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DNA Vaccines Encoding *Ebolavirus* and *Marburgvirus* Wild Type Glycoproteins are Safe and Immunogenic in a Phase I Clinical Trial

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ABSTRACT

Background: *Ebolaviruses* and *Marburgviruses* cause severe hemorrhagic fever with high mortality and are potential bioterrorism agents. There are no available vaccines or therapeutic agents. Previous clinical trials evaluated transmembrane-deleted and point-mutation *Ebolavirus* glycoproteins (GPs) in candidate vaccines. Constructs evaluated in this trial encode wild-type (WT) GP from *Ebolavirus* Zaire and Sudan species and the Marburg virus Angola strain expressed in a DNA vaccine.

Methods: VRC 206 evaluated the safety and immunogenicity of these DNA vaccines administered at 4 mg IM by Biojector® at weeks 0, 4, 8 with a homologous boost at ≥week 32. Safety evaluations included solicited reactogenicity and coagulation parameters. Primary immune assessment was by GP-specific ELISA.

Results: The vaccines were well tolerated and there were no serious adverse events. 80% of subjects had positive ELISA (≥30) at week 12. The fourth DNA vaccination boosted the immune responses.

Conclusions: The investigational *Ebolavirus* and *Marburgvirus* WT GP DNA vaccines were safe, well tolerated, and immunogenic, in this Phase I study. These results will further inform filovirus vaccine research towards a goal of inducing protective immunity utilizing WT GP antigens in candidate vaccine regimens.

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Introduction

Ebolaviruses and *Marburgviruses* belong to the family *Filoviridae* and are known to cause outbreaks of viral hemorrhagic fever- a severe and often fatal disease. Filoviruses are negative strand RNA viruses. A single glycoprotein (GP) facilitates viral entry likely through receptor mediated endocytosis into monocytes and macrophages, endothelial cells, and hepatocytes [1-3]. The wild type (WT) GP antigen is the primary antigen targeted by candidate vaccines.

There are five species of *Ebolavirus*: Zaire (EBOV), Sudan SUDV), Reston (RESTV), Taï Forest (TAFV) and Bundibugyo (BDBV). EBOV and SUDV have been responsible for several human outbreaks with case fatality rates of 41-90% [4]. They are therefore targeted species for vaccine development. *Marburgvirus* has a single species with two viruses that include Marburg virus (MARV) and Ravn virus (RAVV), and several strains, including Angola [5] that are currently targeted for vaccine development.

Outbreaks of *Ebolavirus* and *Marburgvirus* have occurred in Africa and have intermittently re-emerged with varying case fatality rates. In 2014, an outbreak of Ebola virus disease (EVD), species Zaire ebolavirus, in West Africa, including Guinea, Sierra Leone, Liberia, and Nigeria, is the worst outbreak to date and the first to be localized primarily in urban areas [6]. With a case fatality rate ranging from 60-87% reported in the first few months of the outbreak. This outbreak affected community members as well as healthcare workers, and appears to have spread person-to-person through regional and international travel. Marburg virus disease (MVD) has primarily occurred in travelers [4, 5] and similarly to EVD, has case fatality rates of 23-90%[7], the potential to spread internationally with increasing global travel, and the potential threat to be used as a biological weapon.

Several candidate vaccine platforms have been investigated in animal models including vectors such as vesicular stomatitis virus (VSV), DNA plasmids, virus like particles, and recombinant adenovirus (rAd) alone or as part of a prime-boost strategy [2, 5, 8-11]. Research and development toward a vaccine that would provide protective immunity against these infections has been an iterative process requiring

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the clinical evaluation of interim candidate GP vaccine antigens, in part because of theoretical safety concerns.

In 2006, we reported the first clinical trial evaluating a multi-gene DNA vaccine encoding transmembrane-deleted GP from EBOV and SUDV and nucleoprotein (NP) from EBOV [12]. The vaccine was well-tolerated, with no significant adverse events or coagulation abnormalities. The vaccine elicited GP-specific antibody and T cell responses that were not cross reactive, but after further pre-clinical evaluation of GP antigens, we found that a transmembrane deleted GP did not provide optimal protection and that the NP antigen was not required for protection.

The subsequent clinical trial evaluated a rAd5 vector vaccine expressing an EBOV GP with a single amino acid point mutation (PM GP). The product was found to be safe and well tolerated, but elicited modest immunogenicity, possibly due in part to suppression by pre-existing immunity to the Ad5 vector [13]. Non-human primate (NHP) studies have further shown that transmembrane deleted and PM GP antigens are partially protective but WT GP constructs are safe and provide the highest level of protection [14]. Therefore, the WT GP antigen is the current focus of VRC research and development for *Ebolavirus* and *Marburgvirus* vaccines. Here we report the results of a Phase I clinical trial evaluating two DNA vaccines, one that encodes for MARV Angola GP and the second for EBOV and SUDV WT GP.

