Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Regules JA, Beigel JH, Paolino KM, et al. A recombinant vesicular stomatitis virus Ebola vaccine. N Engl J Med 2017;376:330-41. DOI: 10.1056/NEJMoa1414216

Supplemental Materials for

A Recombinant Vesicular Stomatitis Virus Ebola Vaccine

#Jason A. Regules, M.D.[1], John H. Beigel, M.D.[2], Kristopher M. Paolino, M.D. [1], Jocelyn Voell, RN[3], MS, Amy R. Castellano, L.P.N. [1], Zonghui Hu, Ph.D.[3], Paula Muñoz, B.S. [3], James E. Moon, M.D. [1], Richard C. Ruck, M.D. [1], Jason W. Bennett, M.D. [1], Patrick S. Twomey, M.D. [1], Ramiro L. Gutiérrez, M.D. [7], Shon A. Remich, M.D. [1], Holly R. Hack, M.S. [1], Meagan L. Wisniewski, Ph.D. [5], Matthew D. Josleyn, M.S. [5], Steven A. Kwilas, Ph.D. [5], Nicole Van Deusen, B.S. [5], Olivier Tshiani Mbaya, M.D. [4], Yan Zhou, Ph.D. [4], Daphne A. Stanley, M.S. [4], Jing Wang, M.S.[2], Kirsten S. Smith, Ph.D. [5], Meng Shi, M.A. [1], Julie E. Ledgerwood, D.O. [4], Barney S. Graham, M.D. [4], Nancy J. Sullivan, Ph.D. [4], Linda L. Jagodzinski, Ph.D. [1], Sheila A. Peel, M.S.P.H., Ph.D. [1], Judie B. Alimonti, Ph.D. [8], Jay W. Hooper, Ph.D. [5], Peter M. Silvera, Ph.D. [5], Brian K. Martin, Ph.D. [6], Thomas P. Monath, M.D. [6], W. Jay Ramsey, M.D. ,Ph.D. [6], Charles J. Link, M.D. [6], H. Clifford Lane [3], M.D., Nelson L. Michael, M.D., Ph.D.[1], **Richard T. Davey, Jr., M.D. [3], **Stephen J. Thomas, M.D. [1], and the rVSVΔG-ZEBOV-GP Study Group.

*Drs. Regules and Beigel contributed equally to this article
**Drs. Davey and Thomas contributed equally to this article

Affiliations:
[1] Walter Reed Army Institute of Research, Silver Spring, MD
[2] Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD
[3] National Institute of Allergy and Infectious Diseases, Bethesda, MD
[4] Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD
[5] United States Army Medical Research Institute of Infectious Diseases, Frederick, MD
[6] BioProtection Systems/NewLink Genetics Corp., Ames, IA
[7] Naval Medical Research Center
[8] Public Health Agency of Canada

#Corresponding Author, jason.a.regules.mil@mail.mil, 301-619-6731, 1425 Porter Street, Frederick, MD 21702

Table of Contents

The rVSVΔG-ZEBOV-GP Study Group:

Teresa A. Nowak,[2] Robin L. Bliss, PhD[2], Deborah Cebrik, M.S., M.P.H.[2], David M. Hone, Ph.D.[3], Donald G. Heppner, M.D.[3], L. Revell Phillips, Ph.D.[3], Nicole R. Kilgore, M.S.[4], Victor A. Suarez, M.S.[4], Janice M. Rusnak, M.D.[4], Christopher S. Badorrek, Ph.D.[4], Kevin L. Wingerd, Ph.D.[4], Susan B. Cicatelli, R.N.[5], Jakub K. Simon, M.D., MS[6], Ph.D., Beth-Ann G. Coller, Ph.D.[6], Mark B. Feinberg, M.D., Ph.D.[6]

Affiliations:
[1] National Institute of Allergy and Infectious Diseases, Bethesda, MD
[2] Veristat, Holliston, MA
[3] Defense Threat Reduction Agency
[4] Medical Countermeasures Systems-Joint Vaccine Acquisition Program
[5] Walter Reed Army Institute of Research, Silver Spring, MD
[6] Merck

Methods: Statistical analysis

Statistical analyses were performed utilizing R, version 3.3.1. For each serological parameter, summaries were by assessment day, and included calculation of the geometric mean titer (GMT), 95% confidence interval, median, minimum, and maximum values. Two-sample t-test was performed for comparisons in GMT between dose levels and study sites. Paired t-test was used for comparisons between time points within a dose level. All calculations and comparisons were performed on the log10 scale. A positive response for the Kikwit-strain ELISA was defined as a titer ≥ 50, with titers <50 assigned values of 25 for calculation. A positive response for the PsVNA assay was defined as a titer of ≥ 20, with titers <20 assigned values of 10 for calculation. Seroconversion for these assays was defined as a four-fold increase in titer vs. baseline value. Baseline values were subtracted from the post-vaccination values for determination of the Mayinga strain ELISA titers, as previously described.[1,2]

