Phase I Evaluation of the Safety and Immunogenicity of the Live Attenuated Zika Vaccine rZIKV/D4Δ30-713 in Flavivirus-naïve Adults

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List of Abbreviations

AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
CBC	complete blood count
CHIM	controlled human infection model
CIR	Center for Immunization Research
CLIA	Clinical Laboratory Improvement Amendments
CRIMSON	Clinical Research Information Management System of NIAID
CRL	Charles River Laboratories
CSO	Clinical Safety Office
DENV	dengue virus (serotypes DEN1, DEN2, DEN3, and DEN4)
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GBS	Guillain-Barré syndrome
GCP	good clinical practice
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCG	human choriogonadotropin
HCV	hepatitis C virus
HID_{50}	50% human infectious dose
HIV	human immunodeficiency virus
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IND	investigational new drug
IRB	institutional review board
JHSPH	Johns Hopkins Bloomberg School of Public Health
LLN	lower limit of normal
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NP	Nasopharyngeal
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
PBMC	peripheral blood mononuclear cell
PFU	plaque-forming units
PI	principal investigator
$PRNT_{50}$	plaque reduction neutralization titer
PT/PTT	prothrombin time/ partial thromboplastin time
ROP	retro-orbital pain
RT-PCR	reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SE	standard error
SERF	safety expedited report form
SOP	standard operating procedure
SRCP	Safety Review and Communications Plan
SUSAR	suspected unexpected serious adverse reaction
ULN	upper limit of normal
WBC	white blood cell
WHO	World Health Organization
WCG IRB	WIRB- Copernicus Group, Inc.
wt	wild-type
ZIKV	Zika virus

1 PRECIS

Protocol Title: Phase I Evaluation of the Safety and Immunogenicity of the Live Attenuated Zika Vaccine rZIKV/D4Δ30-713 in Flavivirus-naïve Adults

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Subjects: Healthy male and non-pregnant female subjects 18 to 50 years of age, inclusive, with no history of previous flavivirus infection.

Number of Subjects: 56 flavivirus-naïve subjects will be recruited from metropolitan Baltimore/Washington DC and Burlington, Vermont areas.

Study Design: Placebo-controlled, double-blind study evaluating safety and immunogenicity of a single subcutaneous dose of rZIKV/D4Δ30-713. Subjects will be randomly assigned to receive rZIKV/D4Δ30-713 or placebo in a 5:2 ratio.

Table 1: Immunization Schedule

Cohort	# Subjects	Month	Treatment	Dose
1	20	0	rZIKV/D4Δ30-713	10^3 PFU
	8	0	Placebo	0 PFU (diluent)
2	20	0	rZIKV/D4Δ30-713	10^4 PFU
	8	0	Placebo	0 PFU (diluent)

Product Description: The vaccine rZIKV/D4Δ30-713 is a live attenuated chimeric vaccine for the prevention of Zika. rZIKV/D4Δ30-713 is administered in 0.5 mL containing either $10^{3.0}$ plaque-forming units (PFU) or $10^{4.0}$ PFU.

Primary Objectives:

1) Determine the safety and reactogenicity of a single dose of the LA (Live Attenuated) Zika vaccine rZIKV/D4Δ30-713, as assessed by the frequency of adverse reactions (AR) as defined as vaccine-related adverse events (AEs), graded by severity.
    - The frequency of immediate, systemic, and local AEs and ARs following vaccination will be summarized. AEs will be displayed in tabular format, with line listings of individual clinical and laboratory AEs classified as immediate, systemic, and local events. AEs will be summarized by severity and relationship to vaccine by individuals and each group (rZIKV/D4Δ30-713 and Placebo).
2) Determine the immunogenicity of a single dose of rZIKV/D4Δ30-713 vaccine in flavivirus-naïve subjects as assessed by neutralizing antibody titers to ZIKV at 28, 56, and 90 days following vaccination.
3) Determine if 10^4 PFU of vaccine is more infectious and induces a higher proportion of seroconversion to Zika virus than the 10^3 PFU dose while maintaining an acceptable safety profile.

Secondary Objectives:

1) Assess the frequency, quantity, and duration of viremia after a single dose of rZIKV/D4Δ30-713 given at either 10^3 PFU or 10^4 PFU.
2) Evaluate the number of vaccine recipients infected with either 10^3 PFU or 10^4 PFU rZIKV/D4Δ30-713. Infection is defined as recovery of infectious vaccine virus from the blood, serum, or urine of a subject and/or by seroconversion to ZIKV (plaque-reduction neutralization titer [$PRNT_{50}$] ≥ 1:10) within the 90-day period following vaccination.
3) Determine the durability of antibody response 26 weeks after vaccination.
4) Determine the quantity and duration of ZIKV presence as determined by:
    - The peak virus titer in the blood and the duration of viremia induced by the LA Zika vaccine as determined by RT-PCR and virus culture
    - The quantity and duration of possible Zika vaccine shedding in urine determined by RT-PCR and virus culture
    - The quantity and duration of possible Zika vaccine shedding in vaginal secretions determined by RT-PCR and virus culture
    - The quantity and duration of possible Zika vaccine shedding in semen determined by RT-PCR and virus culture

Exploratory objectives:

1) To evaluate the phenotype of peripheral blood mononuclear cells (PBMCs) at primary infection with the rZIKV/D4Δ30-713 vaccine.
2) To evaluate the cellular immune response to primary infection with the rZIKV/D4Δ30-713 vaccine.
3) To evaluate the innate immune response to primary infection with the rZIKV/D4Δ30-713 vaccine.
4) To evaluate B and T cell memory responses following primary infection with the rZIKV/D4Δ30-713 vaccine.
5) To evaluate the antibody response in saliva to infection with rZIKV/D4Δ30-713 as a possible diagnostic tool. This will be done in a subset of subjects (those enrolled at the Center for Immunization Research [CIR]).

Primary Endpoints

Safety

Occurrence, intensity and relationship to vaccination of solicited local and general adverse events (AEs) within the 28-day follow-up period after vaccination.
Occurrence, intensity and relationship to vaccination of unsolicited AEs within the 28-day (Days 0-28) follow-up period after vaccination.

Occurrence of medically-attended adverse events and serious adverse events (SAEs) throughout the entire study period.

Immunogenicity

Determination of the peak neutralizing antibody titer to ZIKV through 90 days following vaccination with either 10^3 PFU or 10^4 PFU of rZIKV/D4Δ30.

Secondary Endpoint

Viremia: Determine the frequency, magnitude, and duration of recovery of vaccine virus from the blood, urine, semen (men), and vaginal secretions (women) of subjects who received the rZIKV/D4Δ30-713 vaccine. Virus titer will be measured by tissue culture (infective virus) and PCR.

Immunogenicity: Determination of the neutralizing antibody titer to ZIKV 6 months following vaccination.

2 INTRODUCTION

2.1 Background – Zika

Zika virus (ZIKV) is a mosquito-borne flavivirus that was first isolated from the blood of a sentinel rhesus macaque in the Zika forest of Uganda in 1947 [1]. Sporadic reports of ZIKV infection and/or serologic evidence of ZIKV infection were reported from 1951 – 1981 from Africa and Asia [2]. The first major outbreak of ZIKV was reported from the Island of Yap in Micronesia in 2007 where it was estimated that 72.6% of the population ≥ 3 years of age was infected, demonstrating the rapid transmission of ZIKV in a naïve population [3]. ZIKV generally causes only a mild infection characterized by rash, low-grade fever, non-purulent conjunctivitis and myalgia with most of those infected not reporting symptoms [3, 4]. Nearly all symptomatic patients from the Yap island outbreak presented with rash (90%), arthritis/arthralgia (65%), and fever (65%). Unlike dengue virus, ZIKV does not cause hemorrhagic manifestations, vascular leak syndrome, or liver function abnormalities. Only approximately 19% of subjects found to be seropositive to ZIKV in a serosurvey recounted being symptomatic with a Zika-like illness. In October 2013, the largest outbreak of ZIKV recorded up to that time began in French Polynesia [5, 6]. It was estimated that 28,000 ZIKV infections occurred (~11% of the population) with most infections presenting with low-grade fever, rash, arthralgia and conjunctivitis. Since its introduction into Brazil, ZIKV has spread to more than 69 countries in the Americas, Caribbean, Asia and Africa [7].

Brazil first identified an outbreak of ZIKV in its northeast region in early 2015. By September increasing numbers of cases of microcephaly were being reported, particularly from regions involved in the ZIKV outbreak [8]. Because of the devastating birth defects caused by congenital ZIKV infection, the World Health Organization (WHO) declared ZIKV a Public Health Emergency of International Concern (PHEIC) on February 1, 2016. Evidence of a link between ZIKV infection in pregnancy, particularly during the first trimester, and microcephaly in the fetus was collected [4, 9, 10] and a causal association between ZIKV infection and microcephaly and/or other birth defects was formally established [11]. Most of the women in whom microcephaly of the fetus was diagnosed reported symptoms of ZIKV infection at some point during their pregnancy, usually in the first or second trimester; rash was the most common symptom reported. However, recent evidence suggests that congenital Zika syndrome (CZS) can occur regardless of symptoms in the mother [12, 13]. Although CZS is most likely to occur with maternal infection in the first or second trimester, there is increasing evidence that congenital anomalies can occur regardless of when during pregnancy the mother was infected with ZIKV. In addition, babies who appeared normal at birth are being diagnosed with developmental delay, hearing problems, and eye abnormalities after birth. Although the PHEIC ended November 11, 2016, concern regarding continued ZIKV transmission and congenital infection remains high and a safe and effective ZIKV vaccine is a public health priority.

Transmission via mosquito vector remains the principal driving force of ZIKV infection and disease. *Aedes* mosquitoes, particularly *Aedes aegypti*, are thought to be the primary vector for ZIKV [14, 15]. Complicating ZIKV control programs, there have been several reports of sexual transmission of ZIKV [16-21]. All but two of these cases involve male-to-female transmission; there has been one report of female-to-male transmission and one report of male-to-male transmission [22, 23]. The cases of sexual transmission have occurred during or just after symptomatic illness in all but one case. In that case, an asymptomatic subject transmitted ZIKV to his partner [24]. ZIKV RNA has been detected in semen by reverse transcriptase – polymerase chain reaction for 2 - 6 months after symptoms have developed [25-27]. However, to date, the longest interval between onset of symptoms and recovery of infectious ZIKV from semen is 69 days [28]. More recent data has found that infectious ZIKV is shed only rarely from ZIKV-infected men; only 4% of semen samples that were positive for ZIKV by PCR contained infectious virus and infectious virus was found only in samples collected within 30 days of symptom onset [29]. It is also not known for how long ZIKV can be sexually transmitted, but all cases of sexual transmission reported in the literature thus far have occurred within 3 weeks of return from an endemic area with all but one of those cases occurring within 2 weeks [21-24, 30]. Although cases of sexual transmission have occurred, it appears to play a minor role in transmission as there have been only 52 documented cases of sexually acquired ZIKV resulting from a total of 5,388 travel-associated ZIKV cases in the United States (< 1% of ZIKV cases) as of March 21, 2018, https://www.cdc.gov/zika/geo/united-states.html, accessed March 26, 2018) and it is not thought to contribute significantly to transmission [31].

The goal of this project is to evaluate the safety and immunogenicity of the first live attenuated ZIKV to be evaluated in humans. The LA ZIKV candidate vaccine rZIKV/D4Δ30-713 is highly attenuated compared to its parent virus (ZIKV Paraiba/2015). The infectious vaccine virus could not be recovered from the blood, saliva, or urine of immunized rhesus macaques following administration of a single dose of $10^{4.0}$ PFU. Should this vaccine have an acceptable safety

profile and prove to be immunogenic in flavivirus-naïve healthy adults, the vaccine will need to be further evaluated for protective efficacy. The ZIKV epidemic has waned considerably with very few cases of ZIKV being reported from Asia and Latin America. For this reason, it is unlikely that efficacy of the vaccine will be able to be assessed in a traditional Phase 3 study. It is likely that, at this time, efficacy of a promising vaccine candidate will only be able to be assessed using a ZIKV controlled human infection model (CHIM) which is currently in development. Should the rZIKV/D4Δ30-713 vaccine prove to be immunogenic and have an acceptable safety profile, we are hoping to include assessment of its efficacy using a ZIKV CHIM as part of our clinical development plan.

2.2 Background – ZIKV strains

2.2.1 rZIKV/D4Δ30-713 Vaccine, Lot ZIKV#118A

The vaccine candidate rZIKV/D4Δ30-713 is a live attenuated virus derived from the Zika virus wild-type (wt) strain ZIKV Paraiba/2015 and assembled on a DEN4Δ30 genetic background using recombinant DNA technology.

A full-length chimeric cDNA clone ZV-Den4Δ30 was constructed to generate a viral transcript expressing the prM and E structural genes of a contemporary ZIKV strain within the genetic background of the DEN4Δ30 vaccine strain. The cDNA plasmid, ZV-Den4Δ30-713 V1 was transfected into qualified Vero cells. Recovered virus was terminally diluted and then amplified by serial passaging in Vero cells. The titer was determined by plaque titration in Vero cells. From initial transfection through final amplification, only serum-free medium was used for Vero cell culture and virus propagation. The rZIKV/D4Δ30-713 seed virus was generated in the Laboratory of Viral Diseases (LVD), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), and the vaccine was manufactured at the Charles River Laboratories (CRL) Biopharmaceutical Services facility in Malvern, PA.

2.2.1.1 Final Container of rZIKV/D4Δ30-713, Lot ZIKV#118A

The Final Drug Product was dispensed as 0.6-mL aliquots of approximately $10^{7.3}$ PFU/mL live recombinant Zika virus rZIKV/D4Δ30-713 Vero-grown virus vaccine into 2.0-mL sterile cryovials and stored at -75°C ± 15°C.

2.2.1.2 Composition of rZIKV/D4Δ30-713, Lot ZIKV#118A

The Final Drug Product composition is a concentration of PFU/mL live attenuated rZIKV/D4Δ30-713 (lot ZIKV#118A) in Leibovitz L-15 medium containing 1X SPG. The potency of ZIKV/D4Δ30-713 (lot ZIKV#118A) is $10^{7.3}$ PFU/mL.