Materials and Methods

Study Design and Procedures

VRC 206 was a single-site, Phase 1, open label study examining the safety, tolerability, and immunogenicity of two investigational DNA vaccines, one (MAR) expressing GP from MARV Angola strain (GP (AN)) and the second (EBO) expressing WT GP from EBOV (GP (Z)) and SUDV (GP (S)) in healthy adults ages 18 to 60 years. The study was conducted at the NIH Clinical Center (CC) by the

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Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland (Clinicaltrials.gov NCT00605514). The study was reviewed and approved by the NIAID Institutional Review Board. U.S. Department of Health and Human Services human experimental guidelines for conducting clinical research were followed. All subjects gave written informed consent prior to participation.

A schema of the study is shown in Figure 1. The study groups were not randomized because approval to proceed with the MAR DNA vaccine was received before approval to proceed with the EBO WT DNA vaccine due to a delay in receiving preclinical data with the EBO WT DNA vaccine. Thus group 1 was fully enrolled first to receive the MAR DNA vaccine. Later group 2 enrolled to receive the EBO WT DNA vaccine. No more than 1 subject per day was administered vaccine for the first 3 injections in each group and safety data through 2 weeks after these injections were reviewed by a protocol safety review team prior to continuing enrollment of that group.

A 4 mg dose of vaccine was administered as 1mL by intramuscular injection in the deltoid muscle using the Biojector® 2000 Needle-Free Injection Management System (Bioject). In both groups, the immunization series was a 3-dose priming regimen with an optional single dose homologous booster. The schedule for the 3-dose priming series was targeted to study days 0, 28 and 56, within permitted windows. Based on results from pre-clinical immunogenicity data available after the VRC 206 study began, an optional homologous booster dose at ≥week 32 was offered to subjects who had completed all 3 injections and remained in clinical follow up.

Laboratory and clinical evaluations were completed at scheduled study visits. Local and systemic reactogenicity was self-reported by subjects using 5-day diary cards following each vaccination. Clinical assessment and laboratory evaluations for creatinine, alanine aminotransferase (ALT), complete blood count, prothrombin time (PT) and partial thromboplastin time (PTT) were completed at scheduled

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study visits. Adverse events (AEs) were reported for the entire duration of the study and coded using the Medical Dictionary for Regulatory Activities (MedDRA), and were severity graded using the Division of AIDS table for Grading the Severity of Adverse Events (December 2004). Subjects were followed for safety and immunogenicity for a period of 32 weeks or for 12 weeks after receipt of the optional fourth study injection.

Vaccines

Both the EBO plasmid DNA vaccine VRC-EBODNA023-00-VP and the MAR plasmid DNA vaccine VRC-MARDNA025-00-VP were developed by the VRC. The EBO plasmid DNA vaccine was manufactured by the VRC/NIAID Vaccine Pilot Plant operated by SAIC-Frederick in Frederick, Maryland and the MAR plasmid DNA vaccine was produced by Althea Technologies in San Diego, California. Both were manufactured under Good Manufacturing Practices (GMP) conditions. The MAR vaccine consists of a single, closed, circular plasmid DNA macromolecule (plasmid VRC 6712) designed to express WT GP of the MARV Angola strain (Genbank accession number DQ447653). The EBO vaccine is composed of two plasmids combined in equal concentrations (mg/mL) in which the amino acid sequences for the GP antigens expressed are identical to WT GP of Ebola virus. These plasmids are VRC 6611, which expresses the GP gene from the Sudan/Gulu (SUDV) strain (Genbank accession number U28134) and VRC 6614, which expresses the GP gene from the Zaire (EBOV) strain (Genbank accession number U23187). The WT GP inserts were codon modified to optimize antigen expression in human cells [15]. The plasmids in both vaccines are incapable of replication in human cells.

The VRC constructed plasmids containing complementary DNAs (cDNAs) that were used to sub-clone the WT GP gene inserts into the CMV/R plasmid DNA expression vector [16]. This CMV/R expression vector has been tested in previous clinical trials of DNA plasmid vaccines for HIV, WNV, SARS and avian influenza conducted by the VRC, NIH [12, 17-20].

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The DNA plasmids were produced in master cell banks (MCB) using plasmid DNA to transform commercially available strains of *Escherichia coli* to produce large volume cultures yielding mass quantities of plasmid DNA. Bacterial cell growth was dependent upon the cellular expression of kanamycin resistance protein encoded in the plasmid DNA. Following the growth of bacterial cells, the bulk plasmid drug substances were filtered and formulated in phosphate buffered saline as a sterile liquid injectable dosage form for intramuscular injection.