Methods: rVSV-ZEBOV-GP surveillance via RT-PCR

A reverse transcriptase – polymerase chain reaction (RT-PCR) assay to measure potential rVSV virus in the plasma, saliva, and urine, via amplification of the Ebola Zaire glycoprotein (GP) gene VSV insert, was performed at the WRAIR. Reagents for amplification of the Ebola Zaire glycoprotein (GP) gene VSV insert, human housekeeping gene, RNase P, and positive Ebola GP and RNase P controls were obtained from the Naval Medical Research Center (NMRC), United States Army Research Institute of Infectious Diseases (USAMRIID) Critical Reagent Program (CRP), Fort Detrick, MD. Total nucleic acid (NA) was purified from EDTA plasma (WRAIR and NIH), EDTA plasma stabilized in PrimeStore (NIH: 4 fold dilution), saliva swab stabilized in PrimeStore, and Urine (WRAIR and NIH: 1.5 ml, centrifuged at 25,000 xg for 60 min), using the QIAamp Viral RNA extraction kit on the QIAcube (Qiagen, Inc. Valencia, CA). All specimens were lysed prior to placement into the QIAcube and NAs were eluted in 50 µl of diluent. Real-time RT-PCR amplification was performed immediately post NA purification following the Ebola Zaire (EZ1) rRT-PCR TaqMan Assay Instruction Booklet version 2 (8-14-2014), except for plate layout and sealing of reaction wells. Day 0 time point specimens were used as negative extraction controls in all assay runs. No Template Controls, NTC1 and NTC2, for both EZ1 and RNase P were employed to monitor contamination: NTC1s were added to reaction plates prior to RNA addition and sealed while NTC2s were added post RNA addition. Optical 8-well strip caps were used to seal reactions. Valid test run criteria required no nucleic acid detection in NTC1 and NTC2 wells and cycle threshold (Ct) values for both Ebola GP and RNase P RNA positive controls. Ct values for participant specimens subjected to EZ1 rRT assay were indicative of VSV viremia (plasma) or VSV in saliva or urine specimens. Specimens with no Ct value by EZ1 rRT-PCR assay, but with a Ct value by human RNase P rRT-PCR assay were scored as virus not detected.

Methods: Measurement of antibody responses to Ebola Glycoprotein via ELISA

For the Zaire-Kikwit GP ELISA, serum samples were added to 96-well microtiter plates pre-coated with recombinant glycoprotein (GP) (Zaire-Kikwit). A reference standard and positive and negative controls were included. EBOV GP-specific antibodies were detected by the addition of an anti-human secondary immunoglobulin G (IgG) antibody conjugated to horse-radish peroxidase, followed by a colorimetric substrate, then optical density (OD) was read at an absorbance wavelength of 450nm. Endpoint titers

were calculated and expressed as the reciprocal of the highest serum dilution that yielded an OD reading greater than the cutoff OD of 0.2.

Methods: Measurement of antibody responses to Ebola Glycoprotein via PsVNA

For the PsVNA, EBOV pseudovirions (PsV) were prepared using methods similar to those described previously.[3,4] The system employs a recombinant Vesicular Stomatitis Virus (rVSV) with the G protein gene deleted and a luciferase gene inserted as a reporter (VSVΔG*rLuc).[3] EBOV PsV were produced using a plasmid expressing the EBOV Zaire 95 Kikwit GP, pWRG7077 $EBOV_{co}$.[5] The PsVNA was performed as previously described.[6] Briefly, an initial 1:10 dilution (in triplicate) of heat-inactivated sera was made followed by five-fold serial dilutions that were mixed with an equal volume of complete EMEM containing EBOV PsV at approximately 4,000 focus-forming units (FFU) per well of a 96-well plate, and 10% (v/v) human complement (Sigma). This mixture, containing 5% human complement, 4,000 FFU of PsV, and sera at a final 1:20-1,562,500 dilution range, was incubated overnight at 4°C. Following this incubation, 50 µl of the PsV + antibody mixture were inoculated onto Vero cell monolayers in a clear bottom black-walled 96-well plate (Corning). Plates were incubated at 37°C for 18-24 hr. Overlying media was removed and the cells were lysed according to the luciferase kit protocol (Promega #E2820). A Tecan M200 Pro microplate reader was used to acquire flash luciferase data. The raw data (relative light units) were exported to GraphPad Prism version 6.04, where the data were baseline-corrected to the untreated PsV signal. The data were fit to a four-parameter logistic equation using GraphPad Prism and then PsVNA 50% ($PsVNA_{50}$) neutralization titers were interpolated from the curves for each sample. The data are reported as the geometric mean titer for replicates.

References

1. Ledgerwood JE, DeZure AD, Stanley DA, et al. Chimpanzee Adenovirus Vector Ebola Vaccine — Preliminary Report. New England Journal of Medicine 2014;141126135947008.

2. Ewer K, Rampling T, Venkatraman N, et al. A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. New England Journal of Medicine 2016;374(17):1635–46.

3. Ray N, Whidby J, Stewart S, Hooper JW, Bertolotti-Ciarlet A. Study of Andes virus entry and neutralization using a pseudovirion system. J Virol Methods 2010;163(2):416–23.

4. Ogino M, Ebihara H, Lee B-H, et al. Use of vesicular stomatitis virus pseudotypes bearing hantaan or seoul virus envelope proteins in a rapid and safe neutralization test. Clin Diagn Lab Immunol 2003;10(1):154–60.

5. Grant-Klein RJ, Van Deusen NM, Badger CV, Hannaman D, Dupuy LC, Schmaljohn CS. A multiagent filovirus DNA vaccine delivered by intramuscular electroporation completely protects mice from ebola and Marburg virus challenge. Hum Vaccin Immunother 2012;8(11):1703–6.

6. Kwilas S, Kishimori JM, Josleyn M, et al. A hantavirus pulmonary syndrome (HPS) DNA vaccine delivered using a spring-powered jet injector elicits a potent neutralizing antibody response in rabbits and nonhuman primates. Curr Gene Ther 2014;14(3):200–10.

Figure S1 – Screening, Enrollment, Vaccinations, and Follow-Up.

The WRAIR and NIAID study sites performed the studies independently. Cohorts 1, 2, and 3 were enrolled sequentially at each site. Four subjects in Cohort 2 were lost to follow-up during the course of the study.