2.2.1.3 Investigational Product Label for rZIKV/D4Δ30-713, Lot ZIKV#118A

Figure 1: Label for Final Vial of rZIKV/D4Δ30-713, Lot (Enlarged Sample)

LIVE RECOMBINANT ZIKA VIRUS VACCINE
rZIKV/D4Δ30-713
VERO GROWN VIRUS
CAUTION: NEW DRUG LIMITED
BY FEDERAL (USA) LAW
TO INVESTIGATIONAL USE
Store at -75 ± 15ºC
Charles River Laboratories, Malvern, PA
Date: 13JUN2017
Vial#: 0000
Lot: ZIKV#118A

2.3 Rationale

2.3.1 Animal Experience

2.3.1.1 Non-human Primate Study

The replication (viremia), immunogenicity, and protective efficacy of the rZIKV/D4Δ30-713 (lot ZIKV#118A) vaccine candidate was studied in rhesus monkeys. The primary purpose of the study was to establish the attenuation phenotype and protective efficacy of the vaccine candidate. Monkeys inoculated with the wt parent virus ZIKV-Paraiba/2015 were viremic (as determined by direct culture in Vero cells) for 4.25 days with a mean peak titer of 2.1 $\log_{10}$ PFU/mL. This was similar to results obtained for an additional wt virus, ZIKV-Nicaragua/2016, in which monkeys were viremic for 4.25 days with a mean peak titer of 2.3 $\log_{10}$ PFU/mL. Replication of the vaccine candidate rZIKV/D4Δ30-713 was below the level of detection (< 0.7 $\log_{10}$PFU/mL) in all animals inoculated, thus demonstrating the attenuation phenotype compared to wt ZIKV (Table 2). Detection of virus genome by quantitative reverse transcriptase – polymerase chain reaction (RT-PCR) in serum also confirmed the attenuation phenotype of rZIKV/D4Δ30-713 (Table 3). rZIKV/D4Δ30-713 was not recovered from the saliva or urine of inoculated monkeys by RT-PCR further confirming its attenuated phenotype. Regardless of the method for measurement of peak virus load following inoculation, the level of replication of rZIKV/D4Δ30-713 was at least 20 - 25 times lower than wt parent ZIKV-Paraiba/2015. Attenuation is likely due to chimerization and the presence of the Δ30 mutation.

Table 2: Replication (Direct Culture in Vero Cells) of rZIKV/D4Δ30-713 and Wild-type ZIKV in Rhesus Monkeys

Virus	N	Dose ($\log_{10}$ PFU)	% with viremia	Mean no. of viremic days per monkey	Mean peak virus titer* ($\log_{10}$ PFU/mL ± standard error [SE])
ZIKV-Paraiba/2015	4	4	100	4.25	2.1 ± 0.2
ZIKV-Nicaragua/2016	4	4	100	4.25	2.3 ± 0.1
rZIKV/D4Δ30-713	4	4	0	0	< 0.7 ± 0.0

* Lower limit of detection = 0.7 $\log_{10}$ PFU/mL

Table 3: Replication (RT-PCR) of rZIKV/D4Δ30-713 and Wild-type ZIKV in Rhesus Monkeys

Virus	N	Dose (log_{10} PFU)	% with genome detected	Mean no. of genome days per monkey	Mean peak titer (log_{10} GE/mL ± SE)
ZIKV-Paraiba/2015	4	4	100	7.75	5.5 ± 0.2
ZIKV-Nicaragua/2016	4	4	100	5.25	5.7 ± 0.1
rZIKV/D4Δ30-713	4	4	100	2.50	4.2 ± 0.2

* Lower limit of detection = 3.2 log_{10} GE/mL

Due to the attenuated nature of rZIKV/D4Δ30-713, the virus was less immunogenic than its wt parent virus (Table 4). However, ***all monkeys inoculated with rZIKV/D4Δ30-713 were protected against challenge with wt ZIKV and none of the monkeys demonstrated any detectable challenge viremia regardless of the detection method*** (tissue culture or PCR, Table 5).

Table 4: Immunogenicity of rZIKV/D4Δ30-713 and Wild-type ZIKV in Rhesus Monkeys

		$PRNT_{50}$ Assay (mean reciprocal titer)[1]			EC_{50} RVP Flow Assay (mean reciprocal titer)[2]		
Virus	N	Day 0	Day 28	Day 42	Day 0	Day 28	Day 42
ZIKV-Paraiba/2015	4	< 10	391	217	< 60	14491	9809
ZIKV-Nicaragua/2016	4	< 10	289	147	< 60	15656	9149
rZIKV/D4Δ30-713	4	< 10	12	15	< 60	871	451

[1] 50% Plaque reduction neutralization mean titer (reciprocal) assayed against ZIKV-Paraiba/2015
[2] 50% Effective serum concentration (mean reciprocal) to block infection of reporter virus particles (RVPs) constructed from ZIKV-Paraiba/2015 structural proteins. Assayed by flow cytometry.

Table 5: Vaccine Candidate rZIKV/D4Δ30-713 Protects Monkeys Against ZIKV Challenge

Primary inoculation	N	Challenge dose[1] (log_{10}PFU)	% with challenge viremia	Mean no. of viremic days per monkey	Mean peak virus titer (log_{10} PFU/mL ± SE)
ZIKV-Paraiba/2015	4	4	0	0	< 0.7 ± 0.0
ZIKV-Nicaragua/2016	4	4	0	0	< 0.7 ± 0.0
rZIKV/D4Δ30-713	4	4	0	0	< 0.7 ± 0.0
Placebo	4	4	100	4.75	2.6 ± 0.2

1. Challenge virus (ZIKV-Paraiba/2015) was administered subcutaneously to all monkeys on day 56 following primary inoculation

2.3.2 Clinical Experience

Prior to initiation of this protocol, rZIKV/D4Δ30-713 had not previously been studied in humans. However, we have studied other recombinant flavivirus vaccines, including chimeric flavivirus vaccines on the rDEN4Δ30 genetic background [32-35]. rDEN4Δ30 has been extensively evaluated in humans as both a monovalent dengue virus (DENV)-4 candidate vaccine and in tetravalent vaccine formulations [36-41]. The vaccine is well tolerated, induces a low-level viremia, and is immunogenic. Chimerization is highly attenuating for flaviviruses and has been used as a strategy for flavivirus vaccine development. The recently licensed live attenuated tetravalent dengue vaccine consists of four chimeric viruses in which the prM and E coding sequence of yellow fever 17D has been replaced with those of DENV-1, DENV-2, DENV-3 or DENV-4. We have used chimerization to attenuate the DENV-2 candidate vaccine rDEN2/4Δ30, the DENV-3 candidate vaccine rDEN3/4Δ30, the candidate West Nile vaccine rWN/DEN4Δ30 and the Langat candidate vaccine rLGT/DEN4 [32-37, 42]. When the prM and E coding sequences of rDEN4Δ30 were replaced with those of either DENV-2 New Guinea C (NGC), DENV-3 Sleman/78, or WNV, the resultant candidate vaccines were less infectious as evidenced by a lower incidence of viremia and/or an increase in the 50% human infectious dose (HID_{50}) of ~10-fold to more than 10,000-fold (Table 6).

The recombinant ZIKV vaccine rZIKV/D4Δ30-713 has been administered at a dose of 10^3 PFU to 20 healthy adult volunteers. The vaccine was well tolerated at this dose (Table 7). The vaccine appears to be over-attenuated at a dose of 10^3 PFU as fewer than 50% of the vaccine recipients were infected with the vaccine, based on virologic and serologic assessments. The vaccine virus was not recovered from any vaccinated subject (serum, urine, semen, or vaginal secretions), as assessed by culture and PCR. Only 9/20 vaccinated seroconverted to ZIKV following vaccination (Table 8), indicating that the vaccine is not sufficiently immunogenic at a dose of 10^3 PFU.

Table 6: Comparison of Chimeric Viruses on a DEN4Δ30 Background with rDEN4Δ30 in Humans

Vaccine Candidate	Dose (log_{10} PFU)	N	% with viremia	HID_{50}	Mean peak titer (log_{10} PFU/mL)	Mean day of onset ± SE	Mean # days of viremia ± SE
rDEN4Δ30 [34]	3	70	28	< 10 PFU	0.6 ± 0.1	10.2 ± 0.6	1.8 ± 0.3
rDEN2/4Δ30 [34]	3	40	60	10 PFU	0.5 ± 0.03	9.2 ± 0.6	3.3 ± 0.6
rDEN3/4Δ30 [34]	3	20	15	> 10^5 PFU	1.0 ± 0.3	12 ± 0	4.3 ± 0.7
WN/DEN4Δ30 [35]	3	19	16	> 10^4 PFU	0.5 ± 0	15.3 ± 1.9	2.3 ± 0.7

Table 7: Adverse events following one dose of ZIKV/DEN4Δ30 or placebo (CIR 318)

		ZIKV/DEN4Δ30 (n=20)	Placebo (n=8)	P value (1-sided)[1]
Local	Erythema	5%	0.0%	0.7143

	ZIKV/DEN4Δ30 (n=20)	Placebo (n=8)	P value (1-sided)[1]
Tenderness	0%	0.0%	n/a
Pain	0%	0.0%	n/a
Pruritus	0%	0.0%	n/a
Induration	0%	0.0%	n/a
Systemic			
Fever	0%	0%	n/a
Headache	50.0%	62.5%	0.8454
Rash	0.0%	0.0%	n/a
Neutropenia[2]	0.0%	0.0%	n/a
Elevated ALT	0.0%	0.0%	n/a
Myalgia	20.0%	25.0%	0.7922
Arthralgia	10.0%	12.5%	0.8120
Retro-orbital Pain	5.0%	12.5%	0.9259
Fatigue	25.0%	25.0%	0.6940
Muscle weakness	5.0%	0.0%	0.7143
Prolonged PT	0.0%	0.0%	n/a
Prolonged PTT	0.0%	0.0%	n/a
Thrombocytopenia	0.0%	0.0%	n/a

1. P value is the probability is greater for rZIKV/DEN4Δ30 than placebo
2. Neutropenia was defined as an ANC ≤ 1,000/mm^3.

Table 8: Serological response to a single dose of ZIKV/DEN4Δ30 (n=20)

Geometric mean neutralizing antibody titer (% seroconverted)				
Day 28	Day 56	Day 90	Peak titer through day 90	# seroconverted (% total)
85.6 (41.2, 20%)	66.8 (40.9, 40%)	71.0 (36.6, 45%)	56.1 (40.9, 45%)	(45%)

1. Seroconversion was defined as a titer of ≥ 10, reciprocal
2. Geometric mean titer calculated at each time point only for those subjects with a titer of ≥ 10, reciprocal
3. One subject had a titer of >5120, reciprocal (the ULN). A value of 10,240 was assigned for calculation purposes

2.3.3 Conclusions

Based on our experience with other chimeric viruses in the rDEN4Δ30 background and the replication data of ZIKV/D4Δ30-713 in NHP (non-human primates), we expected that this candidate vaccine will be attenuated in humans and will induce immune response. For additional safety, we initially administered the vaccine at a dose of 10^3 PFU, a 10-fold lower dose than was administered to NHP. The vaccine was not sufficiently infectious or immunogenic at this dose and for this reason, the protocol is being amended to evaluate a dose of 10^4 PFU. Should a dose of 10^4 be insufficiently immunogenic, the protocol may be further amended to evaluate a dose of 10^5 PFU. Recent data demonstrating a very low incidence of recovery of infectious wild type ZIKV from infected symptomatic men with ZIKV detected by PCR in semen, suggest that the risk of sexual transmission may be much lower than was first thought and is not likely to occur with this highly attenuated vaccine virus [29]. Indeed, vaccine virus was not recovered from the semen of any male volunteer or the cervicovaginal secretions of any female volunteer when administered at a dose of 10^3 PFU.

3 OBJECTIVES

3.1 Primary Objectives

1) Determine the safety and reactogenicity of a single dose of the LA Zika vaccine rZIKV/D4Δ30-713 given at either 10^3 PFU or 10^4 PFU, as assessed by the frequency of adverse reactions (AR) as defined as vaccine-related adverse events (AE)s, graded by severity.
    - The frequency of immediate, systemic, and local AEs and ARs following vaccination will be summarized. AEs will be displayed in tabular format, with line listings of individual clinical and laboratory AEs classified as immediate, systemic, and local events and AEs will be summarized by severity and relationship to vaccine by individuals and each group (rZIKV/D4Δ30-713 and placebo).
2) Determine the immunogenicity of a single dose of rZIKV/D4Δ30-713 vaccine given at either 10^3 PFU or 10^4 PFU as assessed by neutralizing antibody titers to ZIKV at 28, 56, and 90 days following vaccination.

3.2 Secondary Objectives

1) Assess the frequency, quantity, and duration of viremia after a single dose of rZIKV/D4Δ30-713 given at either 10^3 PFU or 10^4 PFU.
2) Evaluate the number of vaccine recipients infected with rZIKV/D4Δ30-713 given at either 10^3 PFU or 10^4 PFU. Infection is defined as recovery of infectious vaccine virus from the blood, serum, or urine of a subject and/or by seroconversion to ZIKV (plaque-reduction neutralization titer [$PRNT_{50}$] ≥ 1:10) within the 90-day period following vaccination.
3) Evaluate the immunogenicity of rZIKV/D4Δ30-713 in flavivirus-naïve subjects as assessed by the $PRNT_{50}$ to ZIKV, for each subject at Study Day 28, 56, and 90 post-administration of LA Zika vaccine.
4) Determine the durability of antibody response 26 weeks after vaccination.
5) Determine the quantity and duration of ZIKV presence as determined by:
    - The peak virus titer in the blood and the duration of viremia induced by the LA Zika vaccine as determined by RT-PCR and virus culture
    - The quantity and duration of possible Zika vaccine shedding in urine determined by RT-PCR and virus culture
    - The quantity and duration of possible Zika vaccine shedding in vaginal secretions determined by RT-PCR and virus culture
    - The quantity and duration of possible Zika vaccine shedding in semen determined by RT-PCR and virus culture

3.3 Exploratory Objectives

The exploratory objectives of this study are:

1) To evaluate the phenotype of peripheral blood mononuclear cells (PBMCs) at primary infection with the rZIKV/D4Δ30-713 vaccine.
2) To evaluate the cellular immune response to primary infection with the rZIKV/D4Δ30-713 vaccine.