Measurement of antibody responses: enzyme-linked immunosorbent assay (ELISA)

Endpoint titers of antibodies directed against MAR GP (AN) and EBO GP antigens (Z and S) were measured throughout the study. Serial dilutions were run in duplicate for pre- and post- vaccine time points. Mean raw optical density (OD) values for each post vaccine time point were corrected for the volunteer and dilution matched samples. Endpoint titers were calculated as the most dilute serum concentration that gave a background corrected OD reading of >0.20.

A titer of ≥30 was determined as a positive result [12, 13]. The primary time points were 4 weeks following the third and the fourth study injections.

Peptides for Evaluation of T cell responses.

Peptides of 15 amino acids in length, overlapping by 11, and corresponding to the vaccine inserts, MAR GP (AN) and EBO GP antigens (Z and S), were synthesized at >80% purity as confirmed by HPLC.

Measurement of T cell responses by ELISpot

Vaccine-induced T cell responses were detected by ELISpot with the following modifications of previously described method [21]. PBMC were stimulated overnight with 2.5 µg/mL peptide at 37°C in triplicate wells at a density of 2×10^5 cells/well. Commercially available ELISpot plates (Mabtech) were

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used with color development with biotinylated IFN-γ detection antibodies, Avidin-HRP solution and AEC substrate solution. The plate was air-dried for a minimum of two hours prior to spot quantitation on a CTL ELISpot image analyzer (Cellular Technology Ltd; Cleveland, OH). Results were expressed as mean spot-forming cells (SFC) per million PBMC. A positive response occurs if the background subtracted number of spots per $1x10^6$ cells exceeds 100 SFC/million PBMC and the non-background corrected mean is at least 4-fold greater than the mean negative stimulation for the sample.

Measurement of T cell responses by intracellular cytokine staining (ICS)

Vaccine-induced T cell responses were detected by ICS with the following modifications of previously described method (16). Antibodies were from BD Biosciences unless otherwise stated: Anti-CD28-Cy5PE, Anti-CD45RA-Cy7PE, Anti-CCR7-Ax680 (ReaMetrix), Anti-IFN-γ-APC, Anti-IL-2-PE, Anti-TNF-α-FITC, Anti-CD4-ECD (Beckman Coulter), Anti-CD3-Cy7APC, Anti-CD8-Pacific Blue, and Aqua-Blue. Cells were stained with Aqua Blue at room temperature for 20 minutes, followed immediately by staining with the surface markers (CD3, CD28, CD45RA, CCR7) for an additional 20 minutes. Cells were washed twice, permeabilized with 100 µL/well CytoFix-CytoPerm reagent (BD) with 20 minute incubation at 2-8°C minutes, and then washed twice with PermWash (BD). Intracellular staining (CD4, CD8, IFN- γ, IL-2, TNF-α) was in a total of 100 µL/well at room temperature for 20 minutes, followed by 3 washes with PermWash. The cells were resuspended in 1% paraformaldehyde and were stored at 4°C for no longer than 36 hours prior to flow cytometry analysis. Multi-parameter flow cytometric analysis was performed on a LSR-II flow cytometer (BDIS). Between 50,000 and 250,000 events are acquired.

Results were analyzed using FlowJo software (Tree Star Software; Ashland, OR). The same gating strategy is used for all clinical testing. A positive response occurs if a Fisher's exact test for the 2x2 table consisting of positive and negative cells by peptide and negative control has a one-sided p-value less than 0.01, and the % positive cells for a peptide minus the % positive cells for the negative

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control (background subtracted percent %) exceeds 0.05 for each combination of cell population and cytokine with the exception of CD8 IFN-γ and CD8 TNF-α where the threshold is greater than 0.08.

Neutralizing Antibody Assay

The measurement of neutralizing antibodies elicited after vaccination was assessed as previously described [13]. In brief, EBOV or MARV GP-specific neutralizing antibody was assessed by using a single-round infection assay with EBOV or MARV GP-pseudotyped lentiviruses, respectively, containing the luciferase reporter gene. The derivative T-Ag-expressing 293T cells were used as infection targets and incubated in a 96 well plate 1 day prior to infection with pseudovirus in the presence of a 1:100 dilution of subject sera. EBOV or MARV GP-pseudotyped lentiviral virions were produced as previously described [15]. Pre- and post-immune sera were tested as indicated in the figure legends. Cells were lysed 72 hours post infection and assayed by Luciferase Assay System (Promega, E1501/E1531) using a Victor X3 Plate Reader from Perkin Elmer for the detection of luciferase activity.

Statistical Methods

Positive response rates are computed along with the exact 95% confidence intervals from binomial distribution. For the magnitude of antibody response, geometric mean and the 95% confidence interval based upon lognormal distribution are reported. Wilcoxon signed-rank test is used for the comparison of antibody response at two time points within a group.