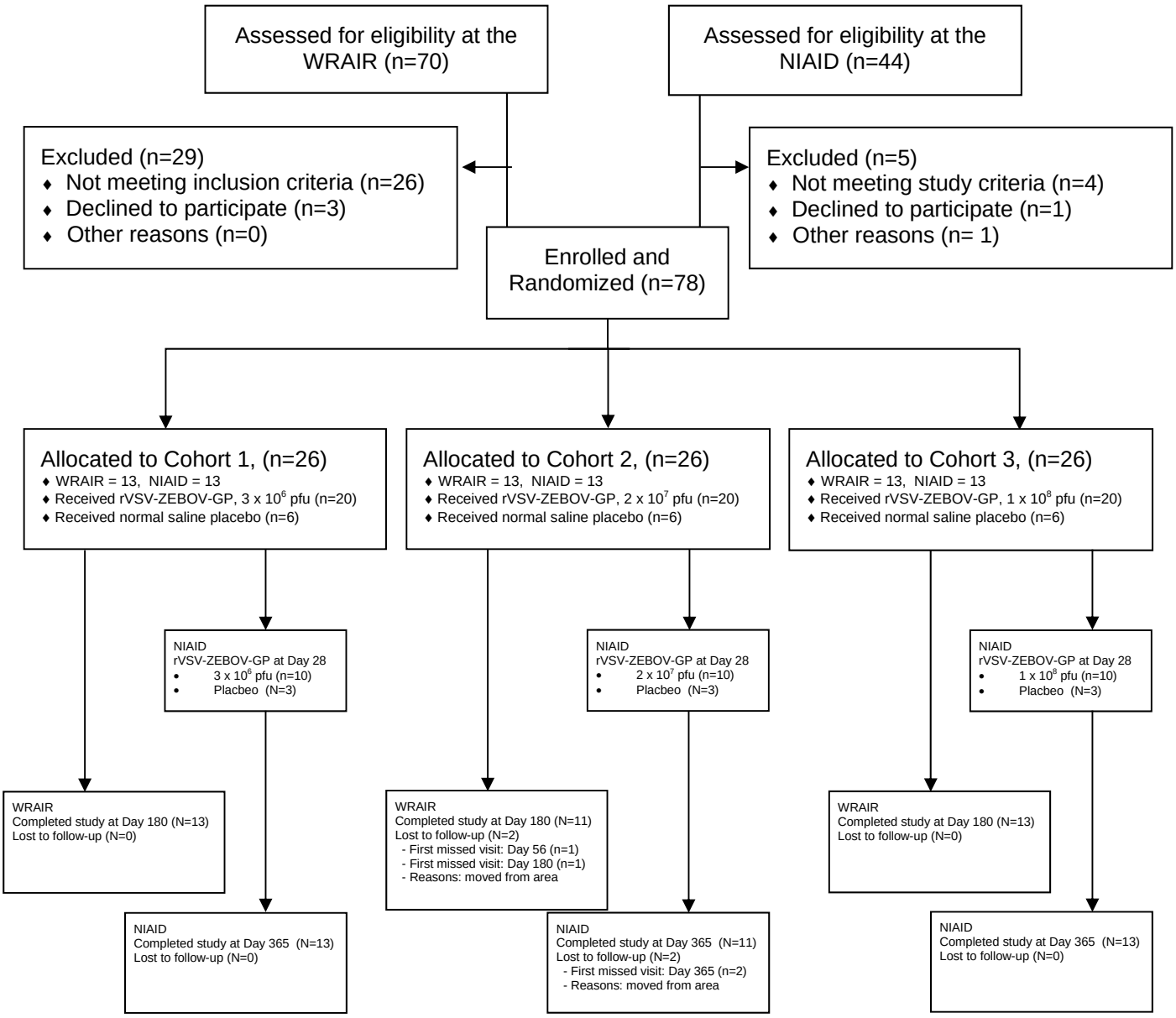

Figure S2: Time to event for all solicited adverse events

Plots depict day after vaccination (x-axis) and proportion of volunteers remaining without first occurrence of given symptom (y-axis).