3) To evaluate the innate immune response to primary infection with the rZIKV/D4Δ30-713 vaccine.
4) To evaluate B and T cell memory responses following primary infection with the rZIKV/D4Δ30-713 vaccine.
5) To evaluate the antibody response in saliva to infection with rZIKV/D4Δ30-713 as a possible diagnostic tool. This will be done in a subset of subjects (those enrolled at the Center for Immunization Research [CIR]).

4 STUDY DESIGN

4.1 Overall Design

This study is a placebo-controlled, double-blind study in normal healthy, flavivirus-naïve adult male and female subjects 18 - 50 years of age, inclusive, recruited from the metropolitan Baltimore/Washington, DC and Burlington, VT areas. The purpose of this study is to evaluate the safety, reactogenicity, and immunogenicity of a single dose of the live attenuated rZIKV/D4Δ30-713 vaccine. Placebo recipients are included in the study as a control to better assess vaccine-associated versus non-vaccine-associated AEs. Cohort 1 (10^3 PFU) will be enrolled and evaluated first. If the the vaccine is not found to induce seroconversion to ZIKV in > 80% of subjects inoculated with 10^3 PFU of the vaccine, a second cohort of volunteers will be enrolled and will be inoculated with 10^4 PFU of vaccine (or placebo).

After providing written informed consent, subjects will undergo eligibility screening, including medical history, physical examination, hematology testing, liver and renal function testing, human immunodeficiency virus (HIV) screening, hepatitis B and C screening, urinalysis, COVID-19 testing (if not fully vaccinated against COVID-19), and serology screening for previous flavivirus infection. Pregnancy testing will be performed on female subjects of reproductive potential. Follicle-stimulating hormone (FSH) levels will be collected for women who report amenorrhea more than 12 months and less than 24 months. All screening tests must be performed within 60 days of inoculation. HIV screening must be performed within 2 weeks of inoculation.

All clinically significant abnormalities will be reviewed with subjects and referral for follow-up care will be provided. Subjects will be determined to be eligible based on the inclusion and exclusion criteria found in Section 5 of this protocol. For subjects who are eligible, the Day 0 visit will be scheduled for receipt of inoculation. Pregnancy testing will be repeated on applicable female subjects on the day of inoculation and during follow-up. Subjects will be considered enrolled in the study only after they receive test article (rZIKV/D4Δ30-713 or placebo). After inoculation, subjects will be evaluated in the clinic for at least 30 minutes. They will return to the clinic on study days 4, 6, 8, 10, 12, 14, and 16 for evaluation by a clinician and to have blood drawn for clinical laboratory studies, virologic assays, and immunologic assays. In addition, urine, vaginal secretions, and semen will be collected at specified time points for additional virologic assays. The subjects will also have a clinical evaluation performed at each specified visit (see Section 7.4 for a detailed description of study procedures). All subjects will be provided with a memory enhancement card on which they will be asked to record their temperature 2 times a day for the first 16 days post-inoculation. They will also return to the clinic on study days 21, 28, 56, 90, 150, and 180.

4.1.1 Study Design

This study will include two cohorts of 28 flavivirus-naïve male and female subjects, 18 - 50 years of age. The live attenuated ZIKV vaccine rZIKV/D4Δ30-713 will be evaluated. Within each cohort, twenty subjects will receive a single dose of either 10^3 PFU or 10^4 PFU of rZIKV/D4Δ30-713 and 8 subjects will receive a single dose of placebo. Subjects will be followed for approximately 26 weeks after receipt of LA Zika vaccine.

4.1.2 Sample Size and Placebo Ratio

Up to fifty-six subjects (40 vaccine recipients and 16 placebo recipients) will be enrolled in the study. Twenty subjects per cohort were chosen to receive vaccine for the following reasons:

- This study is designed to build upon the previous Phase 1 studies of other live attenuated flavivirus vaccines, which used similar cohorts and the same vaccine to placebo ratio.
- Twenty subjects receiving vaccine will provide adequate power to determine seroconversion frequency. We hope to induce a seroconversion frequency of ≥ 80% in those subjects who receive the vaccine. Cohort 1 will be enrolled first. If 10^3 PFU of vaccine does not induce seroconversion to ZIKV in ≥ 80% of vaccinated volunteers, Cohort 2 will be enrolled.
- We have included placebo-recipients so that we can better assess if common AEs are vaccine-related.

4.1.3 Duration of Subject Participation

Subjects will be followed for approximately 26 weeks (approximately 180 days) from the time of the initial inoculation. Subjects will be screened for eligibility up to 60 days prior to inoculation on Study Day 0.

4.1.4 Estimated Duration of the Study

Screening procedures should be completed within 60 days prior to receipt of the test article (except HIV testing, which will be completed within 14 days of inoculation). Enrollment will occur over a period of 4 - 8 weeks. The study will last for a total of approximately 26 weeks from the time the last subject is enrolled (inoculated).

4.1.5 Treatment Assignment

For each cohort, subjects will be randomly assigned to receive rZIKV/D4Δ30-713 or placebo in a 5:2 ratio. Treatment assignment will be done using a random number generator to prepare the sequence in which subjects are assigned to receive vaccine or placebo. Subjects will be centrally randomized by the vaccine preparation study staff at Johns Hopkins Bloomberg School of Public Health (JHSPH) in blocks of 7. There will be 4 blocks of 7 subjects. Each block of 7 will include 5 vaccine recipients and 2 placebo recipients. Subjects will not be enrolled sequentially across sites. Once all 7 subjects in a block have reached Study Day 90, the block may be unblinded to all staff. Enrollment will occur at both sites with the intent of enrolling 2 blocks at CIR and 2 blocks at UVM. Should one site have difficulty enrolling subjects, enrollment may be reassigned to the other site, however, the initial treatment assignment will remain unchanged.

On Study Day 0, once subjects have met all eligibility criteria, study staff will assign the subjects sequential ID study numbers from a prepared list. Subjects will be considered enrolled when they receive the first dose of test article. Clinical staff will remain blinded to treatment assignment until all subjects within a block have reached Study Day 90.

A master log of treatment assignments will be maintained in a record separate from other study records. This log will be maintained by the Investigational Drug Service (IDS) pharmacy. It will be kept in a locked room with limited access. A sealed envelope containing a copy of the treatment assignment will also be kept by the Data and Safety Monitoring Board (DSMB) Executive Secretary. The subjects will be informed of their treatment assignment after their block has completed Study Day 90.

4.1.6 Blinding

This study will be conducted as a double-blind study to avoid biased assessment of AEs. rZIKV/D4Δ30-713 or placebo will be prepared and drawn into syringes by pharmacy personnel who are not involved with the clinical assessment of subjects or performing research assays. Syringes are labeled according to standard operating procedures/recommendations. Because vaccine diluent is used as the placebo, there will be no difference in the appearance of the syringes.

The subject, investigator, and clinical staff will not know which treatment group the subject has been assigned to. In addition, other personnel assigned to monitor the study will not know the treatment assignment of the subject. Clinical staff will remain blinded to treatment assignment until all subjects within a block of 7 subjects have reached study day 90. Once the time point for unblinding has occurred (study day 90 for subjects within a block), the principal investigator (PI) can request treatment assignments in writing from the unblinded study staff.

If it becomes necessary to unblind a specific subject's assignment for emergency medical management prior to all subjects within a block completing study day 90 post-vaccination, the PI will contact the unblinded study staff and obtain the treatment assignment of the subject in question. Only that specific subject's assignment will be unblinded. The sponsor, Office of Clinical Research Policy and Regulatory Operations (OCRPRO) and the DSMB executive secretary will be notified of the event within 2 business days. This will also be documented in the subject's study chart.

5 SELECTION AND ENROLLMENT OF SUBJECTS

Subjects must meet all eligibility criteria to be enrolled in the study.

5.1 Inclusion Criteria

All of the following criteria must be fulfilled for a subject to qualify for inclusion in this study:

1. Adult male or female between 18 and 50 years of age, inclusive.
2. Good general health as determined by physical examination, laboratory screening, and review of medical history.

3. Available for the duration of the study, which is approximately 26 weeks.

4. Willingness to participate in the study as evidenced by signing the informed consent document.

5. Females only: Female subjects of childbearing potential, with the exception noted below, should be willing to use effective contraception and have no plans to undergo IVF (in vitro fertilization) during participation in the trial. Reliable methods of contraception include hormonal birth control, condoms with spermicide, diaphragm with spermicide, surgical sterilization, and intrauterine device. Women must have been on an effective method of birth control for at least 30 days prior to enrollment. All female subjects will be considered as having childbearing potential, except for women who exclusively have sex with women, those who have had a hysterectomy, tubal ligation, or tubal coil (at least 3 months prior to vaccination), or are considered to be post-menopausal, as documented by at least 1 year since last menstrual period with an FSH level in the menopausal range or at least 24 consecutive months of amenorrhea. Transgender men who have internal female organs and have sex with men will be considered of childbearing potential and should be willing to use effective contraception during the trial. Exception: Females who have sex with females (exclusively) and have no intention of conceiving a child during the study and women whose partners have had a vasectomy will not be required to use contraception, however they will be required to use female condoms and/or dental dams for at least 1 month following vaccination. For women whose sexual partner has had a vasectomy, the vasectomy must have been performed 30 days or more prior to enrollment.

6. Males only: Males should be willing to use barrier contraception for the first 3 months following vaccination* and agree to not donate sperm for the duration of the study.

*Based on CDC guidance for men returning from ZIKV-endemic areas

5.2 Exclusion Criteria

A subject will be excluded from the study if any of the following criteria are met:

1. Females only: Currently pregnant, as determined by positive β-human choriogonadotropin (HCG) test, or breast-feeding.

2. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, rheumatologic, autoimmune, or renal disease based on history, physical examination, and/or laboratory studies.

3. History of any neuroinflammatory disorder i.e. Bell's Palsy, transverse myelitis

4. Behavioral, cognitive, or psychiatric disease that, in the opinion of the investigator, affects the subject's ability to understand and cooperate with the requirements of the study protocol.

5. Screening laboratory values of Grade 1 or above for absolute neutrophil count (ANC), alanine aminotransferase (ALT), and serum creatinine, as defined in this protocol.

6. Any other condition that, in the opinion of the investigator, would jeopardize the safety or rights of a subject participating in the trial, or would render the subject unable to comply with the protocol.

7. Any significant alcohol or drug abuse in the past 12 months that has caused medical, occupational, or family problems, as indicated by subject history.

8. History of a severe allergic reaction or anaphylaxis.

9. Severe asthma (emergency room visit or hospitalization within the last 6 months).

10. HIV infection, as indicated by screening and confirmatory assays.

11. Hepatitis C virus (HCV) infection, as indicated by screening and confirmatory assays.

12. Hepatitis B virus (HBV) infection, as indicated by hepatitis B surface antigen (HBsAg) screening.

13. Any known immunodeficiency syndrome.

14. History of Guillain-Barrè syndrome.

15. Current use of anticoagulant medications (this does not include anti-platelet medication such as aspirin or non-steroidal anti-inflammatory medications).

16. Use of immunosuppressive corticosteroids (excluding topical or nasal) or immunosuppressive drugs within 28 days prior to or following inoculation. An immunosuppressive dose of corticosteroids is defined as ≥ 10 mg of a prednisone equivalent per day for ≥ 14 days.

17. Receipt of a live vaccine within 28 days or a killed vaccine within 14 days prior to inoculation, or anticipated receipt of any vaccine during the 28 days following inoculation with the exception of COVID-19 vaccines either licensed or under EUA which can be given at any time, however all effort will be made to avoid giving COVID-19 vaccines within the above windows.

18. Asplenia.

19. Receipt of blood products within the past 6 months, including transfusions or immunoglobulin, or anticipated receipt of any blood products or immunoglobulin during the 28 days following inoculation.

20. History or serologic evidence of previous ZIKV or other flavivirus infection (e.g., dengue, yellow fever virus, St. Louis Encephalitis virus, or West Nile virus).

21. Previous receipt of a flavivirus vaccine (licensed or experimental).

22. Receipt or anticipated receipt of any investigational agent in the 28 days before or after inoculation with the exception of COVID-19 vaccines, either licensed or authorized under EUA.

23. Refusal to allow specimen storage for future research.

24. Is in isolation or quarantine for SARS-CoV-2 infection or exposure and cannot complete screening or enrollment for this reason.

5.3 Subject Withdrawal and Termination Criteria

A subject will not be considered to have completed the trial if any of the following reasons apply. However, any subject who has received vaccine or placebo will be encouraged to remain in the study for periodic safety evaluations for the duration of the study at the discretion of the investigator. Subjects who have been terminated from the study or withdrawn from the study prior to study day 28 may be replaced to ensure sufficient safety data for rZIKV/D4Δ30-713 are collected.

1. ***Research terminated by sponsor or investigator*** – applies if the entire study is terminated by the sponsor or investigator for any reason.

2. ***Withdrawal of consent*** – applies to a subject who withdraws consent to participate in the study for any reason.

3. ***Noncompliant with protocol*** – applies to a subject who does not comply or is not able to comply with protocol-specific visits or evaluations on a consistent basis such that adequate follow-up is not possible, and the subject's safety and the study data's integrity would be compromised by continuing in the trial.

4. ***Withdrawn by PI*** – may occur if the investigator believes that it is in the best interest of the subject to be withdrawn from the study.

5. ***Other*** – a category used when previous categories do not apply and requires an explanation.

5.4 Special Situations:

5.4.1 Pregnancy:

Because this is a live replicating virus, although it is attenuated, there is a perceived risk to the fetus should a woman be pregnant at the time of vaccination or become pregnant during the expected time of vaccine virus replication (expected to be through study day 16 post-vaccination). For this reason, pregnancy is an exclusion criterion for the study and pregnancy prevention counseling will be performed regularly throughout the study. If a subject becomes pregnant prior to study day 28 following rZIKV/D4Δ30-713 or placebo administration, as determined by a positive pregnancy test at the study day 28 visit, the subject will not be included in the per-protocol immunogenicity evaluations from her estimated date of conception. However, she will be encouraged to remain in the study for periodic safety evaluations and will be followed until completion of her pregnancy. The subject will be asked to sign a release of medical information form so that records can be obtained from her obstetrician regarding the outcome of the pregnancy. Should she become pregnant on or after 28 days following administration of rZIKV/D4Δ30-713 /placebo, as determined by positive pregnancy test after study day 28, the study day 28 sample obtained from the subject will be included in the per-protocol analyses of immunogenicity of rZIKV/D4Δ30-713 /placebo. She will also be followed

until completion of her pregnancy. Current evidence does not suggest any risk to the fetus should a woman become pregnant after clearing infectious ZIKV from the blood. For this reason, the vaccine should not pose any risk to the fetus should a subject become pregnant after clearing infectious vaccine virus from the blood (presumed to be approximately day 16 after post-vaccination).