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Results

Study Population

A total of 20 subjects were enrolled between June 4, 2008 and June 17, 2009. Table 1 includes demographic data regarding gender, age, race, ethnicity, body mass index (BMI) and education level at the time of enrollment. All subjects but two completed the 3-dose vaccination series. One subject in group 1 discontinued vaccinations per protocol requirements after one study vaccination due to a medical need for a licensed vaccine and one subject in group 2 was lost to follow up after two vaccinations.

Vaccine Safety

Both vaccines were well tolerated and there were no serious adverse events. Coagulation parameters of study subjects were closely monitored as per protocol due to a theoretical concern over GP-mediated cytopathology [13]. Prothrombin time, partial thromboplastin time and complete blood counts were evaluated at baseline and throughout the study. There were no laboratory or clinical coagulation abnormalities detected. Local and systemic solicited reactogenicity (Table 2) was generally mild in both groups and similar to that seen in previous studies of DNA vaccines with other constructs [12, 17, 20, 22]. One subject reported a moderate local reaction and two subjects reported at least one moderate systemic reaction.

Two adverse events assessed as possibly related to vaccination were mild transient lightheadedness and mild pyuria and one adverse event assessed as definitely related to the study vaccines based on temporal relationship and biologic plausibility was a mild superficial lesion at the injection site that resolved without treatment.

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Vaccine Specific Antibody Responses

EBO and MAR specific antibody responses were detected by ELISA assay. Four weeks after the third vaccination, 80% (8/10) of subjects in group 1 tested positive for GP (AN) antibody. In group 2, 89% (8/9) tested positive for GP (S) and 56% (5/9) tested positive for GP (Z). The frequency of responses decreased over time and 24 weeks post third vaccination 11% (1/9) of subjects were positive for GP (AN) and GP (S) and none were positive for GP (Z).

Seven subjects in group 1 and eight subjects in group 2 received the optional fourth vaccine (homologous boost) between study weeks 32 and 49. Four weeks after the fourth vaccination, 100% (7/7) of subjects were positive for GP (AN), 75% (6/8) for GP (S), and 63% (5/8) for GP (Z). Between 8 and 12 weeks after the boost, 57% (4/7) of subjects in group 1 had sustained positive responses for GP (AN) while 50% (4/8) of subjects in group 2 had sustained positive responses for GP (S) and GP (Z). Cumulative GP-specific response rates in the study were 90% (9/10) for GP (AN), 89% (8/9) for GP (S), and 67% (6/9) for GP (Z).

The magnitude of ELISA titers peaked 4 weeks after the third vaccination and then decreased over the next 24 weeks. Geometric mean titers (GMT) to GP (AN), GP (S), and GP (Z) four weeks post third vaccination were 36.3 (95% CI: 22.3-59.3), 51.1 (95% CI: 29.8-87.8), and 31.8 (95% CI: 16.8-60.2), respectively and then decreased close to baseline levels. Four weeks after the fourth vaccination, GMT to GP (AN), GP (S), and GP (Z) increased to 48.0 (95% CI: 27.9-82.7), 50.1 (95% CI: 21.3-117.7), and 30.4 (95% CI: 16.4-56.1), respectively (Figure 2).

Antibody responses were boosted by the fourth DNA vaccination. Administration of a fourth homologous DNA vaccine at a ≥32 week boost interval demonstrated boosting of waning antibody responses to near peak levels.

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GP (AN)-specific and GP (Z)-specific neutralizing antibody activity was assessed in a reporter virus assay. Results are shown in Figure 3 for each group at baseline (pre-immune) and after the third and fourth DNA vaccinations. No significant MAR neutralizing activity was observed. Low level EBOV neutralizing activity (7% average) was observed after the fourth vaccination, which was statistically improved compared to pre-immune sera or post third vaccination.

Vaccine Specific T Cell Responses

MAR and EBO GP-specific CD4+ T cell responses were seen in all antigen groups by ICS at 4 weeks after the third DNA vaccination. CD4+ T cell responses were detected for GP (AN) in 30% (3/10), GP (S) in 25% (2/8), and GP (Z) in 13% (1/8) of subjects. By week 24, 22% (2/9) still had a CD4+ T cell response for GP (AN) and GP (S) and 11% (1/9) for GP (Z). Four weeks after the fourth DNA vaccination, the frequency was similar at 14% (1/7) for GP (AN), 38% (3/8) for GP (S), and 13% (1/8) for GP (Z) (Figure 4).

CD8+ T cell responses were detected less frequently than CD4+ T cell responses. Four weeks after the third vaccination, CD8+ T cell responses were detected in 13% (1/8) for GP (S) but not detected for GP (AN) or GP (Z). Four weeks after the fourth vaccination, the frequency of measurable CD8+ T cell responses was boosted to 25% (2/8) for GP (S) and 13% (1/8) for GP (Z) although none were seen for GP (AN).