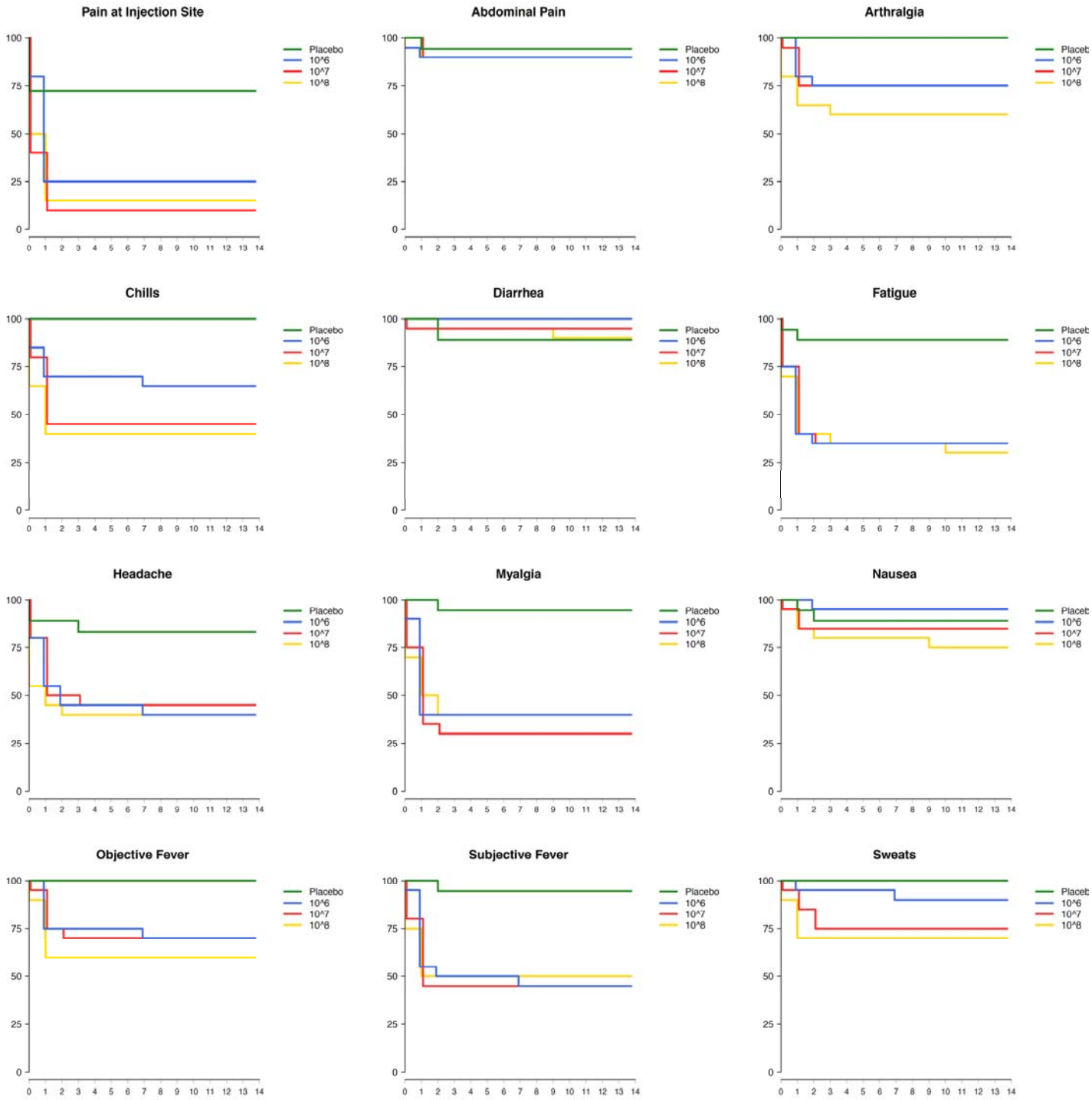

Figure S3: Select Laboratory parameters

Mean and 95% confidence interval by dose level for WBCs (panel A), neutrophils, (panel B), lymphocytes (panel C), and platelets (panel D) in SI units at Study Days 0 (Baseline), 1, 3, 7, and 28. Days 1 and 3 only collected at the WRAIR study site.