5.4.2 Lost to Follow-up:

A subject who is not reachable by telephone or other means of communication, and therefore not able to be located, is considered lost to follow-up. A subject may be considered lost to follow-up and withdrawn from the study once three attempts have been made to contact the subject, followed by no response to a certified letter sent to the subject's last known address requesting that the subject contact the clinical site.

5.4.3 Incarceration:

If a subject becomes incarcerated during the course of the study, he/she may be terminated from the study if his/her period of incarceration will make him/her unable to make scheduled visits.

5.5 Access to Medical Records

The medical history of a subject will be obtained from the subject and recorded. Medical records from an outside institution will not be requested unless there is a need to clarify the subject's medical history. Medical records from an outside institution will not be requested without the medical release form signed by the subject.

6 TEST ARTICLE PREPARATION

6.1 Pre-inoculation Preparation

rZIKV/D4Δ30-713 vaccine virus for this protocol will be stored at a NIAID-contracted repository until requested by the clinical site. Vials of frozen test agent for administration will be formally requested for transfer to the clinical site by the PI/designee after institutional review board (IRB) approval for the study has been granted and the Food and Drug Administration (FDA) has been in receipt of the protocol for at least 30 days without issuing a clinical hold. The test agent may be transferred to the study site prior to IRB approval and FDA review only for the purpose of determining the titer of the test agent.

After transfer to the clinical site, the vaccine virus will be stored in a locked freezer at -75°C ± 15°C until time of use. The vaccine virus is supplied as a concentrate that must be diluted with PlasmaLyte A, pH 7.4. PlasmaLyte is a sterile, nonpyrogenic isotonic intravenous solution for injection Type 1, USP to the proper dose prior to administration. rZIKV/D4Δ30-713 will be diluted to a final concentration $10^{3.3}$ PFU/mL (2000 PFU/mL) or $10^{4.3}$ PFU/mL (20,000 PFU/mL). A single 0.5 mL dose will be administered subcutaneously such that each subject will receive a dose 10^3 PFU (1,000 PFU) or 10^4 PFU (10,000 PFU).

After the test agent has been prepared at the clinical site, an aliquot of thawed, undiluted (if available), and diluted test agent will be titrated. Additionally, the test agent may be titrated periodically to ensure potency. Clinical site pharmacy/lab personnel who are not blinded to treatment assignment will be responsible for preparing the vaccine and placebo. The vaccine and placebo will be prepared as site-of-injection formulations. Prior to inoculation, the PI/designee

will supply a prescription request form to the study staff responsible for test agent preparation that will include the protocol number, date of inoculation, dose, route of administration, test agent name, test agent lot number, test agent titer (concentration), investigational new drug (IND) number, and number of doses to be administered.

The vaccine and placebo will be prepared according to the site's standard operating procedures. rZIKV/D4Δ30-713 will be administered at a dose of 10^3 PFU or 10^4 PFU subcutaneously. Study staff will prepare the correct dose of vaccine (or placebo) for each subject in a biosafety hood using aseptic technique. Test agents will be diluted with PlasmaLyte A, pH 7.4 intravenous solution, USP. The diluted vaccine and placebo will be drawn up to a volume of 0.5 mL in a 1-mL syringe and labeled according to standard operating procedures. The labeled syringes will be transported at room temperature or on wet ice to the clinic for administration. Test agent must be used within 6 hours of being removed from the freezer or refrigerator. Placebo for this study will be the vaccine diluent, PlasmaLyte A, pH 7.4.

6.2 ZIKV Vaccine and Diluent Storage

The rZIKV/D4Δ30-713 vaccine virus should remain frozen at -75°C ± 15°C until just prior to use. Test agent should never be refrozen for reuse in test article preparation. PlasmaLyte will be stored per the manufacturer's recommendation. Vaccine virus and diluent component should be opened from new containers for each use. No component should be reused for future vaccine or placebo preparation.

Vaccine cannot be used until it is verified by the sponsor as acceptable for use. If the sponsor deems any study agent unacceptable for use, it will be quarantined until further directions from the sponsor are received. If a study agent is deemed unacceptable for use, it will be disposed of per the sponsor's instruction and a new shipment will be requested.

6.3 ZIKV Vaccine and Diluent Accountability

The unblinded dispenser personnel will maintain an accurate inventory and accountability record of each study agent for this study. Partially used vials of study agent or placebo components will not be refrozen or reused for future inoculations.

6.4 Storage Disposition of Used/Unused Supplies

After the unblinded dispenser personnel have diluted the study agent and drawn up the syringes for administration, they will remove the label from the test agent vial(s) and place the label on the test agent preparation form. In this manner, monitoring personnel will be able to verify the accountability of all test agent vials used for the study. In addition, the number of test agent vials used will be accounted for in the study-specific drug accountability log.

An aliquot of undiluted (if available) and diluted test agents will be titrated by CIR laboratory personnel after the test agent has been prepared and delivered to the clinical staff. This is done to confirm the potency of the test agent administered to the subjects.

At least 1 aliquot (if available) of diluted test agent will also be frozen and stored at -75°C ± 15°C at the clinical site for future re-titration if needed. After the syringes have been dispensed and aliquots removed for titration, any remaining test agent will be destroyed by the

pharmacy/laboratory personnel per standard operating procedures. Any unused diluent/placebo from opened vials/bottles will also be destroyed.

7 STUDY PROCEDURES

The following sections provide a detailed listing of the procedures and studies to be performed in this protocol at designated time points.

7.1 Recruitment and General Screening

Subjects may be recruited from a variety of sources including, but not limited to, subjects previously enrolled in vaccine trials at the clinical sites, the use of a center-wide IRB-approved screening protocol, and/or by the use of study-specific IRB-approved print, electronic and/or other media advertising.

A screening visit may be scheduled after an initial phone screen by clinic staff using an IRB-approved Pre-Screening Assessment form. The initial phone screen focuses on providing background information of the trial and a review of basic inclusion and exclusion criteria.

During the screening process, which may require more than one visit, the subject will read the consent form, be encouraged to ask questions, and then complete a comprehension assessment. Study staff will review and discuss the answers from the assessment to identify those areas of the informed consent form that need further review with the subject. This procedure will help ensure that the subject has sufficient understanding of the study before the consent form is signed. The subject may either sign the consent form during the screening visit or return after further consideration. The subject will have a medical history review, pregnancy prevention counseling (if female), physical exam and lab specimens drawn during the general screening process.

7.2 Screening Procedures

Subjects will undergo the following screening procedures within 60 days of inoculation:

1. Screening questions for COVID-19.

2. Explain the study and Informed Consent to the subject.

3. Ensure that the subject has successfully completed the Informed Consent comprehension assessment, has signed the Informed Consent, and receives a copy of the signed Informed Consent.

4. Within 14 days of inoculation, ensure that HIV pre-test counseling has been performed and ensure that the subject has agreed to HIV testing (required by MD state law only).

5. Elicit a complete medical history, including menstrual, sexual, and contraceptive history and history of surgical sterilization for all subjects.

6. Perform a complete physical examination, including vital signs (height, weight, blood pressure, respiratory rate, heart rate, and temperature).

7. Obtain approximately 40 mL of blood for the following laboratory screening tests:

   - Prothrombin time/partial thromboplastin time (PT/PTT)
   - Complete blood count (CBC) with differential
   - Alanine aminotransferase (ALT)
   - Aspartate aminotransferase (AST)
   - Total bilirubin
   - Previous flavivirus infection
   - Creatinine
   - Alkaline phosphatase
   - HBV
   - HCV

8. Obtain approximately 5 mL of blood for HIV testing (will be performed within 14 days of inoculation). Alternatively, a rapid HIV test may be used if a Clinical Laboratory Improvement Amendments (CLIA) waiver has been granted to the site.

9. Nasopharyngeal (NP) or mid-turbinate swab to test for SARS-CoV 2 – the virus that causes COVID-19 – will be collected from all volunteers regardless of vaccination status based on guidance from federal, state, local, and/or institutional health officials* or at the discretion of the clinician (may be collected up to 72 hours in advance of stated collection time).

*This guidance may be informed by the local COVID infection rate, severity of infections, vaccination rates, and other factors as the COVID-19 pandemic continues to evolve and change.

10. Obtain urine for urinalysis by dipstick testing (glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, leukocyte esterase).

11. Obtain serum for β-HCG testing in females. A positive β-HCG will exclude the subject from the trial. Obtain FSH for females who have not menstruated for greater than 12 months but less than 24 months.

12. If necessary, obtain medical release form to confirm method of birth control.

13. Counsel all female subjects to avoid becoming pregnant during the study and on pregnancy prevention during the entire study period.

14. Counsel all male subjects to use barrier contraception for the three months following vaccination.

7.3 Inoculation Procedure

Subjects will receive the LA Zika vaccine rZIKV/D4Δ30-713 or placebo on Study Day 0. The vaccine will be kept frozen at -75°C ± 15°C until just before use, whereupon it will be thawed, diluted to the appropriate dose, and drawn up for administration (see Section 6 for test article preparation). The rZIKV/D4Δ30-713 vaccine will be kept on wet ice from the time it is thawed until it is diluted with PlasmaLyte into its final formulation. The vaccine and placebo (PlasmaLyte) will then be delivered at room temperature or on wet ice to clinical staff for administration. A volume of 0.5 mL of test article will be delivered by subcutaneous injection in the deltoid region of the upper arm with a needle of appropriate gauge and length after wiping the injection site with alcohol.

7.4 Detailed Study Procedures

The study procedures to be performed at each visit are listed below. Additional tests may be done at the discretion of the PI/provider to evaluate concomitant illness or further evaluate an adverse event (AE) experienced by a subject. Photographs may be taken of the injection site. In addition, photographs may be taken of other areas of the skin before and/or after inoculation to record the characteristics of any rash that may develop. Possible identifiers, such as tattoos, jewelry, and clothing will not be included in the photographs, to maintain confidentiality. The total volume of blood to be drawn over the 26-week post-vaccination period is approximately 610 mL. This amount is within NIH guidelines (Medical Administrative Policy 95-9) for adult blood donation and should not compromise the health of study subjects. A topical anesthetic cream may be used during blood draw at the phlebotomist's discretion to ease the discomfort of the procedure.

7.4.1 Inoculation and Follow-up

On the day of inoculation with test article, more subjects may be invited to the clinic than will be enrolled. These subjects will be alternates and will be inoculated only if other subjects are not available for inoculation or are found to be ineligible on the day of inoculation. Those subjects who are alternates will be informed that they are alternates when they are invited to the clinic. For study visits with overlapping windows for the visit, subjects will be scheduled so that 2 study visits for an individual do NOT occur on the same day. All subjects invited to the clinic will have to abide by the facility's masking policies and COVID-19 related procedures. At each visit, volunteers will be asked whether or not they have experienced any COVID-19 symptoms (per facility screening guidelines) or if they have had an exposure to someone with COVID-19 prior to coming into the clinic. If the volunteer reports symptoms consistent with COVID-19 and they are already enrolled in the study, an NP or mid-turbinate swab will be collected and tested for COVID-19 on site or at the respective local laboratory. If this is unavailable, the volunteer will be referred for local testing. The volunteer will not come into the clinic until the result is confirmed to be negative (~45 minutes) or per current facility guidelines for COVID-19 screening procedures. Should a volunteer test positive for SARS-CoV-2 at any point during the study, the volunteer will be asked to quarantine according to current CDC guidelines. We will review current CDC guidelines with the volunteer. We will defer all in-person visits until after the volunteer has completed their quarantine or if a COVID-19 positive research space is available they will be brought to that location. If they test positive for SARS-CoV-2 during the first 21-days post-vaccination, scheduled follow-up visits will be done as telehealth visits or at a research space designated for COVID-19 positive patients. Based on COVID-19 testing and

results availability, NP or mid-turbinate swab may be collected up 72 hours prior to scheduled testing visit.

Study Day 0 (Day of Inoculation with Test Article)

1. Screening questions for COVID-19.
2. Verify that Informed Consent was obtained and that the consent form was signed by both the subject and by the study staff.
3. Verify that all applicable eligibility criteria have been met.
4. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints. Focused physical exam will include BP, HR, temperature, respiratory rate, skin examination, eyes, throat, muscle strength, and biceps and patellar deep tendon reflexes. Physical exam may extend beyond these if indicated by complaint or physical finding.
5. Review and document concomitant medications.
6. Nasopharyngeal (NP) or mid-turbinate swab to test for SARS-CoV 2 – the virus that causes COVID-19 – will be collected based on guidance from federal, state, local, and/or institutional health officials* or at the discretion of the clinician *(may be obtained up to 72 hours in advance of study day)*.

*This guidance may be informed by the local COVID infection rate, severity of infections, vaccination rates, and other factors as the COVID-19 pandemic continues to evolve and change.

7. .Obtain approximately 70 mL of blood for CBC with differential, ALT, creatinine, virus isolation and culture (PCR/culture), and immunology (PBMCs, plasma, and PAXGene). These laboratory studies are drawn as baseline values and will not determine eligibility.
8. Obtain 5 mL of clean-catch urine for virus isolation & culture.
9. Obtain vaginal secretion sample (women) for virus isolation and culture (may be collected at home by the volunteer).
10. Obtain semen sample (men) for virus isolation and culture (may be collected at home by the volunteer).
11. Obtain oral fluid for serology (CIR site only).
12. Perform serum or urine β-HCG testing for female subjects. Ensure the test is negative before proceeding; a positive test will exclude the subject from the study as per protocol, Section 5.2.
13. Review pregnancy prevention.
14. Record vital signs (blood pressure, temperature, heart rate, and respiratory rate).
15. Administer the test article.
16. Observe for at least 30 minutes after inoculation and evaluate for immediate hypersensitivity.