GP-specific T cell responses were also assessed by ELISpot at baseline and scheduled intervals throughout the study. Four weeks after the third DNA vaccination, GP-specific T cell responses were present at a frequency of 40% (4/10) for GP (AN), 63% (5/8) for GP (S), and 25% (2/8) for GP (Z). At 24 weeks, 22% (2/9) were positive for GP (AN), 44% (4/9) were positive for GP (S), and 22% (2/9) were positive for GP (Z). Four weeks after the fourth vaccination, the frequency of T cell responses increased

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to 57% (4/7) for GP (AN), 50% (4/8) for GP (S), and 33% (3/8) for GP (Z) indicating a slight boost in T cell responses with the fourth DNA vaccination.

Discussion

There are currently no available effective vaccines or therapies against filoviruses. An effective vaccine would be a key preventive health measure for limiting the spread of infection, protecting health care workers and military troops, and containing natural outbreaks.

This is the first clinical trial report of an *Ebolavirus* WT GP construct and the first report of a *Marburgvirus* vaccine clinical trial. We have previously shown that earlier generation gene-based constructs were safe and immunogenic including a DNA vaccine with an EBOTM deleted GP and a rAd5 vector encoding EBO GP containing a point mutation. NHP studies have clearly demonstrated that full length WT GP constructs are safe and protective in cynomolgous macaques [14] and WT GP is considered the antigen most likely to induce protective antibody and T cell responses. In this (VRC 206) Phase I study, both the EBO and MAR WT GP vaccines were well tolerated. The WT GP constructs evaluated in the current study were immunogenic and induced both humoral as well as T cell responses to all three GP immunogen inserts.

In addition we saw that administration of a fourth dose of DNA as a homologous boost improved the otherwise waning antibody titers and T cell responses. It is known that DNA provides a priming response for numerous antigens typically with a protein or more potent vector boost and that a prolonged boost interval improves the overall effect of DNA priming [23], but this report is unique in that homologous DNA vaccine induced a demonstrable boost of pre-existing memory B cells.

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In the current trial, antibody responses dominated the immune response and CD4+ T cell responses were more frequent than CD8+ responses. The induction of T cell responses by the vaccine is significant as recent NHP studies suggest that CD8+ T cell responses play an important role in protection induced by an EBO GP construct vaccine and are known to be important for efficient viral clearance [24, 25].

VRC 206 demonstrated that WT GP DNA vaccines for *Ebolavirus* and *Marburgvirus* were safe and immunogenic in humans. The results from this study paved the way for further evaluation of these two candidate vaccines in the first clinical trial of candidate *Ebolavirus* and *Marburgvirus* vaccines in Africa. A Phase Ib clinical trial evaluating these vaccines opened to accrual in Kampala, Uganda following interim safety analysis of the VRC 206 study. The successful evaluation of DNA vaccines targeting *Ebolavirus* and *Marburgvirus* reported here provides the opportunity to further explore wild type filovirus GP antigen delivery in other vaccine platforms with greater immunogenicity and potential for protective immunity. These results will guide further filovirus vaccine research and development and also provide important generalizable data regarding DNA-based priming and boosting in humans.

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Figure Legends

Figure 1. Schematic diagram of study design, vaccination schedule and follow up. Screening, enrollment and follow up is shown. The original protocol design included 3 study vaccinations at day 0, weeks 4 and 8 with 32 weeks of follow-up. An optional fourth vaccination was added by amendment with 12 weeks of additional follow-up.

Figure 2. GP-specific mean antibody responses assessed by ELISA. GP-specific antibody responses were assessed by ELISA for GP (AN), GP (S) and GP (Z). The mean titers with the upper 95% confidence intervals are shown for 3 time points including: 4 weeks and 24 weeks following the third vaccination and 4 weeks after the fourth DNA vaccination. The threshold for positivity in this assay was a reciprocal dilution of 30 and is indicated by a dashed line.

Figure 3. *Ebolavirus* and *Marburgvirus* neutralization as percent infection in the presence of subject sera relative to control infection. Neutralization is represented as percent infection in the presence of subject sera relative to control infection in the absence of sera. Results are shown for 9/10 EBO vaccinees in panel A and for 9/10 MAR vaccinees in panel B at 3 time points, pre-immune, post third DNA and post fourth DNA vaccinations.

Figure 4. Frequency of CD4+ and CD8+ T cell responses by ICS and ELISpot analysis.

Percent responders is shown for each vaccine antigen at four time points for CD4+ and CD8+ ICS and ELISpot.