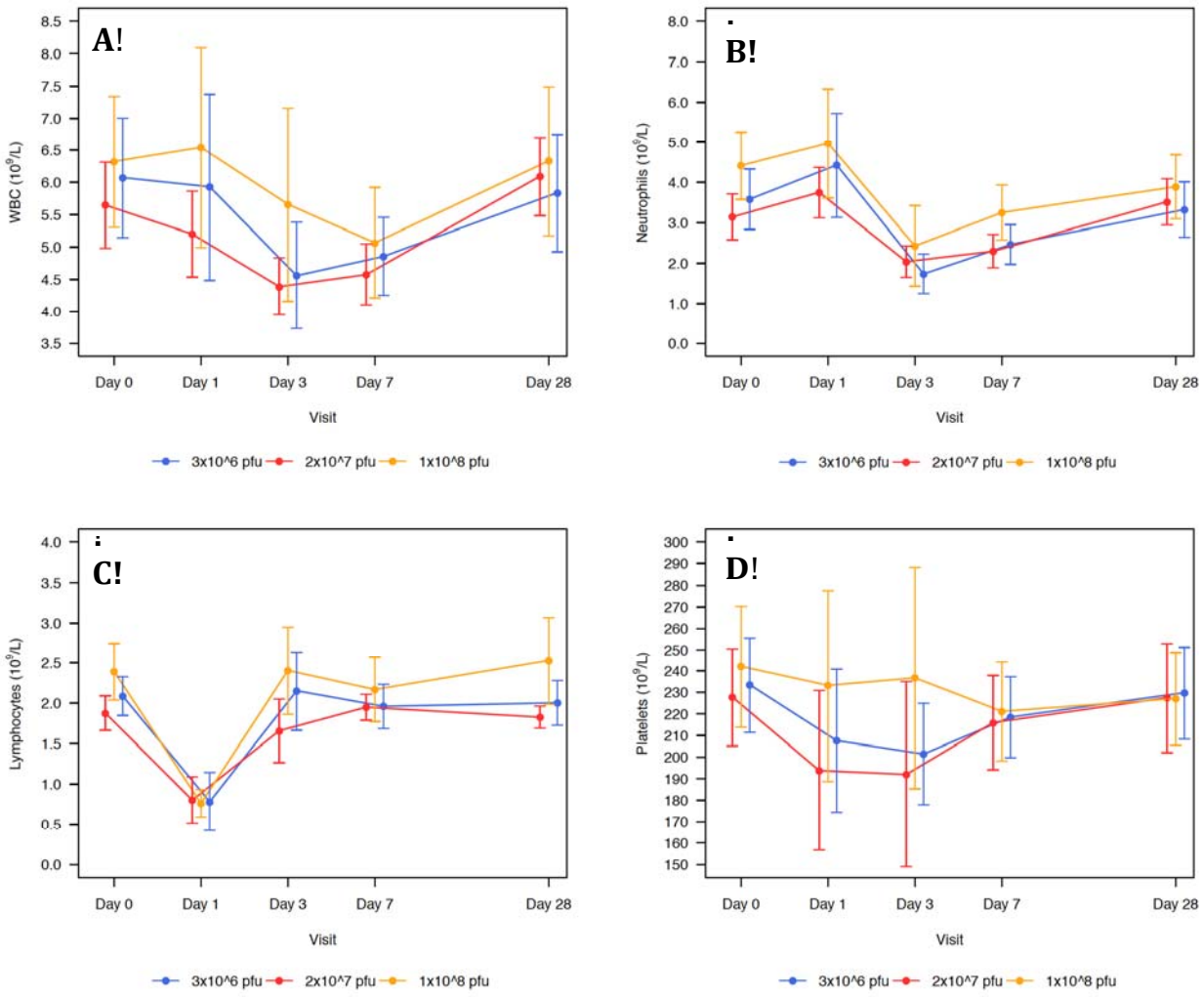

Table S1: ELISA against the Zaire-Kikwit strain GP

Antibody titers as assessed at Day 0, and 7, 14, and 28 days after vaccination, as measured by ELISA against the Zaire-Kikwit strain GP. For each parameter, summaries were performed by assessment day, and included calculation of the geometric mean titer (GMT), 95% confidence interval of the GMT, median, and minimum and maximum values. Not all samples have been tested at Day 7 due to laboratory capacity (WRAIR data presented on day 7) . Titers of <50 are given a value of 25, titers > 25600 are given a value of 25600 for calculation. *Analysis of variance (ANOVA) models were performed for each parameter to compare observed GMT values between dose levels at Day 14 and Day 28. **Additional comparisons were performed between Day 14 and Day 28 using paired t-tests by dose level.

Vaccine Dose	2nd Dose?*		Day 0	Day 7	Day 14	Day 28	Day 56	Day 84	Day 180
Placebo*	N/A	N	18	9	18	18	18	18	17
		GMT	28.06	34.02	29.16	29.16	30.31	27	30.65
		lower	21.99	19.82	22.66	22.66	24.86	22.95	19.9
		upper	35.81	58.39	37.53	37.53	36.95	31.77	47.22
		median	25	25	25	25	25	25	25
		min	25	25	25	25	25	25	25
		max	200	200	200	200	100	100	800
3x10^6 pfu	Y	N	10	NA	10	10	10	10	9
		GMT	25	NA	200	1299.6	4222.43	2985.71	3200
		lower	25	NA	56.8	577.33	2477.85	1823.49	1878.27
		upper	25	NA	704.21	2925.49	7195.31	4888.68	5451.83
		median	25	NA	200	1600	6400	3200	3200
		min	25	NA	25	200	800	800	800
		max	25	NA	12800	12800	6400	6400	6400
	N	N	10	10	10	10	10	10	10
		GMT	25	25	400	1299.6	2599.21	2262.74	2785.76
		lower	25	25	237.17	731.34	1537.15	1484.66	1248.05
		upper	25	25	674.61	2309.42	4395.07	3448.59	6218.08
		median	25	25	400	1200	3200	2400	2400
		min	25	25	200	400	800	800	400
		max	25	25	1600	6400	12800	6400	25600
2x10^7 pfu	Y	N	10	NA	10	10	10	10	10
		GMT	28.72	NA	1131.37	5198.42	7351.67	4222.43	3675.83
		lower	23.3	NA	662.23	2477.73	4971.88	3268.58	2485.94
		upper	35.4	NA	1932.87	10906.57	10870.55	5454.63	5435.28
		median	25	NA	800	3200	6400	3200	3200
		min	25	NA	400	1600	3200	3200	1600
		max	50	NA	3200	25600	12800	6400	12800
	N	N	10	10	10	10	10	10	9

		GMT	25	25	649.8	3200	3732.89	2743.18	2539.84
		lower	25	25	234.27	1724.13	2085.24	1634.49	1195.55
		upper	25	25	1802.41	5939.24	6682.45	4603.91	5395.66
		median	25	25	800	2400	6400	3200	1600
		min	25	25	50	1600	800	800	800
		max	25	25	12800	12800	6400	6400	12800
1x10^8 pfu	Y	N	10	NA	10	10	10	10	10
		GMT	28.72	NA	649.8	3675.83	11143.05	7351.67	5571.52
		lower	20.99	NA	298.77	2330.6	8143.45	4971.88	3338.63
		upper	39.3	NA	1413.28	5797.53	15247.53	10870.55	9297.8
		median	25	NA	800	3200	12800	6400	4800
		min	25	NA	50	1600	6400	3200	3200
		max	100	NA	1600	12800	25600	25600	25600
	N	N	10	10	10	10	10	10	10
		GMT	28.72	30.78	1212.57	4525.48	4525.48	3939.66	2785.76
		lower	23.3	24.22	342.89	2141.22	1932.63	1500.69	1169.06
		upper	35.4	39.11	4288.05	9564.64	10596.97	10342.54	6638.23
		median	25	25	2000	4800	4800	6400	3200
		min	25	25	50	800	400	200	200
		max	50	50	6400	12800	25600	25600	12800

Table S2: Dose comparisons for ELISA against the Zaire-Kikwit strain GP

Analysis comparing the ELISA response at different vaccine doses, by study day and by those subjects that received a second dose or not. All p values based on two-sample t-test.

Day	2nd Dose?	10^6 vs 10^7	10^6 vs 10^8	10^7 vs 10^8
		p-value	p-value	p-value
14	*N/A*	0.008	0.015	0.934
28	*N/A*	0.001	<0.001	1
56	Yes	0.075	0.003	0.078
	No	0.306	0.229	0.677
84	Yes	0.181	0.005	0.017
	No	0.519	0.256	0.466
180	Yes	0.638	0.105	0.163
	No	0.85	1	0.857

Table S3: Day comparisons for ELISA against the Zaire-Kikwit strain GP

Analysis comparing the ELISA response at different study days, by vaccine doses and by those subjects that received a second dose or not. All p values based on two-sample t-test.

