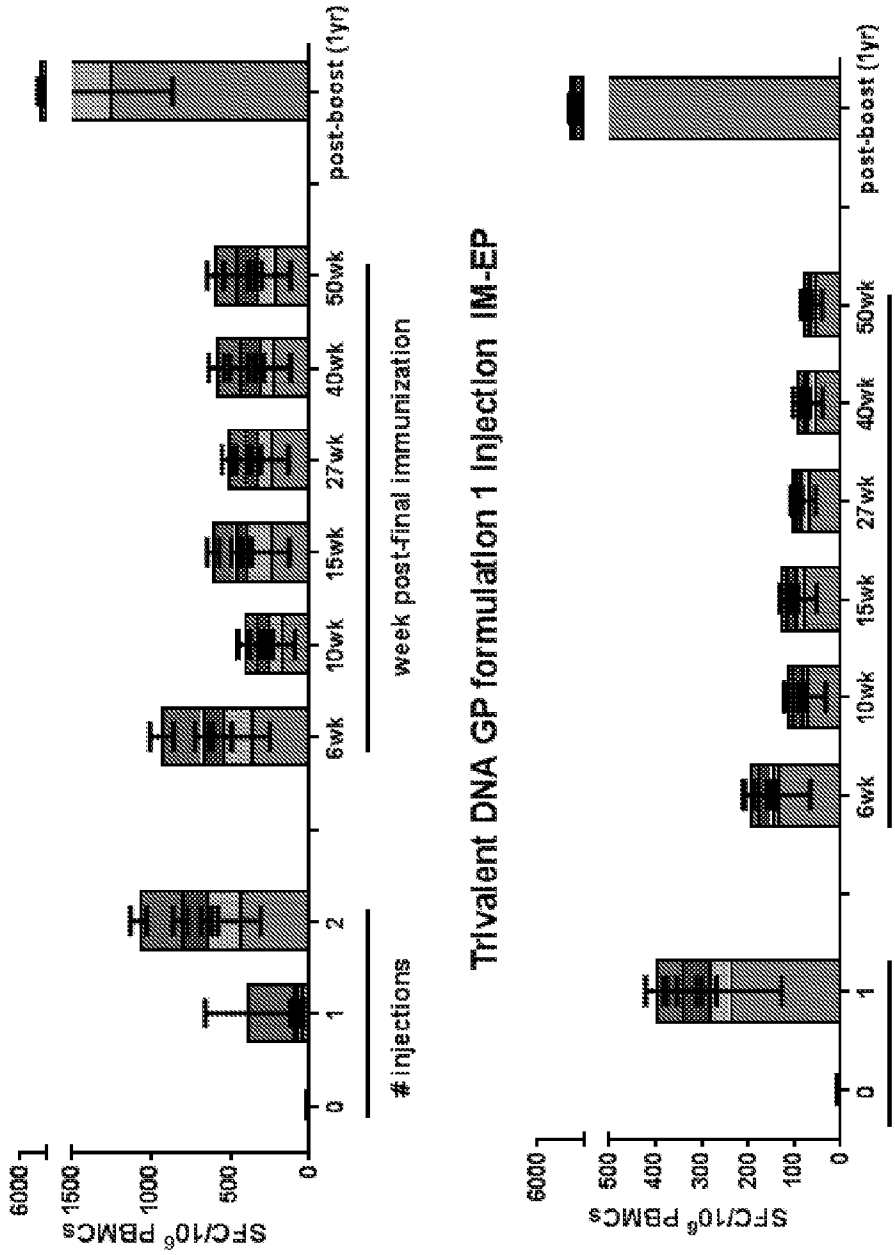
Increase in total IgG antibody responses following the 1 year boost