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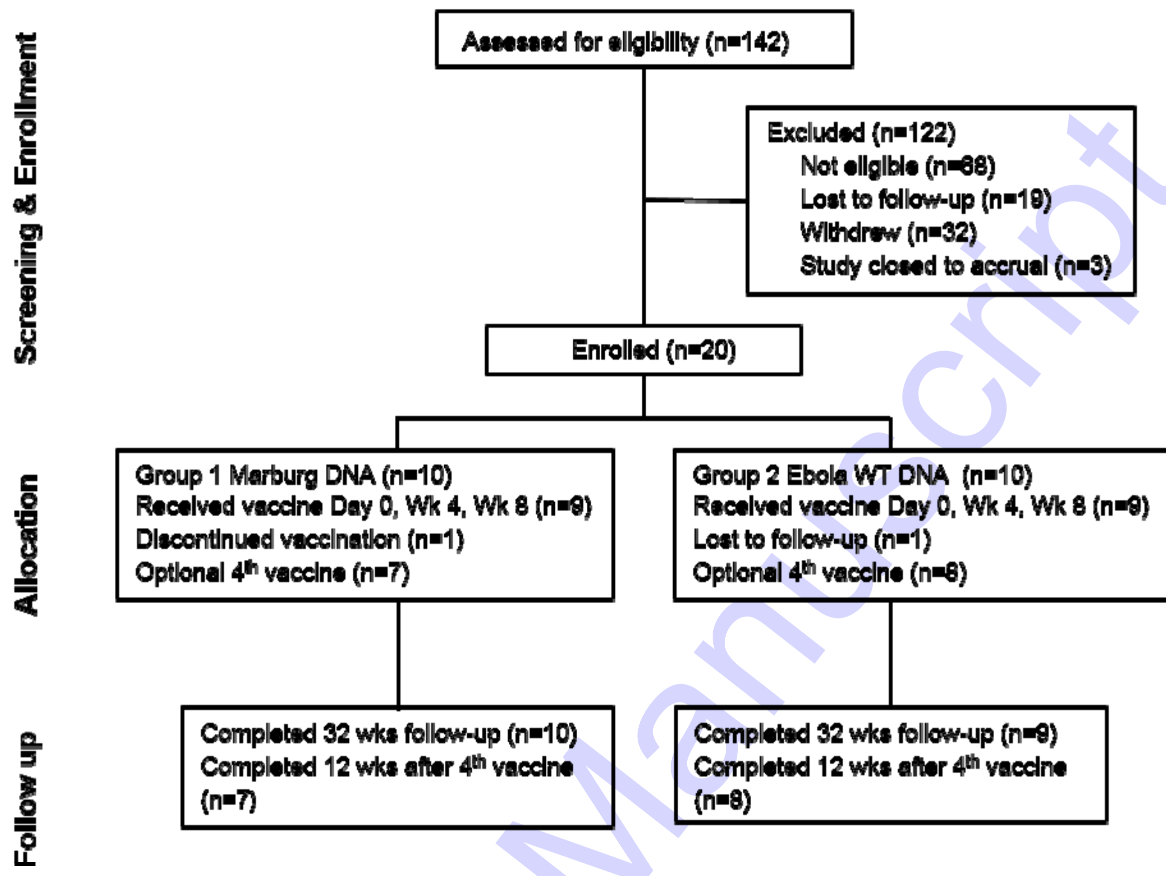

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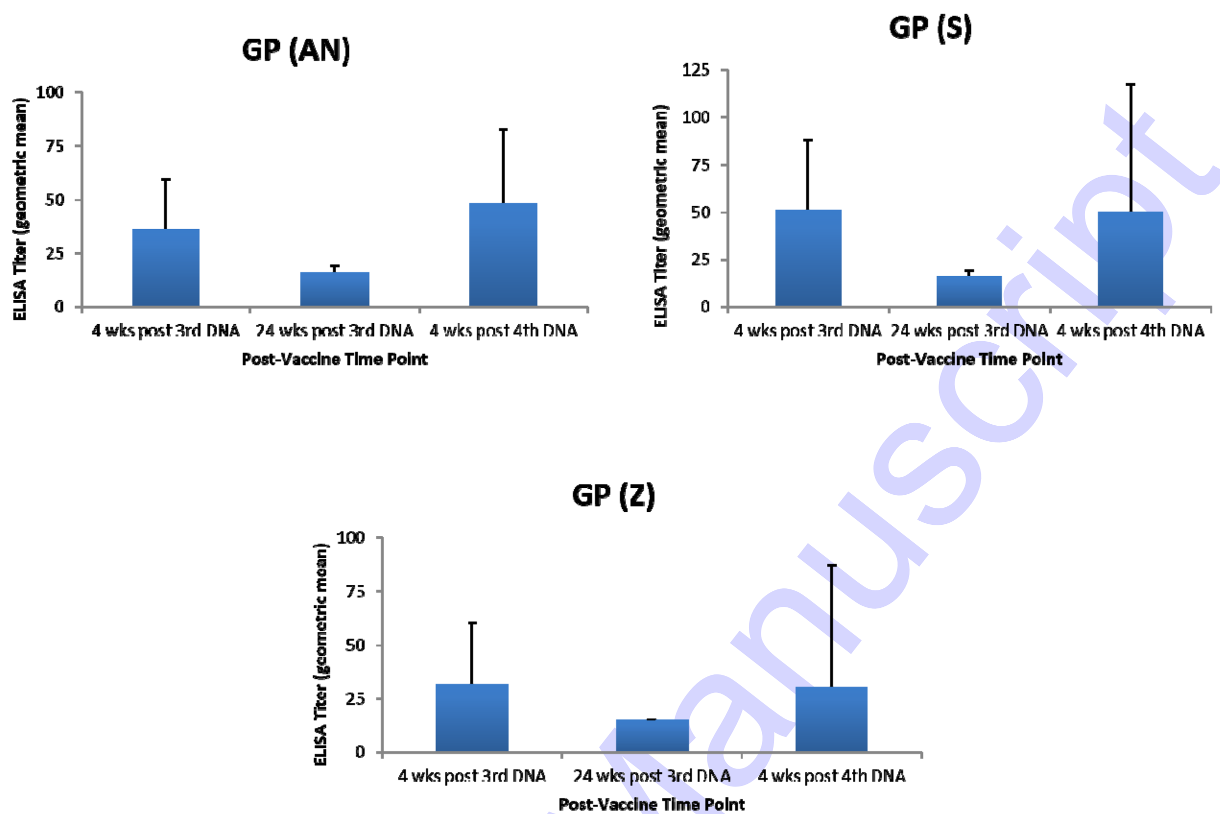