Dose	2nd Dose?	Day 28 vs. 56	Day 28 vs. 84
		p-value	p-value
3x10^6 pfu	Yes	<0.001	0.018
	No	<0.001	0.003
2x10^7 pfu	Yes	0.272	0.520
	No	0.799	0.499
1x10^8 pfu	Yes	<0.001	0.023
	No	1	0.726

Table S4: Regimen comparisons ELISA against the Zaire-Kikwit strain GP

Analysis comparing the ELISA response of those subjects that received 2 vaccinations vs 1 vaccination, by vaccine dose and study day. All p values based on two-sample t-test.

Dose	Day	2 vaccinations vs 1 vaccination
		p-value
3x10^6 pfu	28	1
	56	0.16
	180	0.748
2x10^7 pfu	28	0.271
	56	0.043
	180	0.337
1x10^8 pfu	28	0.599
	56	0.045
	180	0.141

Table S5: Pseudovirion Neutralization Assay (PsVNA) against the Zaire-Kikwit strain GP

Antibody titers as assessed at Day 0, and 7, 14, and 28 days after vaccination, as measured by the PsVNA. For each parameter, summaries were performed by assessment day, and included calculation of the geometric mean titer (GMT), 95% confidence interval of the GMT, median, and minimum and maximum values. Not all samples have been tested at Day 7 and 14 due to laboratory capacity (WRAIR data presented where n<20). For the day 14 Titers of <20 are given a value of 10 for calculation. *Analysis of variance (ANOVA) models were performed for each parameter to compare observed GMT values between dose levels at Day 14 and Day 28. **Additional comparisons were performed between Day 14 and Day 28 using paired t-tests by dose level.

Vaccine Dose	2nd Dose*		Day 0	Day 7	Day 14	Day 28	Day 56	Day 84	Day 180
Placebo	N/A	N	18	9	18	18	18	9	18
		GMT	10	10	10	10	10	10	10
		lower	10	10	10	10	10	10	10
		upper	10	10	10	10	10	10	10

		median	10	10	10	10	10	10	10
		min	10	10	10	10	10	10	10
		max	10	10	10	10	10	10	10
3x10^6 pfu	Y	N	10	NA	10	10	10	10	9
		GMT	10	NA	32.46	222.23	344.13	32.65	36.09
		lower	10	NA	15.8	139.65	203.11	15.39	16.17
		upper	10	NA	66.7	353.66	583.05	69.26	80.55
		median	10	NA	36.5	239.5	346.5	32.5	60
		min	10	NA	10	94	125	10	10
		max	10	NA	143	519	999	223	118
	N	N	10	10	10	10	10	NA	10
		GMT	10	10	45.97	222.81	137.72	NA	26.19
		lower	10	10	21.86	96.96	74.14	NA	9.61
		upper	10	10	96.65	512.03	255.82	NA	71.38
		median	10	10	45	207	141.5	NA	10
		min	10	10	10	30	44	NA	10
		max	10	10	282	2461	462	NA	538
2x10^7 pfu	Y	N	10	NA	10	10	10	10	10
		GMT	10	NA	10	415.85	653	47.31	34.85
		lower	10	NA	10	156.16	468.14	27.64	17.36
		upper	10	NA	10	1107.38	910.85	80.96	69.99
		median	10	NA	10	293	615	49	42
		min	10	NA	10	56	242	10	10
		max	10	NA	10	2396	1474	134	148
	N	N	10	10	10	10	10	NA	10
		GMT	10	10	216.51	468.03	170.34	NA	22.95
		lower	10	10	92.35	174.75	105.59	NA	10.13
		upper	10	10	507.59	1253.55	274.81	NA	52.02
		median	10	10	242.5	377	135	NA	10
		min	10	10	10	83	70	NA	10
		max	10	10	635	4910	434	NA	130
1x10^8 pfu	Y	N	10	NA	10	10	10	10	10
		GMT	10	NA	91.53	475.54	669.02	89.71	46.8
		lower	10	NA	34.75	239.56	418.04	46.59	15.57
		upper	10	NA	241.08	943.98	1070.7	172.73	140.71
		median	10	NA	66.5	461.5	661.5	99.5	52.5
		min	10	NA	10	88	252	21	10
		max	10	NA	777	2075	1644	341	832
	N	N	10	10	10	10	10	NA	10
		GMT	10	10	175.9	446.96	218.63	NA	46.23
		lower	10	10	64.79	262.88	98.29	NA	20.79

		upper	10	10	477.6	759.95	486.31	NA	102.8
		median	10	10	127.5	562.5	188	NA	68.5
		min	10	10	10	138	22	NA	10
		max	10	10	1334	908	1636	NA	224

Table S6: Dose Comparison for PsVNA against the Zaire-Kikwit strain GP

Analysis comparing the PsVNA response at different vaccine doses, by study day and by those subjects that received a second dose or not. All p values based on two-sample t-test

Day	2nd Dose?*	10^6 vs 10^7	10^6 vs 10^8	10^7 vs 10^8
		p-value	p-value	p-value
14	N/A	0.687	0.004	0.054
28	N/A	0.068	0.012	0.901
56	Y	0.034	0.048	0.925
	N	0.544	0.316	0.552
84	Y	0.377	0.034	0.105
	N**	N/A	N/A	N/A
180	Y	0.941	0.67	0.616
	N	0.819	0.33	0.18

Table S7: Day Comparison for PsVNA against the Zaire-Kikwit strain GP

Analysis comparing the PsVNA response at different study days, by vaccine doses and by those subjects that received a second dose or not. All p values based on two-sample t-test. N/T represents samples not tested.

Dose	2nd Dose?	Day 28 vs. 56	Day 28 vs. 84
		p-value	p-value
3x10^6 pfu	Yes	0.082	<0.001
	No	0.059	N/A
2x10^7 pfu	Yes	0.326	0.003
	No	0.008	N/A
1x10^8 pfu	Yes	0.187	<0.001
	No	0.017	N/A

Table S8: Regimen Comparison for PsVNA against the Zaire-Kikwit strain GP

Analysis comparing the PsVNA response of those subjects that received 2 vaccinations vs 1 vaccination, by vaccine dose and study day. All p values based on two-sample t-test.