Figure 50



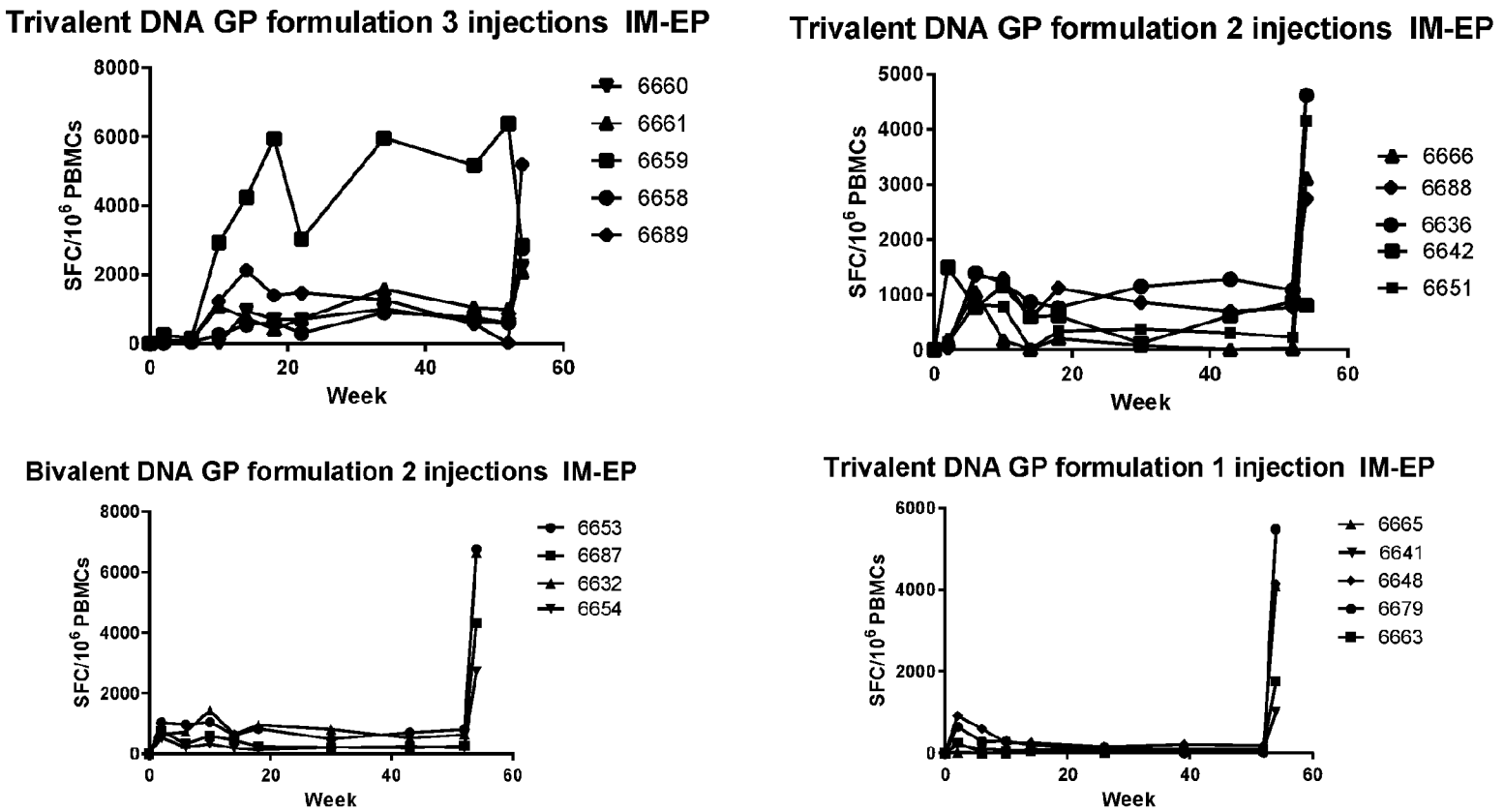
Increase IFNγ ELISPOT responses following the 1 year boost. Magnitude of boost is not as high in the 3 injection group.

Figure 51



Increase IFNγ ELISPOT responses following the 1 year boost. Remarkably strong boost in single immunization group.

Figure 52



- 17/19 animals had increased T cell responses following the boost.
- 1/19 animals maintained the same level of T cell responses.
- 1/19 animals had worse T cell responses following the boost. This was the animal that has been consistently high over the past year in the 3 injection group.