17. Provide education by study staff describing proper use of thermometer, the signs and symptoms of potential AEs, and how and when to contact study staff (see Section 7.5.1).

Study Day 4 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Obtain approximately 40 mL of blood for CBC with differential, virology, and immunology (PBMCs, plasma, and PAXGene).
5. Obtain 5 mL of clean-catch urine for virus isolation & culture.
6. Review pregnancy prevention.

Study Day 6 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Obtain approximately 20 mL of blood for CBC with differential, ALT, virology, and PAXGene.
5. Obtain 5 mL of clean-catch urine for virus isolation & culture.
6. Review pregnancy prevention.

Study Day 8 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Nasopharyngeal (NP) or mid-turbinate swab to test for SARS-CoV 2 – the virus that causes COVID-19 – will be collected based on guidance from federal, state, local, and/or institutional health officials* or at the discretion of the clinician *(may be obtained up to 72 hours in advance of study day).*
5. Obtain approximately 50 mL of blood for CBC with differential, virology, and immunology (PBMCs, plasma, and PAXGene).
6. Obtain urine sample for virus isolation and culture.
7. Obtain vaginal secretion sample (women) for virus isolation and culture. Vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic.
8. Obtain semen sample (men) for virus isolation and culture, may be collected at home by the volunteer prior to presentation to the clinic.
9. Obtain oral fluid for serology (CIR subjects only).
10. Review pregnancy prevention.

*This guidance may be informed by the local COVID infection rate, severity of infections, vaccination rates, and other factors as the COVID-19 pandemic continues to evolve and change.

Study Day 10 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Obtain approximately 20 mL of blood for CBC with differential, virology, and PAXGene.
5. Obtain 5 mL of clean-catch urine for virus isolation & culture.
6. Review pregnancy prevention.

Study Day 12 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Obtain approximately 20 mL of blood for CBC with differential, virology, and PAXGene.
5. Obtain urine sample for virus isolation and culture.
6. Obtain oral fluid for serology (CIR subjects only).
7. Review pregnancy prevention.

Study Day 14 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Review pregnancy prevention with the subject.
5. Nasopharyngeal (NP) or mid-turbinate swab to test for SARS-CoV 2 – the virus that causes COVID-19 – will be collected based on guidance from federal, state, local, and/or institutional health officials* or at the discretion of the clinician *(may be obtained up to 72 hours in advance of study day).*

*This guidance may be informed by the local COVID infection rate, severity of infections, vaccination rates, and other factors as the COVID-19 pandemic continues to evolve and change.

6. Obtain approximately 50 mL of blood for CBC with differential, ALT, creatinine, virology, and immunology (PBMCs, plasma, and PAXGene).
7. Obtain 5 mL of clean-catch urine for virus isolation & culture.

Study Day 16 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Obtain approximately 20 mL of blood for CBC with differential, virology, and PAXGene.
5. Obtain urine sample for virus isolation and culture.
6. Obtain vaginal secretion sample (women) for virus isolation and culture. Vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic.
7. Obtain semen sample (men) for virus isolation and culture, which may be collected at home by the volunteer prior to presentation to the clinic.
8. Obtain oral fluid for serology (CIR subjects only).
9. Review pregnancy prevention.

Study Day 21 (± 1 day)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Review pregnancy prevention with the subject.
5. Obtain approximately 40 mL of blood for immunology (PBMCs, serum, plasma, PAXGene), and virology*. CBC and/or ALT or creatinine will be done if prior white blood cell (WBC), ANC, platelet count, hemoglobin, or ALT or creatinine results are Grade I or above and unresolved, as defined by protocol.
6. For females, perform β-HCG testing. A positive test will exclude the subject from the per-protocol analysis, as per Section 5.
7. Obtain urine sample for virus isolation and culture.*

   *Virology will be done on Study Day 21 only if virus was recovered from the sample drawn at the previous time-point (Day 16)

Study Day 28 (± 2 days)

1. Screening questions for COVID-19.
2. Perform interim history and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Obtain approximately 50 mL of blood for immunology (PBMCs, serum, plasma, PAXGene and virology*.
5. For females, perform β-HCG testing. A positive test will exclude the subject from the per-protocol analysis, as per Section 5.
6. Review pregnancy prevention with the subject.
7. Obtain urine sample for virus isolation and culture.*
8. Obtain vaginal secretion sample (women) for virus isolation and culture.* Vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic.

9. Obtain semen sample (men) for virus isolation and culture, which may be collected at home by the volunteer prior to presentation to the clinic.*
10. Obtain oral fluid for serology (CIR site only).

* Virology will be done on Study Day 28 only if virus was recovered from the sample drawn at the previous time-point (Day 21)

Study Day 56 (+ 14 days)

1. Screening questions for COVID-19.
2. Perform interim history for interim complaints (physical exam is optional – will be done should subject have any medically-attended AEs since last visit).
3. Record vital signs.
4. Obtain approximately 50 mL of blood for virology* and immunology (PBMCs, serum, and plasma).
5. For females, perform β-HCG testing.
6. Review pregnancy prevention with the subject.
7. Obtain urine sample for virus isolation and culture.*
8. Obtain vaginal secretion sample (women) for virus isolation and culture.* Vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic.
9. Obtain semen sample (men) for virus isolation and culture, which may be collected at home by the volunteer prior to presentation to the clinic.*
10. Obtain oral fluid for serology (CIR site only).

*Virology will be done on Study Day 56 only if virus was recovered from the sample collected at the previous time-point (Day 28).

Study Day 90 (+ 10 days)

1. Screening questions for COVID-19.
2. Perform interim history for interim complaints (physical exam is optional - will be done should subject have any medically-attended AEs since last visit).
3. Record vital signs.
4. Obtain approximately 40 mL of blood for virology* and immunology (PBMCs, serum, and plasma).
5. For females, perform β-HCG testing.
6. Review pregnancy prevention with the subject.
7. Obtain urine sample for virus isolation and culture.*
8. Obtain vaginal secretion sample (women) for virus isolation and culture. Vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic.*
9. Obtain semen sample (men) for virus isolation and culture, which may be collected at home by the volunteer prior to presentation to the clinic.*
10. Obtain oral fluid for serology (CIR site only).

*Virology will be done on Study Day 90 only if virus was recovered from the sample collected at the previous time-point (Day 56).

Study Day 150 (+ 14 days)

1. Screening questions for COVID-19.
2. Perform interim history for interim complaints (physical exam is optional- will be done should subject have any medically-attended AEs since last visit or at the discretion of the investigator).
3. Record vital signs.
4. Obtain approximately 50 mL of blood for virology* and immunology (PBMCs, serum, and plasma).
5. For females, perform β-HCG testing.
6. Review pregnancy prevention with the subject.
7. Obtain urine sample for virus isolation and culture.*
8. Obtain vaginal secretion sample (women) for virus isolation and culture. Vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic.*
9. Obtain semen sample (men) for virus isolation and culture, may be collected at home by the volunteer prior to presentation to the clinic.*
10. Obtain oral fluid for serology (CIR site only).

*Collection of urine, vaginal secretion, and semen samples and virology (urine, vaginal secretion, semen, blood) will be done on Study Day 150 only if virus was recovered from the sample collected at the previous time-point (Day 90) or if virology results are not available.

Study Day 180 (+ 28 / - 14 days)

1. Screening questions for COVID-19.
2. Perform interim history for interim complaints and focused physical exam, concentrating on any acute complaints.
3. Record vital signs.
4. Obtain approximately 50 mL of blood for immunology (PBMCs, serum, and plasma).
5. For females, perform β-HCG testing.
6. Obtain urine sample for virus isolation and culture.*
7. Obtain vaginal secretion sample (women) for virus isolation and culture. Vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic.*
8. Obtain semen sample (men) for virus isolation and culture, may be collected at home by the volunteer prior to presentation to the clinic. *
9. Obtain oral fluid for serology (CIR site only).

*Collection of urine, vaginal secretion, and semen samples and virology (urine, vaginal secretion, semen) will be done on Study Day 180 only if virus was recovered from the sample collected at the previous time-point (Day 150) or if virology results are not available.

7.4.1.1 Persistence of Zika Vaccine in Bodily Fluids

If Zika vaccine is detected in any bodily fluid at Study Day 180, the subject will continue to be followed until Zika vaccine is no longer detected in the bodily fluid. To achieve this, the subject

will be brought back for unscheduled visits for collection of the specimen of interest (the bodily fluid that was positive for Zika vaccine at Study Day 180) and for review of clinical symptoms. If clinical symptoms are reported, the subject will undergo clinical evaluation. Persistent shedding of Zika vaccine in bodily fluids at Study Day 180 will be reported to the DSMB and the FDA as soon as it is detected. The DSMB and the FDA will be asked to provide additional guidance as to follow-up procedures, the interval of specimen collection, and the duration of specimen collection.

7.5 Subject Temperature Memory Card

Subjects will be provided with a thermometer and a Temperature Memory Card to use as a memory aid and will be asked to record temperatures 2 times a day from study day 0 through study day 16 post-inoculation. Study staff will review the subjects' temperature cards on study day visits 4 through 21 to assess for any fevers and compare subject recordings to the study visit temperatures. Staff will record all temperatures recorded by the subject and will also separately record the maximum temperature for each date (T_{max}) on the source document. Subject memory cards will not be collected by the study staff as subject's recordings will not be considered required data.

Staff will instruct subjects on how to use the thermometers, to take their temperatures at approximately the same time each day, and to take additional temperatures if they feel they have elevated temperatures. Subjects will be asked to wait at least 15 minutes after eating, drinking, and smoking before taking their temperatures. They will be asked to confirm an elevated temperature (≥ 100.4°F) by retaking the temperature after a 20-minute interval and at 1 hour. Temperatures not documented to last at least 1 hour will not be considered an AE or included in the analysis.

7.5.1 Notification of Staff of Zika-like Symptoms

Subjects will be asked to call or otherwise contact the study staff should they experience any symptoms of Zika illness. The Zika-like illness symptoms that study staff will review with the subjects are the systemic reactogenicity signs included in Table 9. Once notified, study staff will arrange to evaluate the subject at the earliest convenience; this may result in an unscheduled visit for the subject.

7.5.2 Notification of Staff of COVID-19-like symptoms

Subjects will be asked to call or otherwise contact study staff should they experience any COVID-19 symptoms. In addition, at each visit, volunteers will be asked whether or not they have experienced any COVID-19 symptoms (per facility guidelines) or if they have had an exposure to someone with COVID-19 prior to coming into the clinic. If the volunteer reports symptoms consistent with COVID-19 and they are already enrolled in the study, an NP or mid-turbinate swab will be collected and tested for COVID-19 on site or at a local laboratory at any time. The volunteer will not come into the clinic until the result is confirmed to be negative. Should a volunteer test positive for SARS-CoV-2 at any point during the study, the volunteer will be asked to quarantine according to current CDC guidelines. We will review current CDC guidelines with the volunteer. We will defer all in-person visits until after the volunteer has completed their quarantine or see them in a COVID-19 positive research space if it is available. If they test positive for SARS-CoV-2 during the first 21-days post-vaccination, scheduled follow-up visits will be done as telehealth visits or in a COVID-19 positive research space.

7.6 Clinical Laboratory Testing

Using standard techniques, Quest Diagnostics or other CLIA-certified laboratories will perform the following tests:

1. CBC plus WBC differential
2. PT/PTT
3. ALT, AST, alkaline phosphatase, total bilirubin, creatinine
4. HIV assay (screening antibody assay with confirmation for positive antibody assays).
5. Hepatitis B screening by testing for HBsAg
6. Hepatitis C screening by testing for hepatitis C antibody. If positive, the test is confirmed by testing for hepatitis C RNA. If hepatitis C antibody is positive but RNA is negative, the subject is assessed as having a past infection that has cleared. In this case, the subject is eligible for the study.
7. Urinalysis (in the event of an abnormal urine dipstick test)
8. SARS-CoV-2 PCR testing
9. Serum β-HCG, if required

Urine and serum β-HCG testing will be performed at the clinical trial site using an FDA-approved pregnancy test kit. Urine dipstick testing will be performed at the clinical trial site using an FDA-approved product. Rapid HIV testing may be performed at the clinical trial site using an FDA-approved test kit. Determination of vaccine virus titer, plaque reduction neutralization antibody assays, and cellular immune studies will be done at the clinical trial site laboratory.

7.7 Medical History and Concomitant Medications

A complete medical history will be collected during screening. Any changes reported in medical history during the 28-day post-inoculation period will be assessed as possible AEs. After study day 28, the medical history will be updated for any new or changed significant or chronic conditions, and to identify if any medically attended AEs have occurred.

Study staff will collect current medications as part of the medical history, including over-the-counter medications and herbal supplements, at the time of enrollment. All changes or updates to medications will be collected through study day 28 following inoculation. After study day 28, concomitant medications will be collected to identify new or changed significant or chronic conditions. Medications taken for AEs continuing after study day 28 will be recorded throughout the trial.

7.8 Immunology Testing

7.8.1 Antibody Testing

Serum antibody levels to ZIKV will be measured by plaque reduction neutralizing antibody assay using standard laboratory protocols. The $PRNT_{50}$ is defined as the highest dilution of antibody that reduces the number of foci or plaques by 50%, compared to the plaque titer of the virus alone.

7.8.2 Other Immunological Assays

Cytokine, T-cell and B-cell stimulation assays, and PBMC phenotyping may be performed on PBMCs. RNA may be isolated from cells and run on microarrays to identify immune pathways of interest, such as defining the innate immune response to the vaccine virus. In addition, human leukocyte antigen (HLA) typing of samples may be performed as part of assays to map the Zika virus epitopes that induce T- or B-cell responses. In addition, an exploratory neutralization capacity assay may be performed using target cells that express CD32a (FcγRIIa). This assay will evaluate the effect of neutralizing and enhancing antibody in the same assay. ZIKV specific antibodies will be measured in oral fluids as an exploratory assay for detection of Zika.

7.9 Retention of Study Subjects

We will employ several strategies aimed at retaining subjects through study completion. During screening, we will obtain detailed primary locator information, as well as secondary contact information. Subjects will also provide information for people who may be contacted if primary and secondary means of contact fail. Locator information will be reviewed with subjects at each visit (i.e., addresses, phone numbers, email addresses). In addition, birthday cards/holiday cards may be mailed to check addresses, and reminders may be sent using various methods (including, but not limited to, phone, email, text messaging, electronic media, and postal mail). All data will be maintained and updated in a password-protected locator database.