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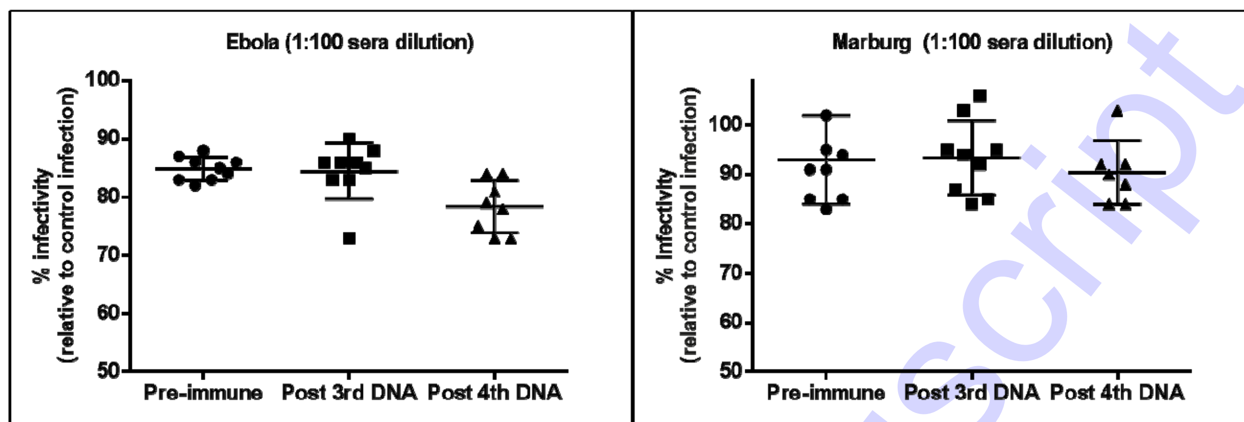

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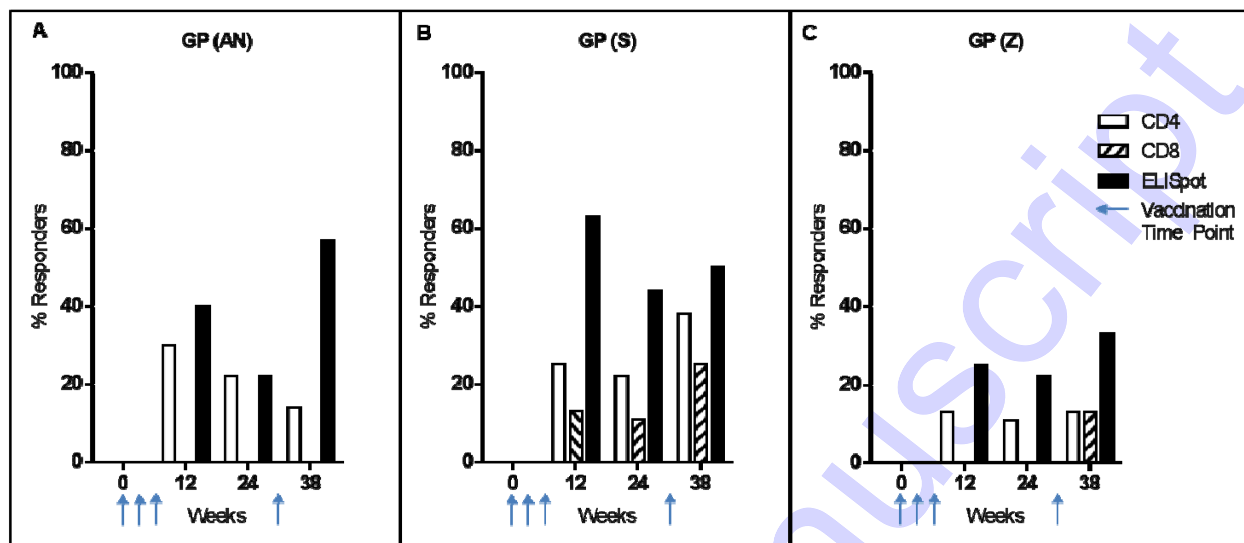