Figure 53

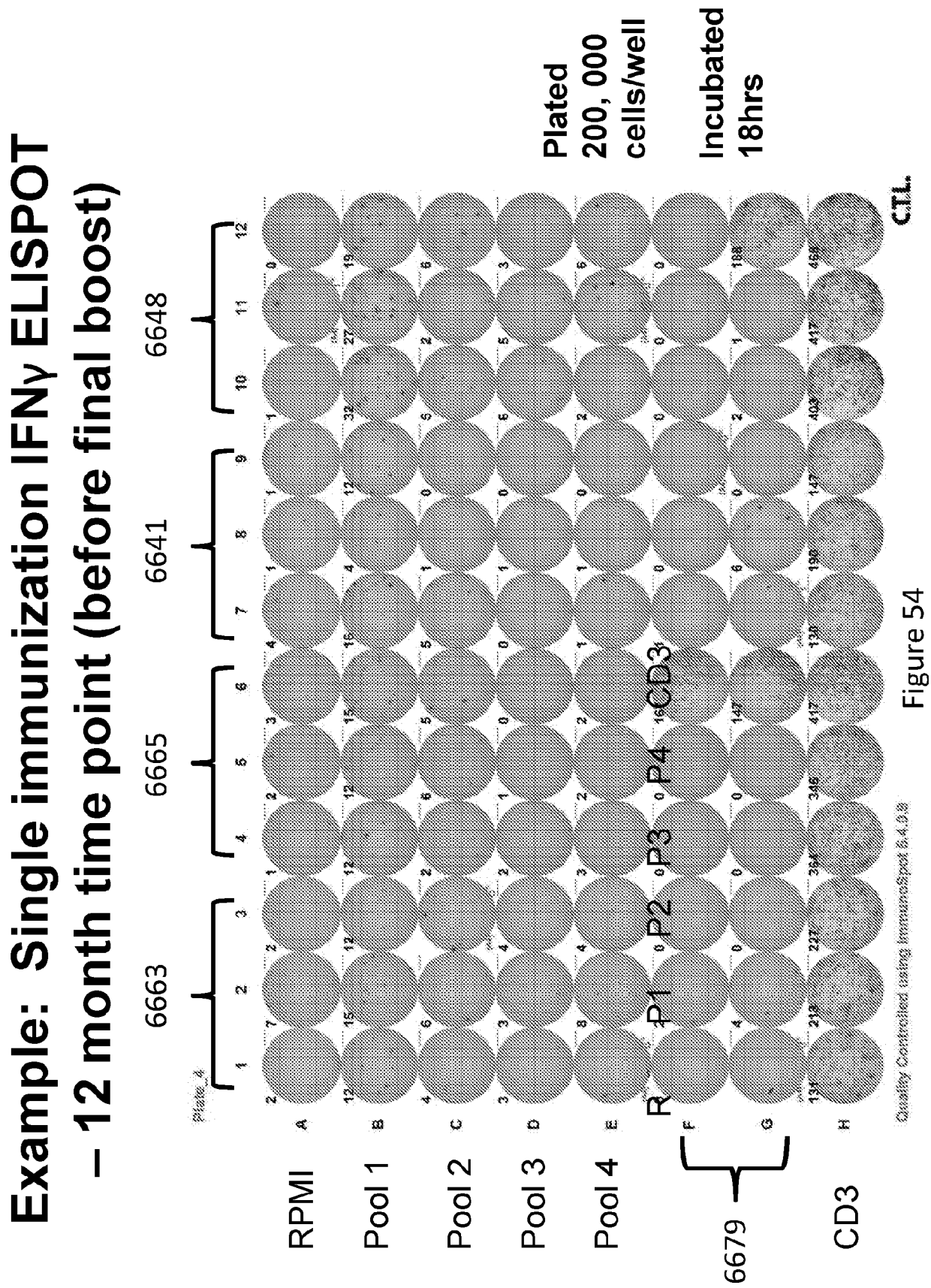

Example: Single immunization IFNγ ELISPOT – 12 month time point (before final boost)
6663
6665
6641
6648
Plated 200, 000 cells/well
Incubated 18hrs
RPMI
Pool 1
Pool 2
Pool 3
Pool 4
6679
CD3
R
P1
P2
P3
P4
CD3
CTL.
Quality Controlled using ImmunoSpot 6.4.0.13