8 ADVERSE EVENT MONITORING

8.1 Definitions

8.1.1 Adverse Event

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not it is considered related to the research.

All AEs will be evaluated for severity, action taken, seriousness, outcome, and relationship to the investigational vaccine as described in Section 8.2 in this protocol. If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE. AEs will be collected through the 28-day period following inoculation, and any test agent–related AEs (adverse reactions, see below) identified in the 28-day post-inoculation period will be followed until resolution.

8.1.2 Medically-attended Adverse Event

A medically attended AE is defined as any visit (other than hospitalization, visit for routine health maintenance, or visit for a pre-existing condition that has not worsened) to an emergency room (ER), urgent care facility, doctor, nurse, or any other health care provider. Medically attended AEs from days 29 through 180 will be collected. Medically attended AEs that are found to be unrelated to the study product will be followed until resolution or the end of the study, whichever occurs first.

8.1.3 Adverse Reaction

An adverse reaction (AR) is an AE that is caused by an investigational agent (drug or biologic). AEs that are deemed possibly, probably, or definitely related to study article will be classified as adverse reactions. ARs are categorized as solicited ARs and other ARs. Solicited ARs include local reactogenicity, systemic reactogenicity, and laboratory events. Solicited ARs are those events that the clinician is specifically evaluating during the 28-day post-inoculation period, listed in Table 9. All AEs are evaluated using the Adverse Event Grading Table in Section 14.1, Appendix 1**.**

Table 9: Solicited Adverse Events/Adverse Reactions

Systemic Reactogenicity	Laboratory Events	Local Reactogenicity
Fever	Decreased Hemoglobin	Injection Site Pain
Headache	Neutropenia	Injection Site Erythema
Retro-orbital Pain (ROP)	Elevated ALT	Injection Site Tenderness
Photophobia	Thrombocytopenia	Injection Site Induration
Nausea	Leukocytosis	Injection Site Pruritus
Fatigue		
Myalgia		
Arthralgia		
Non-purulent Conjunctivitis		
Muscle Weakness		
Zika Virus-Like Rash		

8.1.4 Adverse Event of Special Interest

Guillain-Barré syndrome (GBS) is an AE of special interest. Subjects will be examined for signs of muscular weakness at each visit that includes a physical exam and muscle strength will be reported as in Section 8.2.2. Should a subject present to an outside physician for evaluation of GBS, this will be captured and reported. The Brighton Criteria for diagnosis of GBS will be used (Section 14.2, Appendix 2) and the WHO Guillain-Barré Syndrome Disability Scale will be used (Section 14.3, Appendix 3).

8.1.5 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be serious adverse events (SAEs). Events that meet SAE criteria during pregnancy, delivery, or in the neonate (e.g., congenital anomaly/birth defect) are reportable to the Clinical Safety Office (CSO) per the sponsor's reporting guidelines. Pertinent obstetrical information for all

pregnancies will be reported to the CSO via the REDCap reporting system within 1 business day from site awareness of the pregnancy on the Pregnancy Notification and Outcome Form.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness on a protocol-specified form. If a subject becomes pregnant within 28 days of test article administration, the subject will be unblinded to determine if she received ZIKV. If she did receive ZIKV, she will be referred to an OB/GYN experienced in caring for ZIKV-infected pregnant women (Dr. Jeanne Sheffield at JHU).

If the pregnancy occurs prior to study day 28 after rZIKV/D4Δ30-713 administration, as determined by a positive pregnancy test at the study day 28 visit or before, the subject will not be included in the per-protocol analysis. The subject will remain on the study for safety follow-up.

- If unblinding the treatment assignment would help the subject determine whether she wishes to continue her pregnancy, her treatment assignment will be unblinded.
- The research subject will be advised to notify her obstetrician of study participation and study agent exposure.
- The pregnancy will be reported to the DSMB (if applicable) and/or IRB (if applicable).

If the pregnancy occurs after study day 28 following rZIKV/D4Δ30-713 administration, as determined by a positive pregnancy test after the study day 28 visit, the subject will be included in the per-protocol immunogenicity analysis.

8.1.6 Serious Adverse Event

A serious adverse event (SAE) is an AE that is determined to be "serious," whether considered related to the investigational vaccine or not. SAEs will be collected for the duration of the trial. An SAE results in one or more of the following outcomes:

- Death during the period of protocol-defined surveillance
- A life-threatening event, defined as an event that places a subject at immediate risk of death at the time of the event and does not refer to an event that hypothetically might have caused death were it more severe
- Inpatient hospitalization or prolongation of existing hospitalization, defined as at least an overnight stay in the hospital or emergency room for treatment that would have been inappropriate if administered in the outpatient setting
- Congenital anomaly or birth defect
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Other medically important events*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately

life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed above. These will also usually be considered serious.

Each AE will be classified by the investigator/designee as serious or non-serious. An SAE needs to meet only one or more of the above criteria to be considered serious.

8.1.7 Unexpected Adverse Events

An AE is considered unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. "Expected" does not mean that the event is expected with pharmacologically similar drugs, the underlying disease(s), or concomitant medications. It is the responsibility of the IND sponsor to make this determination.

8.1.8 Serious and Unexpected Suspected Adverse Reaction (SUSAR)

A serious and unexpected suspected adverse reaction (SUSAR) is a suspected adverse reaction (SAR) that is serious and unexpected.

8.1.9 Unanticipated Problem

An unanticipated problem is any event, incident, experience, or outcome that is:

1. Unexpected in terms of nature, severity, or frequency in relation to
    a. The research including but not limited to risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
    b. The characteristics of the subject population being studied;
2. Possibly, probably, or definitely related to participation in the research; and
3. Places subjects or others at a greater risk of harm (including physical, psychological, economic, or social) than was previously known or recognized. (An AE with a serious outcome will be considered increased risk).

8.1.10 Unanticipated Problem that is not an Adverse Event (UPnonAE)

This is an unanticipated problem that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered non-serious unanticipated problems. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

8.1.11 Protocol Deviation

A protocol deviation is any change, divergence, or departure from the study design or procedures in an IRB-approved research protocol that has an impact on the subject's rights, safety, or well-being, and/or the completeness, accuracy, or reliability of the study data. Protocol deviations are designated as serious or non-serious and further characterized as:

1. Those that occur because a member of the research team deviates from the protocol

2. Those that are identified before they occur, but cannot be prevented
3. Those that are discovered after they occur

8.1.12 Serious Protocol Deviation

A serious protocol deviation is any change, divergence, or departure from the study design or procedures in an IRB-approved research protocol that meets the definition of a Serious Adverse Event or compromises the subject's rights, safety, or well-being, and/or the completeness, accuracy, or reliability of the study data.

Non-compliance is the failure to comply with applicable NIH Human Research Protection Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as

1. Serious: Non-compliance that:
    a. Increases risks or causes harm to subjects
    b. Decreases potential benefits to subjects
    c. Compromises the integrity of the NIH HRPP
    d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that is neither serious nor continuing

8.1.13 Pre-existing Conditions, Worsening of Pre-existing Condition

Stable chronic conditions that are present prior to enrollment and do not worsen are not considered AEs and will be accounted for in the subject's medical history. Exacerbation or worsening of pre-existing conditions are defined as AEs and are evaluated using the same criteria described in Section 8.2 of this protocol.

8.2 Assessment of Adverse Events

8.2.1 Identification of Adverse Events

All AEs occurring from the time the subject is enrolled (at administration of investigational agent on day 0) through the Study Day 28 will be documented, recorded, and reported. The Investigator will assess all AEs with respect to **Seriousness**, **Severity**, and **Causality** (relationship to study agent and relationship to research) according to the following guidelines. An assessment of safety will include clinical observations and monitoring of hematological, blood chemistry, and immunologic parameters. Safety will be evaluated by monitoring the subjects for local and systemic adverse events during the course of the trial. Subjects will be closely monitored for 30 minutes following inoculation. Additionally, subjects will return to the clinic on study days 4, 6, 8, 10, 12, 14, 16, 21, and 28 post-inoculation and may be asked to return more often if warranted. It is during this time period that we anticipate AEs related to infection with the vaccine virus to manifest themselves; therefore, the subjects will be seen frequently. Study staff will review subjects' reported temperatures and clinical temperatures for the first 16 days following inoculation to assess for AEs. At each visit through study day 28 following inoculation, subjects will be queried about adverse events, including possible vaccine-related AEs (solicited AEs) and will have a focused physical exam performed. A study clinician will be available to subjects by telephone or pager 24 hours a day during the study evaluation period.

All AEs will be recorded during the period after the subject receives the study vaccine through and including post-inoculation study day 28.

All SAEs will be assessed appropriately as AEs and reported following SAE reporting guidelines outlined in Section 8.3.2 of this protocol.

8.2.2 Protocol Specific Adverse Event Definitions

Fever: Temperature ≥ 100.4°F lasting at least 1 hour
ZIKV-like Rash: Areas of **confluent** macular or maculo-papular rash over trunk and extremities, blanches when compressed.
Non-purulent conjunctivitis: Bilateral reddening of the sclera of the eyes without evidence of exudate or crusting of the eyelids
Headache: A pain located in the head, over the eyes, at the temples, or at the base of the skull.
ROP: Bilateral pain situated behind the orbits of the eye
Photophobia: An abnormal sensitivity or intolerance to light
Nausea: Discomfort in the stomach with an urge to vomit
Fatigue: Excessive tiredness following minimal exertion
Myalgia: Pain in the muscles that is found in ≥ 2 muscle groups
Arthralgia: Pain in a joint that is found in ≥ 2 joints.
Muscle Weakness: Grade 0 - 3 out of 5 on the Oxford Scale. The Oxford Scale grading is as follows:

- **0/5**: No contraction
- **1/5**: Visible/palpable muscle contraction but no movement
- **2/5**: Movement with gravity eliminated
- **3/5**: Movement against gravity only
- **4/5**: Movement against gravity with some resistance
- **5/5**: Movement against gravity with full resistance

8.2.3 Determination of Severity

The investigator/designee will assess all AE severity using the following classifications.

Table 10: Severity Definitions

Severity	Defined
Grade 1 (Mild)	An event that is easily tolerated and may require 1 dose of medication/treatment
Grade 2 (Moderate)	An event that interferes with daily activity or requires > 1 dose of medication/treatment
Grade 3 (Severe)	An event that prevents daily activity and requires medical intervention
Grade 4 (Life-threatening)	An adverse event that is deemed by the study clinician, the medical monitor, or an outside clinician caring for the subject to be life-threatening
Grade 5 (Death)	Any adverse event that results in the death of the subject

Solicited AE severity grading classifications are listed in Table 10 and

Table 11. All other AEs will be graded on severity using the Adverse Event Grading Table in Section 14.1, Appendix I.

Table 11: Assessment of Solicited Adverse Events

Local Reactogenicity	Grade	Severity
Injection Site Tenderness, Pruritis, and Pain	1	An event that is easily tolerated and may require 1 dose of medication
	2	An event that interferes with daily activity or requires > 1 dose of medication
	3	An event that prevents daily activity and requires medical intervention
	4	A life-threatening event
Injection Site Induration and Erythema	1	> 0 – 20 mm
	2	> 20 – 50 mm
	3	> 50 mm
	4	Life-threatening
Systemic Reactogenicity	**Grade**	**Severity**
Fever (Oral)	1	100.4°F – 101.4°F
	2	101.5°F – 102.4°F
	3	≥ 102.5°F
	4	Life-threatening
ZIKV-Like Rash	1	A rash that is present but asymptomatic
	2	A rash that is symptomatic (pruritus/pain) but does not interfere with function
	3	A rash that is symptomatic and interferes with function
	4	A rash that is life-threatening
Headache ROP Photophobia Nausea Fatigue Myalgia Arthralgia Conjunctivitis Muscle weakness	1	An event that is easily tolerated and may require 1 dose of medication/treatment
	2	An event that interferes with daily activity or requires > 1 dose of medication/treatment
	3	An event that prevents daily activity and requires medical intervention
	4	A life-threatening event
Solicited Laboratory AEs	**Grade**	**Severity**
Hemoglobin (Women)	1	9.5 – 10.7 gm/dL
	2	8.0 – 9.4 gm/dL
	3	< 7.9 gm/dL
	4	Life-threatening
Hemoglobin (Men)	1	11 – 12.5 gm/dL
	2	9.0 – 10.9 gm/dL
	3	< 8.9 gm/dL

	4	Life-threatening
Neutropenia (Reduced ANC)	1	750 – 999/mm^3
	2	500 – 749/mm^3
	3	< 500 mm^3
	4	Life-threatening
Leukocytosis (Increased WBCs)	1	11,500 – 13,000/mm^3
	2	13,001 – 15,000/mm^3
	3	15,000 or < 1,000/mm^3
	4	Life-threatening
Thrombocytopenia (Decreased Platelets)	1	≥ 100,000 – 120,000/mm^3
	2	≥ 75,000 – 99,999/mm^3
	3	< 74,999/mm^3
	4	Life-threatening
ALT	1	> 1.25 – 2.5 × ULN
	2	> 2.5 – 5.0 × ULN
	3	> 5.0 × ULN
	4	Life-threatening

1. Any adverse event resulting in death will be given a severity grade of 5.

8.2.4 Relationship with Receipt of Test Article

The clinical investigator will assess all AEs for their relationship to the study agent using the following classifications:

Definitely related: Clear-cut temporal association, with no other possible cause

Probably related: Reasonable temporal association, and a potential alternative etiology is not apparent

Possibly related: Less clear temporal association; other etiologies also possible

Unlikely related: Temporal association between the AE and the study agent or the nature of the event is such that the study agent is not likely to have had any reasonable association with the observed illness/event (cause and effect relationship improbable but not impossible)

Unrelated: The AE is completely independent of study agent administration, and/or evidence exists that the event is definitely related to another etiology

The degree of certainty with which an AE can be attributed to study agent administration will be determined by how well the event can be understood in terms of one or more of the following:

- A reaction of a similar nature having previously been observed with this type of vaccine and/or formulation or naturally occurring Zika illness
- The event having often been reported in the literature for similar types of vaccine products

All local injection-site reactions will be considered causally related to inoculation. Causality assessment is based on information available at the time of the AE assessment. The investigator may revise the causality assessment as additional information becomes available.