Dose	Day	2 vaccinations vs 1 vaccination
3x10^6 pfu	28	0.995
	56	0.02
	180	0.577
2x10^7 pfu	28	0.85
	56	<0.001
	180	0.387
1x10^8 pfu	28	0.873
	56	0.016

	180	0.984

Table S9: ELISA against the Zaire-Mayinga strain GP

Antibody titers as assessed at Day 0, and 28 days after vaccination, as measured by ELISA against the Zaire- Mayinga strain GP. For each parameter, summaries were performed by assessment day, and included calculation of the baseline-subtracted geometric mean titer (GMT), 95% confidence interval of the GMT, median, and minimum and maximum values. *Analysis of variance (ANOVA) models were performed for each parameter to compare observed GMT values between dose levels at Day 28.

Dose Level	Statistic	Day 0	Day 28	Change from Day 0
Placebo	N	11	11	11
	GMT	86.35	93.46	4.03
	95% CI	51.18-145.69	62.38-140.02	1.57-10.37
	Median	114.00	92.00	4.00
	Min-Max	21.00-259.00	31.00-245.00	1.00-21.00
3x10^6 pfu	N	20	20	20
	GMT	108.03	707.35	282.52
	95% CI	54.83-212.82	414.62-1206.78	95.99-831.51
	Median	95.95	555.63	380.50
	Min-Max	6.00-3097.00	148.00-12910.00	1.00-12351.00
2x10^7 pfu	N	20	20	20
	GMT	117.96	1697.63	1428.89
	95% CI	78.78-176.62	1007.42-2860.73	808.18-2526.32
	Median	98.95	1469.91	1025.36
	Min-Max	25.00-950.00	414.00-17769.00	271.00-17581.00

Comparison	Subset	p value
2x10^7 vs 3x10^6 *	Day 28	0.0084

Table S10: Number of Subjects with Solicited, Unsolicited, and Laboratory Adverse Events

All adverse events graded for relatedness and severity based upon the FDA's Guidance "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". AEs judged not related are not presented. Cohort 1= 3 x 10^6 pfu dose group, Cohort 2= 2 x 10^7 pfu dose group, and Cohort 3= 1 x 10^8 pfu dose.

All Subjects - NIH and WRAIR combined		Adverse Events After First Vaccination (Study days 0-28)											
	AE Term	Cohort 1			Cohort 2			Cohort 3			Placebo		
		Severity			Severity			Severity			Severity		
		1	2	3	1	2	3	1	2	3	1	2	3
Solicited AE	Abdominal pain	2	0	0	1	1	0	1	1	0	1	0	0
	Arthralgia	3	2	0	2	3	0	3	4	1	0	0	0
	Chills	3	3	1	6	3	2	5	5	2	0	0	0
	Diarrhea	0	0	0	1	0	0	1	1	0	1	1	0
	Fatigue	10	2	1	6	6	1	7	4	3	2	0	0
	Headache	7	4	1	7	3	1	7	3	2	3	0	0
	Injection-Site Pain	12	3	0	15	3	0	15	4	0	9	0	0
	Injection-Site Redness	0	0	0	0	0	0	1	0	0	0	0	0
	Injection-Site Swelling	1	0	0	0	0	0	1	0	0	0	0	0
	Myalgia	10	3	0	9	4	1	7	4	1	1	0	0
	Nausea	0	1	0	2	1	0	3	2	0	2	0	0
	Objective Fever	4	2	0	4	2	0	3	3	2	0	0	0
	Subjective Fever	5	4	2	6	4	1	5	3	2	1	0	0
	Sweats	2	0	0	5	0	0	2	2	2	0	0	0
	Vomiting	0	0	0	0	0	0	1	0	0	0	0	0
Unsolicited AE	Anaemia	0	0	0	0	0	0	0	1	0	0	0	0
	Chest discomfort	0	0	0	1	0	0	0	0	0	0	0	0
	Conjunctivitis	0	0	0	1	0	0	0	0	0	0	0	0
	Dizziness postural	0	0	0	0	0	0	1	0	0	0	0	0
	Injection site bruising	1	0	0	0	0	0	0	0	0	1	0	0
	Injection site pruritus	0	0	0	1	0	0	0	0	0	0	0	0
	Mouth ulceration	0	0	0	1	0	0	0	0	0	0	0	0
	Oral disorder	1	0	0	0	0	0	1	0	0	0	0	0
Laboratory AE	Activated partial thromboplastin time prolonged	0	0	0	1	0	0	0	0	0	0	0	0
	Alanine aminotransferase increased	2	0	0	0	0	0	0	1	0	2	0	0
	Aspartate aminotransferase increased	1	0	0	0	0	0	1	1	0	1	0	0
	Decreased appetite	1	0	0	0	0	0	0	0	0	0	0	0
	Eosinophilia	0	0	0	1	0	0	0	0	0	0	0	0
	Haematuria	0	0	0	0	0	0	1	0	0	0	0	0
	Haemoglobin decreased	1	0	0	0	0	0	1	0	0	0	1	0
	Lymphadenopathy	0	0	0	2	0	0	0	0	0	1	0	0
	Lymphocyte count decreased	0	0	0	2	3	3	3	3	0	1	0	0
	Lymphopenia	1	2	4	0	0	0	0	0	2	0	0	0
	Neutropenia	3	0	0	0	0	0	0	0	0	0	0	0
	Neutrophil count decreased	0	1	0	4	0	0	0	4	0	0	0	0
	Platelet count decreased	1	0	0	0	1	0	0	0	0	0	0	0
	Prothrombin time prolonged	0	1	0	1	0	0	0	0	0	2	0	0
	White blood cell count decreased	4	0	0	3	0	0	3	0	0	1	0	0