Figure 54

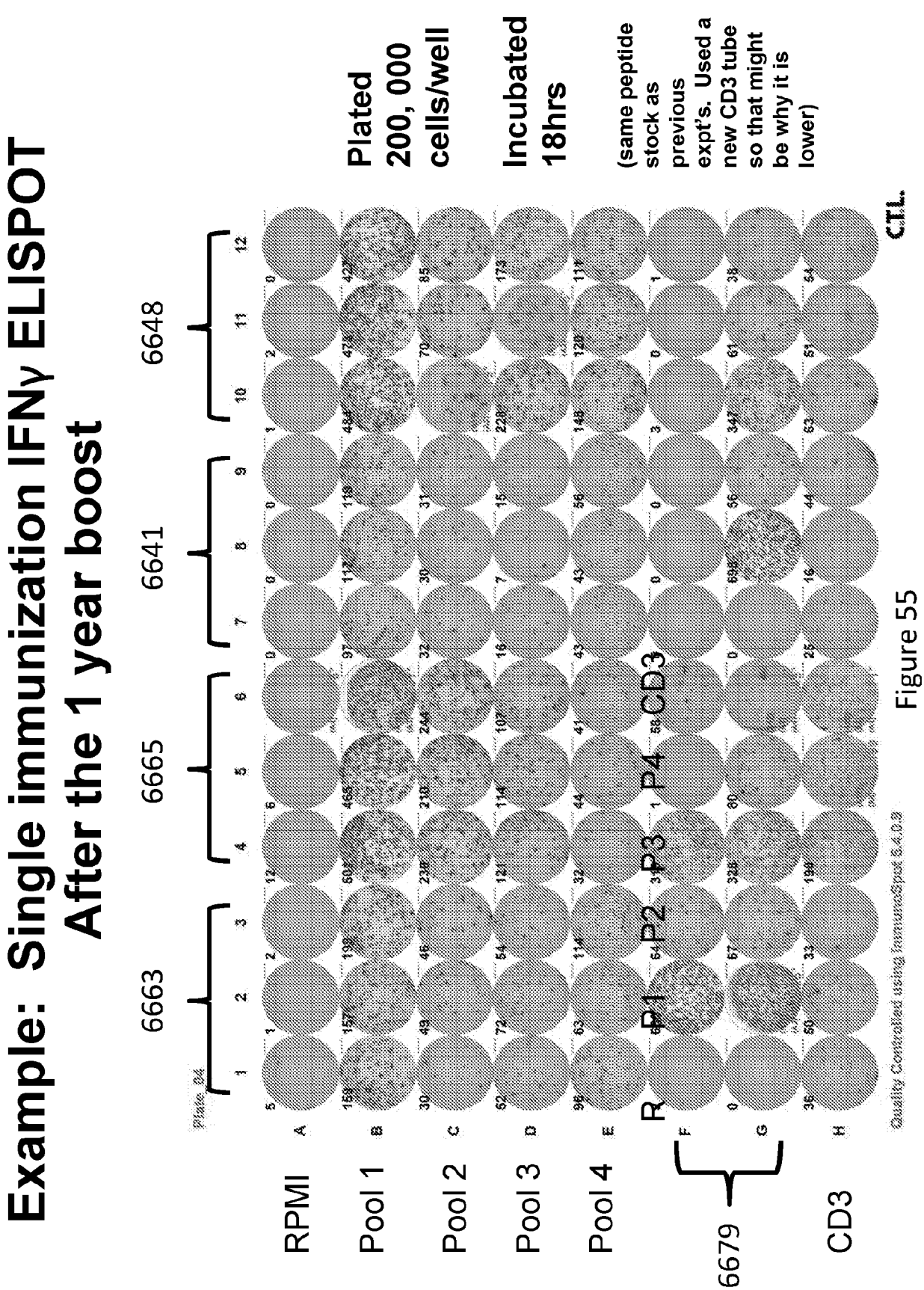

Example: Single immunization IFNγ ELISPOT
After the 1 year boost
6663
6665
6641
6648
Plated 200, 000 cells/well
Incubated 18hrs
(same peptide stock as previous expt's. Used a new CD3 tube so that might be why it is lower)
RPMI
Pool 1
Pool 2
Pool 3
Pool 4
6679
CD3
P1
P2
P3
P4
CD3
CTL.
Quality Controlled using ImmunoSpot 5.4.0.3