8.2.5 Adverse Event Action Taken

The investigator/designee will assess the action taken by the subject or the study staff in relation to the AE, using the following classifications:

Action
1 = None
2 = Remedial therapy (more than 1 dose of medication required)
3 = Discontinued study
4 = Hospitalization
5 = Other

8.2.6 Adverse Event Outcome

The investigator/designee will assess the outcome of the AE, either at resolution or the end of the study period, using the following classifications:

Outcome
1 = Resolved
2 = Continuing
3 = Continuing Chronic Condition
4 = Unknown, Off-Study before could confirm resolution of AE
5 = Death
6 = Unknown

8.2.7 Adverse Event Seriousness

The investigator/designee will categorize all AEs as either serious or non-serious using the criteria defined in Section 8.1.6 of this protocol.
Any events defined as serious will also be reported following SAE reporting guidelines outlined in Section 8.3 of this protocol.

8.3 Adverse Event Reporting

8.3.1 Non-Serious Adverse Events

Non-serious AEs will be followed to resolution or until the study ends, and will be reported to the sponsor as requested, to the IRB according to IRB policies, to the DSMB as required, and to the FDA at least annually in the annual report.

AEs meeting the pausing criteria outlined in Section 8.5 of this protocol will be reported to the sponsor following the SAE reporting guidelines. AE data will be submitted to the IND Sponsor when requested for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

8.3.2 Serious Adverse Events

Office of Clinical Research Policy and Regulatory Operations Clinical Safety Office
All SAEs (regardless of relationship and whether or not they are also unanticipated problems) must be reported according to the sponsor's reporting plan by using the REDCap system. Deaths and immediately life-threatening SAEs must be reported to the CSO within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 working days of notification of the SAE occurrence to:

- REDCap website: https://crimsonredcap.cc.nih.gov/redcap/index.php
- OCRPRO CSO Phone: 301-846-5301, Fax: 301-846-6224, E-mail: rchspsafety@mail.nih.gov

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE case report form and according to the sponsor's reporting guidelines.

SAEs that occur after the study follow-up period (study day 180) that are reported to and are assessed by the investigator to be possibly, probably, or definitely related to study drug must be reported to the CSO.

Data and Safety Monitoring Board
All SAEs must be reported by telephone (followed by written report), email, or fax within 1 working day of notification of the SAE occurrence to:

- The DSMB executive secretary – Phone: 301-846-5301, Fax: 301-846-6224, Email: niaiddsmbia@mail.nih.gov

Johns Hopkins University Biosafety Committee
All SAEs must be reported by telephone (followed by written report), email, or fax within 1 working day of notification of the SAE occurrence to:

- The JHU Institutional Biosafety Committee: Phone: 410-955-5918, Fax 410-955-5929

Institutional Review Board Reporting
All SAEs will be reported to WIRB-Copernicus Group, Inc (WCG IRB) and UVM IRB as per guidelines, respectively, for JHU and UVM as below:

- WCG IRB **Guidelines:**
  WCG IRB Phone: 800-562-4789, Fax: 360-252-2498
  www.wcgirb.com for updated reporting guidelines
- **UVM IRB Guidelines:**
  - Investigators are required to report AEs that fit the following criteria:
    - Report timelines are based on of the time the investigator or site personnel becomes aware of them:
      - A local death report within 48 hours
      - All other local AEs should be reported promptly, not to exceed 7 days
  - Local AEs (whether or not they are serious) that are unexpected, and possibly, probably, or definitely related to study participation:
    - Local AE: A negative side effect resulting from the study intervention that occurred to a subject enrolled at UVM or another research site under the jurisdiction of the UVM IRB.
    - Unexpected: An event does not meet the criteria of unexpected if it is (1) included in the current protocol, drug/device brochure, or the informed consent; or (2) due to the subject's underlying disease or predisposing risk factors
    - Related: An AE is considered to be related if there is a reasonable possibility that the event was caused by the protocol or study

interventions. A related event has a strong temporal relationship to the drug, device, or intervention, and an alternative cause is unlikely. If it cannot be determined whether an event is related, it should be reported as "possibly related."

- At UVM, all SAEs must be reported to the UVM IRB according to UVM IRB guidelines.
- UVM IRB Phone: 802-656-5040, Fax: 802-656-5041

8.3.3 Unanticipated Problems

Unanticipated problems that are also AEs must be reported to the CSO and sent sent by using the REDCap system no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the Sponsor CSO. Unanticipated problems that are not AEs are not reported to the sponsor CSO. Report unanticipated problems that are also AEs to the CSO according to the sponsor's reporting guidelines.

8.4 Sponsor's Reporting Responsibilities

SUSARs, as defined in 21 *Code of Federal Regulations* (CFR) 312.32 and determined by the IND sponsor, must be reported to the FDA and all participating investigators as IND Safety Reports. IND Safety Reports will also be sent to other investigators conducting research under the OCRPRO IND and will be shared with other stakeholders per applicable agreements (e.g. CRADAs and CTAs).

The sponsor will also submit a brief report of the progress of the investigation to the FDA on an annual basis, as defined in 21 CFR 312.33.

AEs that are also unanticipated problems will be summarized by the IND sponsor and distributed to investigators.

8.5 Halting Rules

"Halting" is discontinuation of study agent for all subjects in a study and suspension of enrollment until a decision is made to either resume or permanently discontinue such activity. Subjects are still followed for safety.

8.5.1 Halting Rules for the Study

If the LA Zika vaccine is considered unacceptably reactogenic, as defined below, additional inoculations will be suspended for all subjects and enrollment will be suspended (halted) until the DSMB and study sponsor have reviewed the data and recommend that enrollment and study agent administration can continue. The PI will notify the DSMB at the time that halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

The study PI/protocol chair and/or CSO will determine if the study should be halted. In addition, the FDA may halt the study at any time following review of any safety concerns. A local IRB may halt the study at its site.

8.5.1.1 Halting for Unacceptable Reactogenicity

Following administration of rZIKV/D4Δ30-713, the following criteria will be used to define unacceptable reactogenicity of the rZIKV/D4Δ30-713 vaccine:

1. One or more subjects experience an SAE (as defined in Section 8.1.6 of this protocol) that is determined to be possibly, probably, or definitely related to the administered vaccine (as defined in Section 8.2.4. of this protocol);
2. One or more subjects experience anaphylaxis that is possibly, probably or definitely related to the administered vaccine;
3. One or more subjects experience a Grade 4 adverse event that is definitely, probably, or possibly related to the administered vaccine;
4. Two or more subjects experience the same objective physical finding of severity Grade 3 that is definitely, probably, or possibly related to the administered vaccine, with the exception of Grade 3 erythema at the injection site, as defined in Section 8.2.3. of this protocol;
5. Two or more subjects experience the same Grade 3 laboratory abnormality that is possibly, probably, or definitely related to the administered vaccine;
6. Two or more subjects experience an ANC $\geq 500/mm^3$ but $< 750/mm^3$ for > 5 days duration[1];
7. Two or more subjects experience an ANC $< 500/mm^3$ for any duration[1]; or
8. Two or more subjects experience a vaccine-associated Zika-like syndrome, defined as an infection[2] associated with fever and 2 or more of the following symptoms:
    a. Grade 2 or greater headache lasting ≥ 12 hours
    b. Grade 2 or greater photophobia lasting ≥ 12 hours
    c. Grade 2 or greater generalized myalgia lasting ≥ 12 hours

8.5.1.2 Halting for Shedding of Zika vaccine in Semen or Vaginal Secretions

1. Should the Zika vaccine be detected in either semen or vaginal secretions following vaccination, the study enrollment will be halted pending discussion with the DSMB and the FDA. Subjects already enrolled will continue to be followed and additional specimens collected per the protocol.

8.5.2 Reporting a Study Halt

If halting criteria are met, a description of the AE(s) or safety issue(s) must be reported by the PI within 1 business day to the CSO and the local IRB according to their requirements.

- The PI will notify the DSMB
- The CSO or designee will notify all other site Investigators by email or through the specified pathway

8.5.3 Resumption of a Halted Study

The IND sponsor, in collaboration with the PI and DSMB, and in the case of a study halt due to shedding of Zika vaccine in semen or vaginal secretions, with the FDA, will determine if it is safe to resume the study. The CSO or designee will notify the site investigators of the decision to resume the study. The site investigators will notify their local IRB(s) of the decision.

8.6 Safety Oversight

8.6.1 Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the IND sponsor CSO that delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

8.6.2 Sponsor Medical Monitor

A medical monitor, Shirley Jankelevich, MD, representing the IND sponsor (OCRPRO), has been appointed for safety oversight in this clinical study. The sponsor medical monitor will be responsible for performing safety assessments as outlined in an SRCP, as defined in Section 8.6.1.

8.6.3 NIAID Intramural Data and Safety Monitoring Board

The NIAID intramural DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The NIAID intramural DSMB is constituted to review the safety data of NIAID intramural clinical studies that require DSMB oversight, and consists of experts in infectious diseases, biostatistics, and clinical trials. The PI's designee will provide the DSMB executive secretary with blinding codes in a sealed envelope in case the DSMB requires this information to make its recommendations. The DSMB will review the study prior to initiation and twice a year thereafter. The board may convene additional reviews as necessary. Prior to each review, the PI will submit a summary of cumulative safety data in a format acceptable (by unblinded cohort if requested) to the board. The board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. Reports of SAEs will be submitted by the PI to the board at the same time they are reported to the sponsor and IRB. All unanticipated problems will be submitted to the DSMB at the same time they are submitted to the IRB or IND sponsor. IND Safety Reports will be submitted to the DSMB by the investigator after their receipt. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the board at the time halting criteria are met, and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

9 Data Collection and Monitoring

9.1 Source Documentation and Data Collection

Complete source documentation (laboratory test reports, hospital or medical records, progress notes, observations, etc.) is required for every study subject for the duration of the study. The subject's study record must record his/her participation in the clinical trial and, after unblinding, the randomization treatment received (with doses and frequency) or other concomitant medications or interventions administered, as well as any adverse reactions experienced during the trial.
Data from source documentation for subjects enrolled in the study will be entered into the Clinical Research Information Management System of NIAID (CRIMSON) Data System. The data entry is to be completed on an ongoing basis during the study. Data entry into CRIMSON will be performed by authorized individuals, and each individual entering data into CRIMSON will have a unique user

ID and password. Corrections to the data system will be tracked electronically (password protected) with time, date, individual making the correction, and what was changed.

Corrections to the source document must be made by striking through the incorrect entry with a single line (taking care not to obliterate or render the original entry illegible) and entering the correct information adjacent to the incorrect entry. Corrections must be initialed and dated by the person making the correction whenever possible. Source documentation should support the data collected in CRIMSON and must be signed and dated by the person recording and/or reviewing the data.

The investigator is responsible for the accuracy, completeness, and timeliness of the data reported to the sponsor in CRIMSON. All data entered into CRIMSON should be reviewed by the investigator/designee, and signed as required with written or electronic signature, as appropriate. Data reported in CRIMSON should be consistent with source documents or the discrepancies should be explained. Source documentation will be made available for review or audit by the sponsor or designee and any applicable federal authorities.

9.2 Study Documentation

Study-related documentation will be completed as required by the IRBs, the sponsor, and regulatory authorities. Continuing review documentation will be submitted by the investigator to the IRBs by the anniversary date of initial review as specified by each IRB. An annual report will be submitted by the sponsor to the FDA according to regulations. These reports will provide a brief description of the progress of the investigation as outlined in 21 CFR 312.33, and will include any protocol revisions if not previously submitted.

The PI will maintain adequate records to account for the disposition of the investigational products, including dates of receipt and quantity, current inventory, and dispensation to subjects. If the study is terminated, suspended, or completed, the PI will return all unused supplies of the investigational product to a NIAID-approved repository or destroy according to the sponsor's recommendation.

9.3 Retention of Specimens

All specimens collected as part of this trial will be stored for future research as part of our approved biosample repository for vaccine research. These samples may be used to learn more about flavivirus infection and other diseases. These samples will not be sold or used to make commercial products. All samples stored in the repository will be labeled with the study subject ID numbers, which, by themselves, cannot identify study subjects, but are linkable to other research databases (e.g., from questionnaires, clinical assessments, logbooks, etc.) generated by the main study. The repository database will contain only the study subject ID numbers. A master log linking the study subject ID numbers to the names of the subjects will be maintained in a password-protected database system with limited access to authorized research team members.

9.4 Retention of Records

The PI is responsible for retaining all essential documents listed in the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines. Trial-related documents will

be maintained by the investigator in a secure storage facility for a period of 2 years after final marketing approval of the vaccine or if 2 years have elapsed since the formal discontinuation of the product's clinical development. These records are also to be maintained in compliance with IRB, state, and federal medical records' retention requirements, whichever is longest. The sponsor is required to inform the investigator as to when such documents need no longer be retained. Storage of all trial-related documents will be such that confidentiality will be strictly maintained to the extent provided by federal, state, and local laws.

It is the PI's responsibility to retain copies of source documents until receipt of written notification to the contrary from NIAID OCRPRO. Study documents should not be destroyed without prior written agreement between NIAID OCRPRO and the PI. Should the PI wish to assign the study records to another party and/or move to another location, the PI must provide written notification of such intent to NIAID OCRPRO, with the name of the person who will accept responsibility for the transferred records and/or the new location. NIAID must be notified in writing, and written permission must be received by the site prior to destruction or relocation of research records.

9.5 Protocol Compliance

The PI will conduct the trial in compliance with the protocol agreed to by the sponsor. The investigator will not implement any deviation from, or changes to, the protocol without agreement, prior review, and documented approval by the sponsor and the IRB that granted original approval for the study. The DSMB will be made aware of all protocol revisions (other than administrative) and will review any changes to the protocol that involve DSMB oversight or involve changes to the study's data and safety monitoring plan. However, the investigator may implement a deviation from, or change in, the protocol to eliminate an immediate hazard(s) to subjects without prior IRB or sponsor approval, or when the change(s) involves only logistical or administrative aspects of the trial (i.e., change of telephone number[s]). In the event of a medical emergency, the PI will perform any medical procedures that are deemed medically appropriate.

As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted to the Sponsor, IRB, DSMB, and the regulatory authorities.