Only subjects enrolled at WRAIR		Adverse Events After First Vaccination (Study days 0-28)												All Adverse Events in Study (Study days 0-180)											
	AE Term	Cohort 1			Cohort 2			Cohort 3			Placebo			Cohort 1			Cohort 2			Cohort 3			Placebo		
		Severity			Severity			Severity			Severity			Severity			Severity			Severity			Severity		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Solicited AE	Abdominal pain	2	0	0	1	1	0	0	1	0	1	0	0	2	0	0	1	1	0	0	1	0	1	0	0
	Arthralgia	1	0	0	0	1	0	2	2	1	0	0	0	1	0	0	0	1	0	2	2	1	0	0	0
	Chills	1	1	1	2	0	2	1	4	2	0	0	0	1	1	1	2	0	2	1	4	2	0	0	0
	Diarrhea	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
	Fatigue	4	2	0	2	1	1	4	1	2	1	0	0	4	2	0	2	1	1	4	1	2	1	0	0
	Headache	1	3	1	2	2	1	4	2	1	2	0	0	1	3	1	2	2	1	4	2	1	2	0	0
	Injection-Site Pain	6	2	0	7	1	0	9	1	0	4	0	0	6	2	0	7	1	0	9	1	0	4	0	0
	Injection-Site Swelling	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Myalgia	4	2	0	1	2	1	4	1	1	0	0	0	4	2	0	1	2	1	4	1	1	0	0	0
	Nausea	0	1	0	1	1	0	1	1	0	1	0	0	0	1	0	1	1	0	1	1	0	1	0	0
	Objective Fever	2	1	0	2	1	0	2	2	1	0	0	0	2	1	0	2	1	0	2	2	1	0	0	0
	Subjective Fever	1	1	2	1	1	1	3	2	1	0	0	0	1	1	2	1	1	1	3	2	1	0	0	0
	Sweats	0	0	0	1	0	0	1	2	1	0	0	0	0	0	0	1	0	0	1	2	1	0	0	0
Unsolicit	Anaemia	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Dizziness postural	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	Injection site bruising	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Laboratory AE	Alanine aminotransferase increased	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Aspartate aminotransferase increased	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
	Haemoglobin decreased	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0
	Lymphocyte count decreased	0	0	0	1	3	3	2	3	0	0	0	0	0	0	0	1	3	3	2	3	0	0	0	0
	Lymphopenia	1	2	4	0	0	0	0	0	2	0	0	0	1	2	4	0	0	0	0	0	2	0	0	0
	Neutropenia	3	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
	Neutrophil count decreased	0	1	0	2	0	0	0	3	0	0	0	0	0	1	0	2	0	0	0	3	0	0	0	0
	Platelet count decreased	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
	Prothrombin time prolonged	0	1	0	1	0	0	0	0	0	2	0	0	0	1	0	1	0	0	0	0	0	2	0	0
	White blood cell count decreased	2	0	0	2	0	0	3	0	0	1	0	0	2	0	0	2	0	0	3	0	0	1	0	0

Only subjects enrolled at NIH		Adverse Events After First Vaccination (Study days 0-28)												Adverse Events After Second Vaccination (Study days 29-56)												All Adverse Events in Study (Study days 0-180)											
	AE Term	Cohort 1			Cohort 2			Cohort 3			Placebo			Cohort 1			Cohort 2			Cohort 3			Placebo			Cohort 1			Cohort 2			Cohort 3			Placebo		
		Severity			Severity			Severity			Severity			Severity			Severity			Severity			Severity			Severity			Severity			Severity			Severity		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Solicited AE	Abdominal pain	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	Arthralgia	2	2	0	2	2	0	1	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	2	2	0	3	2	0	1	3	0	0	0	0
	Chills	2	2	0	4	3	0	4	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	2	0	4	3	0	4	1	0	0	0	0
	Diarrhea	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0
	Fatigue	6	0	1	4	5	0	3	3	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	6	0	1	4	5	0	3	3	1	1	1	0
	Headache	6	1	0	5	1	0	3	1	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	7	1	0	6	1	0	3	1	1	1	0	0
	Injection-Site Pain	6	1	0	8	2	0	6	3	0	5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	7	1	0	8	2	0	6	3	0	5	0	0
	Injection-Site Redness	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	Injection-Site Swelling	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	Myalgia	6	1	0	8	2	0	3	3	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	6	1	0	8	2	0	3	3	0	1	1	0
	Nausea	0	0	0	1	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	0	1	0	0
	Objective Fever	2	1	0	2	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	2	1	0	1	1	1	0	0	0
	Subjective Fever	4	3	0	5	3	0	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0	5	3	0	2	1	1	1	0	0
	Sweats	2	0	0	4	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	4	0	0	1	0	1	0	0	0
	Vomiting	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Unsolicited AE	Aphthous stomatitis	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Chest discomfort	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Conjunctivitis	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Injection site pruritus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Mouth ulceration	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Oral disorder	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	2	0	0	0	0	0	2	0	0	1	0	0
	Oral pain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Rhinorrhoea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Laboratory AE	Activated partial thromboplastin time prolonged	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
	Alanine aminotransferase increased	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0
	Aspartate aminotransferase increased	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
	Decreased appetite	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Eosinophilia	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	Haematuria	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
	Haemoglobin decreased	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
	Lymphadenopathy	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	1	0	0	1	0	0
	Lymphocyte count decreased	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0
	Lymphocyte percentage decreased	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Neutrophil count decreased	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	2	0	0	1	1	0	0	0	0
	White blood cell count decreased	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0