Figure 55

Summary – Long Term Memory

- EBOV-GP DNA vaccine elicits long-term immune responses
- Strong recall response following a 1 year boost
  - This response is remarkably high in the group that received a single IM injection
- Next steps: memory study with intradermal delivery
  - Working on securing a challenge slot for this study

Figure 56

EBOV-001: INO-4212 DNA Vaccine Clinical Trial

- Initial study enrolled 75 healthy volunteers across 5 cohorts
- 3 dose regimen (weeks 0, 4, 12)
- 2-8°C stable vaccine formulation: Does not require frozen cold-chain
  - 1 year stability at room temperature (25°C)

Cohort descriptions

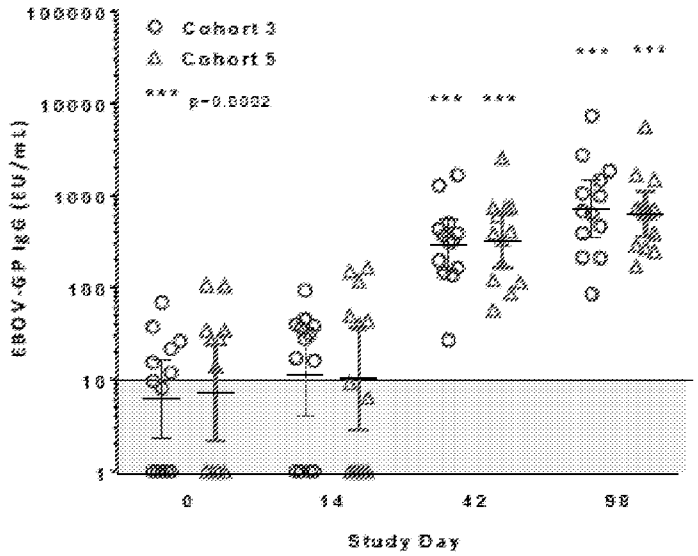
Cohort	Vaccine		Vac. Schedule (Weeks)	# Volunteers (actual)	Vac. Route	Dose
1	INO-4201	Pre-2014 consensus	0-4-12	15 (14)	IM	2 mg
2	INO-4202	2014 Guinea	0-4-12	15 (15)	IM	2 mg
3	INO-4201	Pre-2014 consensus	0-4-12	15 (13)	ID	2 mg
4	INO-4212	(4201 + 4202)	0-4-12	15 (14)	IM	4 mg
5	INO-4212 + INO-9012	4212 + human IL-12	0-4-12	15 (14)	IM	4 mg + 1 mg

Figure 57

Comparison of Binding Antibodies in EBOV-001 to rVSV EBOV

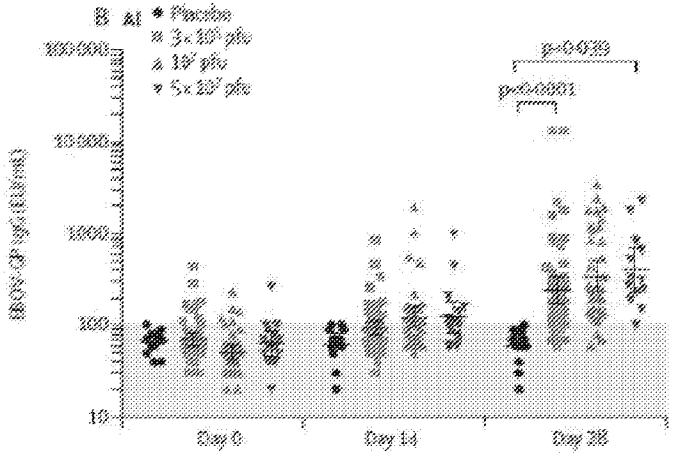
All 5 EBOV-001 cohorts had significant increases at both Weeks 6 and 14 compared to Week 0