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Table 1. Baseline demographics

		Marburg (N=10)	*Ebola WT (N=10)*	*All Subjects (N=20)*
Characteristic		Number (%) subjects		
GENDER	Male	5 (50)	5 (50)	10 (50)
	Female	5 (50)	5 (50)	10 (50)
AGE - *years*	Mean [S.D.]	34.0 [9.7]	36.6 [11]	35.3 [10]
	Range	[24, 52]	[24, 59]	[24, 59]
RACE	White	9 (90)	7 (70)	16 (80)
	Black or African American	1 (10)	1 (10)	2 (10)
	Asian	0	1 (10)	1 (5)
	All other races combined	0	1 (10)	1 (5)
ETHNICITY	Non-Hispanic/Latino	10 (100)	10 (100)	20 (100)
	Hispanic/Latino	0	0	0
BODY MASS INDEX	Mean [S.D.]	24.6 [2.4]	25.8 [4.7]	25.2 [3.7]
	Range	[21.4, 28.5]	[20.0, 33.4]	[20.0, 33.4]
EDUCATION	Less than high school	0	0	0
	High school/GED	0	1 (10)	1 (5)
	College/University	2 (20)	3 (30)	5 (25)
	Advanced degree	8 (80)	6 (60)	14 (70)

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Table 2. The local and systemic reactogenicity tables show the maximum subject local or systemic reactogenicity by vaccine group and overall over the 5-day period of solicited reactogenicity for any of the vaccinations received by the subject for each parameter and for any of the parameters on the table.

Table 2a. Maximum local reactogenicity by group

Local Symptoms Intensity	*Marburg (N=10)*	*Ebola WT (N=10)*	*All Subjects (N=20)*
	Number of subjects (%)		
PAIN/TENDERNESS			
None	1 (10)	3 (30)	4 (20)
Mild	8 (80)	7 (70)	15 (75)
Moderate	1 (10)	0	1 (5)
Severe	0	0	0
SWELLING			
None	5 (50)	7 (70)	12 (60)
Mild	5 (50)	3 (30)	8 (40)
Moderate	0	0	0
Severe	0	0	0
REDNESS			
None	6 (60)	6 (60)	12 (60)
Mild	4 (40)	4 (40)	8 (40)
Moderate	0	0	0
Severe	0	0	0
ANY LOCAL SYMPTOM			
None	1 (10)	3 (30)	4 (20)
Mild	8 (80)	7 (70)	15 (75)
Moderate	1 (10)	0	1 (5)
Severe	0	0	0

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Table 2b. Maximum systemic reactogenicity by group

Systemic Symptoms Intensity	*Marburg (N=10)*	*Ebola WT (N=10)*	*All Subjects (N=20)*
	Number of subjects (%)		
MALAISE			
None	4 (40)	8 (80)	12 (60)
Mild	4 (40)	2 (20)	6 (30)
Moderate	2 (20)	0	2 (10)
Severe	0	0	0
MYALGIA			
None	7 (70)	10 (100)	17 (85)
Mild	2 (20)	0	2 (10)
Moderate	1 (10)	0	1 (5)
Severe	0	0	0
HEADACHE			
None	6 (60)	9 (90)	15 (75)
Mild	4 (40)	1 (10)	5 (25)
Moderate	0	0	0
Severe	0	0	0
CHILLS			
None	9 (90)	9 (90)	18 (90)
Mild	1 (10)	1 (10)	2 (10)
Moderate	0	0	0
Severe	0	0	0
NAUSEA			
None	8 (80)	10 (100)	18 (90)
Mild	2 (20)	0	2 (10)
Moderate	0	0	0
Severe	0	0	0
TEMPERATURE			
None	10 (100)	10 (100)	20 (100)
Mild	0	0	0
Moderate	0	0	0
Severe	0	0	0
ANY SYSTEMIC SYMPTOM			
None	3 (30)	7 (70)	10 (50)
Mild	5 (50)	3 (30)	8 (40)
Moderate	2 (20)	0	2 (10)

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Systemic Symptoms Intensity	*Marburg (N=10)*	*Ebola WT (N=10)*	*All Subjects (N=20)*
Severe	0	0	0

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