ADI ELISA from EBOV-001



		Day 42	Day 98
Cohort 3	INO-4201 ID	290.8	726.6
Cohort 5	INO-4212 + IL12 IM	320.6	629.6

ADI ELISA from rVSV-EBOV



Huttner, A., *et al.* 2015. *Lancet Infect. Dis.* 15:1156-66

	Day 28
3x10^5 PFU	241.4
1x10^7 PFU	342.3
5x10^7 PFU	392.8

Figure 58

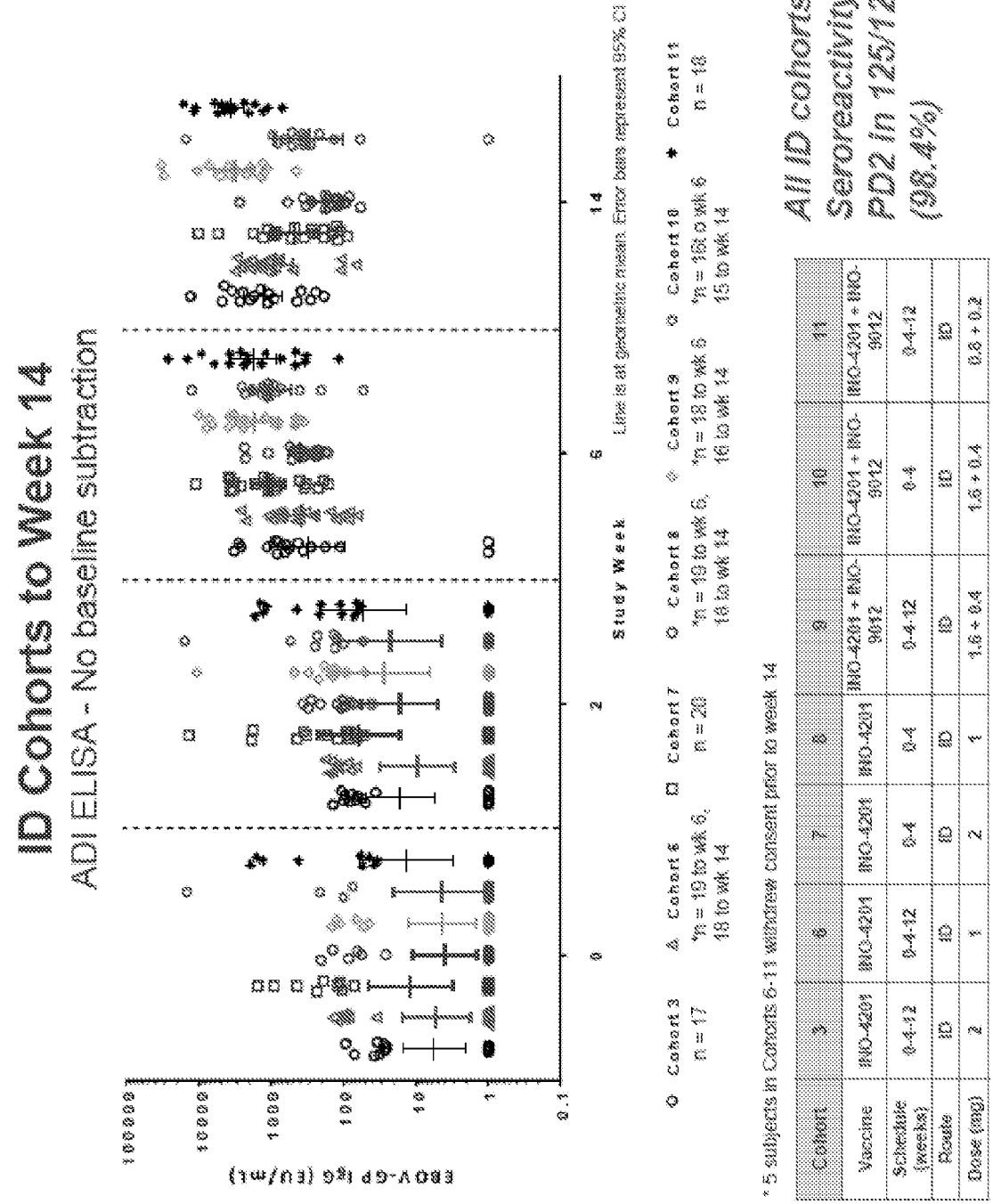
EBOV-001: ID Delivery Expansion Cohorts

Based on the seroconversion observed cohort 3 (ID delivery) 6 additional ID cohorts were added to explore different doses, dose regimens and impact of IL-12 DNA as an immuno-adjuvant

Cohort	Vaccine	Dose Schedule (Weeks)	# Volunteers	Vac. Route	Dose (mg)
1	INO-4201	0-4-12	15	IM	2
2	INO-4202	0-4-12	15	IM	2
3	INO-4201	0-4-12	20*	ID	2
4	INO-4212	0-4-12	15	IM	4
5	INO-4212 + INO-9012	0-4-12	15	IM	4 + 1
6	INO-4201	0-4-12	20	ID	1
7	INO-4201	0-4	20	ID	2
8	INO-4201	0-4	20	ID	1
9	INO-4201 + INO-9012	0-4-12	20	ID	1.6 + 0.4
10	INO-4201 + INO-9012	0-4	20	ID	1.6 + 0.4
11	INO-4201 + INO-9012	0-4-12	20	ID	0.8 + 0.2
		TOTAL	200		

* Part 2 enrolled an additional 5 subjects will be enrolled in cohort 5

Figure 59



Cohort	3	6	7	8	9	10	11
Vaccine	INO-4201	INO-4201	INO-4201	INO-4201	INO-4201 + INO-9012	INO-4201 + INO-9012	INO-4201 + INO-9012
Schedule (weeks)	0-4-12	0-4-12	0-4	0-4	0-4-12	0-4	0-4-12
Route	ID	ID	ID	ID	ID	ID	ID
Dose (mg)	2	1	2	1	1.6 + 0.4	1.6 + 0.4	0.8 + 0.2

* 5 subjects in Cohorts 6-11 withdrew consent prior to week 14

All ID cohorts; Seroreactivity PD2 in 125/127 (98.4%)

Figure 60

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/31215

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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/31215

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.:
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, 8, 11, 14, 17-20, (21-22) (in part), limited to ZEBOVCON (SEQ ID NOs: 1 and 69)

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 17/31215

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 35/76, A61K 39/12, A61K 48/00, A61P 31/14, A61P 37/04 (2017.01)
CPC - A61K 39/12, A61K 2039/53, A61K 35/76, C12N 2760/14134, C12N 2760/14234, C12N 7/00

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 --- Y	US 2015/0335726 A1 (WEINER et al.) 26 November 2015 (26.11.2015) para [0009], [0013], [0014], [0015], [0051], [0061], [0116], [0126], [0131], [0167]; SEQ ID NO: 1	1-3, 8, 11, 17-20, (21-22)/(1-3,8,11,17-20) ---------- 14, (21-22)/14
Y	US 2016/0045589 A1 (WEINER et al.) 18 February 2016 (18.02.2016) para [0015], [0140], SEQ ID NO: 14	14, (21-22)/14

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2017	02 OCT 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/31215

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 1-22, drawn to compositions comprising isolated nucleic acid molecule comprising a nucleic acid sequence encoding an ebolavirus envelope glycoprotein immunogen. The compositions will be searched to the extent that the ebolavirus envelope glycoprotein immunogen encompasses a first consensus Zaire ebolavirus envelope glycoprotein immunogen (ZEBOVCON) comprising an amino acid sequence with homology to SEQ ID NO: 1, and a nucleic acid sequence with homology to SEQ ID NO: 69. It is believed that claims 1-3, 8, 11, 14, 17-20, (21-22) (in part), limited to ZEBOVCON (SEQ ID NOs: 1 and 69), encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass the ebolavirus envelope glycoprotein immunogen of ZEBOVCON (SEQ ID NOs: 1 and 69). Additional ebolavirus envelope glycoprotein immunogen sequences will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected ebolavirus envelope glycoprotein immunogen sequences. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be wherein the ebolavirus envelope glycoprotein immunogen encompasses a second consensus Zaire ebolavirus envelope glycoprotein immunogen (ZEBOVCON2) comprising an amino acid sequence with homology to SEQ ID NO: 67, and a nucleic acid sequence with homology to SEQ ID NO: 70, i.e. claims 1, 6-7, 10, 13, 16-20, (21-22) (in aprt), limited to ZEBOVCON2 (SEQ ID NOs: 68 and 70).

Group II: Claims 23-28, drawn to methods of inducing an immune response against a filovirus such as Ebolavirus, methods of treating an individual who has been diagnosed with Ebolavirus, and methods of preventing Ebolavirus infection in an individual.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept 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+ requires compositions of matter, not required by the methods of Group II. Further, the technical feature of each of the inventions listed as Group I+ is the specific ebolavirus envelope glycoprotein immunogen sequences recited therein. Each invention requires ebolavirus envelope glycoprotein immunogen sequences not required by any of the other inventions.

Group II requires method steps for inducing an immune response in an individual and treating or preventing Ebolavirus infection in an individual, not required by the compositions of Group I+.

Common Technical Features

The feature shared by Groups I+ and II is an isolated nucleic acid molecule comprising one or more nucleic acid sequences encoding an amino acid sequence of an ebolavirus envelope glycoprotein immunogen (claims 1-10), wherein the ebolavirus envelope glycoprotein immunogen is linked to an IgE signal peptide (claims 11-13), nucleic acids encoding the ebolavirus envelope glycoprotein immunogen (claims 14-16), compositions that can comprise two or three nucleic acid molecules (claims 17-19), wherein the nucleic acid molecule is a plasmid (claim 20).

The feature shared by the inventions listed as Groups I+ is the isolated nucleic acid molecule of claim 1 and the composition of claim 17.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2015/0335726 A1 to Weiner et al. (hereinafter 'Weiner').

Weiner discloses [claims 1-10] an isolated nucleic acid molecule comprising one or more nucleic acid sequences selected from the group consisting of a nucleic acid encoding a first consensus Zaire ebolavirus envelope glycoprotein immunogen (ZEBOVCON), wherein the ZEBOVCON comprises an amino acid sequence that is at least 95% homologous to SEQ ID NO: 1 (para [0009] "A composition comprising a nucleic acid sequence that encodes a consensus Zaire ebolavirus envelope glycoprotein immunogen . . . The amino acid sequence of the consensus Zaire ebolavirus envelope glycoprotein immunogen may be SEQ ID NO:1 (ZEBOV CON)"; para [0061] "The antibody may be an antibody isolated from the serum sample of mammal, . . . affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom"; SEQ ID NO: 1, amino acids 1-676, exhibits 100% identity to claimed SEQ ID NO: 1).

Weiner further discloses [claims 11-13] wherein the ebolavirus envelope glycoprotein immunogen is linked to an IgE signal peptide (para [0116] "The amino acid sequence for immunogens include SEQ ID NO:1-6, . . . optionally including a signal peptide such as for example an IgE or IgG signal peptide").

-------please see continuation on next extra sheet-------

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

Weiner further discloses [claims 14-16] nucleic acids encoding the ebolavirus envelope glycoprotein immunogen (para [0118] "Nucleic acid sequence may encodes a full length consensus Zaire ebolavirus envelope glycoprotein immunogen"; para [0013] "Each of the different nucleic acid sequences may be on a single nucleic acid molecule, may each be on a separate nucleic acid molecules or various permutations. Nucleic acid molecules may be plasmids").

Weiner further discloses [claims 17-19] compositions that can comprise two or three nucleic acid molecules (para [0009] "A composition comprising a nucleic acid sequence that encodes a consensus Zaire ebolavirus envelope glycoprotein immunogen"; para [0051] "In some embodiments, the strategy employs coding sequences for three filovirus immunogens"; para [0126] "A single plasmid may contain coding sequence for a single filoprotein immunogen, coding sequence for two filoprotein immunogens, coding sequence for three filoprotein immunogens").

Weiner further discloses [claim 20] wherein the nucleic acid molecule is a plasmid (para [0013] "Nucleic acid molecules may be plasmids").

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.

Groups I+ and II therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.