9.6 Clinical Investigator's Brochure

Investigators will receive the current versions of the Clinical Investigator's Brochure for the live attenuated Zika vaccine rZIKV/D4Δ30-713, which comprehensively describes all the available preclinical experience with the experimental vaccine. If relevant new information becomes available during the course of the trial, the investigators will receive a revised brochure or an amendment to the current version.

9.7 Study Monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." The sponsor will monitor all aspects of the study,

with respect to current GCP, for compliance with applicable government regulations. Prior to the start of the study, the investigator will be informed of the frequency of monitoring visits and will be given reasonable notification prior to each visit. The objectives of a monitoring visit will be to verify the prompt and accurate recording of all monitored data points, and prompt reporting of SAEs; to check the availability of signed informed consent forms and documentation of the informed consent process for each monitored subject; to compare CRIMSON reports and line listings with source data for completeness and accuracy; and to help ensure investigators are in compliance with the protocol. The monitors will also inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections, FDA) and applicable guidelines (ICH GCP) are being followed. During the monitoring visit, the investigator (and/or designee) and other study personnel should be available to discuss the study. Study documents must be available for review throughout the course of the study. The sponsor will retain original copies of Form FDA 1572 and copies of other study documents as deemed necessary.

The investigator (and/or designee) will make study documents (e.g., consent forms, CRIMSON, and pertinent hospital or clinical records) readily available for inspection by the local IRB, the FDA, the site monitors, and NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

10 STATISTICAL CONSIDERATIONS

10.1 General Design

The goal of the Phase 1 vaccine trial is to determine the safety and immunogenicity of rZIKV/D4Δ30-713, a live attenuated Zika vaccine candidate in healthy human subjects. The purpose of the Phase 1 trial is to evaluate the reactogenicity, immune response to Zika vaccine and the persistence of antibody following subcutaneous administration of a single dose of rZIKV/D4Δ30-713.

10.2 Statistical Methods

This study, like other Phase 1 studies, is exploratory rather than confirmatory; its purpose is to estimate event frequencies and patterns of immune responses rather than to test formal statistical hypotheses. Descriptive approaches will be used to meet the protocol objectives as stated in Section 3 of this protocol, as outlined below. Results will be presented in tabular format, as well as graphically where appropriate.

Primary Objective 1: Determine the safety and reactogenicity of a single dose of the LA Zika vaccine rZIKV/D4Δ30-713, as assessed by the frequency of adverse events (AEs) and adverse reactions (AR) as defined as vaccine-related adverse events (AE)s, graded by severity.

The frequency of immediate, systemic, and local AEs and ARs following vaccination will be summarized. AEs and ARs will be displayed in tabular format, with line listings of individual clinical and laboratory AEs and ARs classified as immediate, systemic, and

local events and AEs and ARS will be summarized by severity and relationship to vaccine by individuals and each group (rZIKV/D4Δ30-713 and placebo).

Primary Objective 2: To determine the immunogenicity of a single dose of rZIKV/D4Δ30-713, as assessed by:

- Determination of the serum plaque reduction neutralization titer 50% ($PRNT_{50}$) to ZIKV for each subject at Study Day 28, 56, and 90 post-inoculation. Seroconversion will be defined as achieving a $PRNT_{50} \geq 1:10$ at any time-point through Study Day 90 (Day 28, 56, or 90). The peak $PRNT_{50}$ to ZIKV through Study Day 90 will be calculated for each subject included in the per-protocol an intent-to-treat analysis and the geometric mean peak titer for vaccinated subjects will be calculated.

Secondary Objective 1: Assess the frequency, quantity, and duration of viremia (virus in the blood) induced by a single dose of the rZIKV/D4Δ30-713 vaccine. The mean peak viremia, mean day of onset of viremia, and mean duration of viremia will be calculated. Viremia will be detected by culture (infectious virus) and by RT-PCR.

Secondary Objective 2: Determine the number of vaccinees infected with rZIKV/D4Δ30-713. Infection is defined as recovery of infectious vaccine virus from the blood, serum or urine of a subject and/or by seroconversion to ZIKV. Seroconversion will be defined as achieving a $PRNT_{50} \geq 1:10$ by Study Day 90 post-vaccination.

Secondary Objective 3: Evaluate the immunogenicity of rZIKV/D4Δ30-713 in flavivirus-naïve subjects as assessed by the $PRNT_{50}$ to ZIKV, for each subject at Study Day 28, 56, and 90 post-administration of LA Zika vaccine.

Secondary Objective 4: Determine the durability of antibody response 26 weeks after vaccination.

Secondary Objective 5: Determine the quantity and duration of ZIKV presence as determined by:

- The peak virus titer in the blood and the duration of viremia induced by the LA Zika vaccine as determined by RT-PCR and virus culture
- The quantity and duration of possible Zika vaccine shedding in urine determined by RT-PCR and virus culture
- The quantity and duration of possible Zika vaccine shedding in vaginal secretions determined by RT-PCR and virus culture
- The quantity and duration of possible Zika vaccine shedding in semen determined by RT-PCR and virus culture

Exploratory Objective 1: To evaluate the phenotype of peripheral blood mononuclear cells (PBMCs) at primary infection with the rZIKV/D4Δ30-713 vaccine.

Exploratory Objective 2: To evaluate the cellular immune response to primary infection with the rZIKV/D4Δ30-713vaccine.

Exploratory Objective 3: To evaluate the innate immune response to primary infection with the rZIKV/D4Δ30-713 vaccine.

Exploratory Objective 4: To evaluate B and T cell memory responses following primary infection with rZIKV/D4Δ30-713 vaccine.

Exploratory Objective 5: To evaluate the antibody response in saliva to infection with rZIKV/D4Δ30-713 as a possible diagnostic tool. This will be done in a subset of subjects (those enrolled at the Center for Immunization Research [CIR]).

10.3 Safety Endpoint

The primary safety endpoint is the frequency of rZIKV/D4Δ30-713-related AEs, as classified by both severity and seriousness, through active and passive surveillance. Separate assessments of systemic and local reactions will be performed.

10.4 Immunogenicity

Zika virus neutralizing antibody titers will be measured on study day 0 (prior to inoculation) and Study Days 28, 56, 90, and 180 following vaccination with rZIKV/D4Δ30-713. Seropositivity to ZIKV will be defined as a $PRNT_{50}$ of ≥ 1:10. Seroconversion will be defined as a $PRNT_{50}$ of ≥ 1:10 to ZIKV by study day 90. Seroconversion will be defined as a $PRNT_{50}$ of ≥ 1:10 to ZIKV or following administration of rZIKV/D4Δ30-713.

10.5 Per Protocol Analysis

10.5.1 Safety Data

Subjects who have completed ≥ 5 visits through Study Day 28 will be included in the per-protocol analysis of the AE / adverse reaction data. All SAEs and AEs of special interest will be included in the per-protocol analysis.

10.5.2 Virology

Subjects who have completed ≥ 5 visits through Study Day 28 will be included in the per-protocol analysis of the virology data.

10.5.3 Immunogenicity Data

Subjects who have completed at least 2 of the Study Day 28, Study Day 56, and Study Day 90 visits will be included in the per-protocol analysis of the immunogenicity data.

10.6 Intent-to-treat analysis

10.6.1 Safety Data

All subjects, regardless of the number visits that they missed, will be included in the safety analysis. This will include subjects who were replaced.

10.6.2 Virology

All subjects, with the exception of those who were replaced, regardless of the number visits that they missed, will be included in the virology analysis.

10.6.3 Immunogenicity Data

All subjects, with the exception of those subjects who were replaced, who have had at least one specimen collected at an immunogenicity time-point, regardless of the number visits that they missed, will be included in the immunogenicity analysis.

11 PROTECTION OF HUMAN SUBJECTS

11.1 Institutional Review Boards

The PI will be responsible for obtaining IRB approval for the study. Before the start of the study, the appropriate documents (including, but not limited to, the protocol, Investigator's Brochure, Informed Consent Form, information sheets, and advertisements) will be submitted to the IRB for approval. A copy of the study approval (including approval of the informed consent form) is to be maintained in the investigator study document binder and a copy will be supplied to the sponsor. During the study, the investigator is responsible for providing the IRB with all documents subject to review (e.g., protocol amendments, informed consent form updates, advertisements, and any written information that may be provided to the subject). Annual reports on the progress of the study will be made to the IRB by the investigator in accordance with IRB guidelines and government regulations.

11.2 Data Safety Monitoring Board

The Sponsor has convened a DSMB to review this study at regular intervals. The DSMB is comprised of individuals with expertise in clinical flavivirus vaccine studies, statistics, clinical trials, and infectious diseases. The study team will provide the DSMB with all requested data/materials they have requested for review at regular intervals determined by the DSMB. If a Halting Rule is triggered, the DSMB will review the event and decide, in collaboration with the PI and Sponsor, whether or not the study should continue (Section 8.5).

11.3 Informed Consent

In obtaining and documenting informed consent, the investigator and study staff must comply with the applicable regulatory requirements, GCP guidelines, and ethical principles. The written informed consent form must be approved by the IRB prior to its use. The subject may withdraw consent at any time during the course of the trial. A copy of the informed consent document will be given to the subject for his or her records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.4 Risks

Risks to the subjects are associated with venipuncture, topical anesthetic cream, and study agent(s). These risks are outlined below. In addition, questions asked regarding a subject's use of contraception may make the subject uncomfortable. These questions will be asked in a private area and the subject will be made aware that he or she does not have to answer any question that they do not feel comfortable answering. Because this is a live vaccine, there is an assumed risk to the fetus should the mother receive the vaccine when pregnant. The vaccine virus is highly attenuated and is expected to have only minimal replication in the host. Nevertheless, female subjects will be cautioned of the unknown risk of study agents to the fetus and will be advised to use effective birth control methods for the duration of the study. Current evidence does not suggest any risk to the fetus should a woman become pregnant after clearing infectious ZIKV

from the blood. For this reason, the vaccine should not pose any risk to the fetus should a subject become pregnant after clearing infectious vaccine virus from the blood (presumed to be approximately day 16 and after post-vaccination).

11.4.1 Venipuncture

The total amount of blood to be drawn throughout the 6-month duration of the primary study is approximately 500 mL. Risks occasionally associated with venipuncture include excessive bleeding, pain, bruising, or hematoma at the site of venipuncture, lightheadedness, and syncope (rarely). Infection may occur rarely.

11.4.2 Risk of Use of Vaginal Cup

The vaginal cup is a commercially-available product used during menstruation. It is contra-indicated in women with an intrauterine device (IUD) in place. For women who have an IUD in place, vaginal secretion specimens will be collected by use of vaginal swab.

11.4.3 Topical Anesthetic Cream

Risks occasionally associated with the use of topical anesthetic cream include temporary skin discoloration, skin irritation, rash, hives, and rarely, dizziness or drowsiness.

11.4.4 Inoculation

Possible local vaccine reactions include pain, swelling, or erythema for 2 to 3 days, lymphadenopathy, or pruritus at the injection site. Potential systemic reactions that may occur include symptoms of ZIKV such as rash, fever, headache, non-purulent conjunctivitis, generalized myalgias, and arthralgias. Immediate hypersensitivity reactions including urticaria, anaphylaxis, or other IgE-mediated responses are possible, as with any vaccine. Guillain-Barre syndrome has been reported in persons infected with wt ZIKV.

11.4.5 Other Risks

Subjects may be asked to defer routine immunization (such as influenza) with live vaccines 28 days prior or killed vaccines 14 days prior to inoculation or until after 28 days following inoculation, with the exception of COVID-19 vaccines. This may increase the risk that the subject will be infected with an influenza virus during this period. As with any investigational vaccine, there is a theoretical possibility of risks about which we have no present knowledge. Subjects will be informed of any such risks should further information become available.

11.5 Benefits

Subjects will not receive any direct benefit from participation in this study. They will receive a physical examination and laboratory screening for HIV infection, hepatitis B infection, and hepatitis C infection. They may potentially develop antibodies against ZIKV virus. It is hoped that information gained in this study will contribute to the development of a safe and effective vaccine for the prevention of ZIKV infection and to a better understanding of the protective immune response to ZIKV infection.

11.6 Compensation

Subjects will be compensated up to $80 for screening, up to $150 for inoculation day, and up to $80 for each completed scheduled follow-up visit. They will also receive up to a $250 bonus if all study visits are completed on time. Subjects will be compensated for the screening only if

they are enrolled in the study. Subjects will only be compensated for the visits that they complete. In addition, should subjects be asked to return to the clinic after Study Day 180 for collection of vaginal secretions (women) or semen (men), they will be compensated up to $80.00 for each additional visit. Alternates who come to the clinic on vaccination day but who are not vaccinated due to fulfillment of enrollment for that day will be compensated up to $150. Subjects enrolled in the study will receive a maximum total compensation of up to $1,520 if they complete all scheduled visits. Subjects will receive additional compensation, described above, if they complete additional visits as required by the protocol.

11.6.1 Compensation for Injury

The services at the Johns Hopkins Hospital, the Johns Hopkins Bayview Medical Center, or the University of Vermont Medical Center will be available to subjects who require inpatient care for any injury resulting from participation in the trial. This short-term medical care will be paid for through our contract with NIH. Short-term medical care will be given at a facility determined by JHU/UVM and NIH. No long-term medical care or financial compensation for research-related injuries will be offered by the Johns Hopkins University, Johns Hopkins Hospital, the NIH, or the federal government.

11.7 Confidentiality

All study-related information will be stored securely at the study site. All subject information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, and process and administrative forms will be identified by coded number only to maintain subject confidentiality. Computer entry will be done using a study ID number for each subject and all local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointments books, and any other listings that link subject study ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. A subject's study information will not be released without the written permission of the subject, except as necessary for monitoring by the Sponsor and/or its contractors and the FDA.

11.8 Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel during the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the NIH.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72. Please also refer to individual carrier guidelines (e.g., Federal Express, Airborne Express) for specific instructions.

12 Publication Policy

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials' registration policy as a condition for publication. This policy requires that all clinical trials be registered prior to enrollment of any subject in a public trials registry, such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention

or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity, would be exempt from this policy.

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14 APPENDICES

14.1 Appendix 1: Adverse Event Grading Table[1]

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
General Severity Grading	**Event that is easily tolerated, may require 1 dose of medication/treatment**	**Event that interferes with daily activity or requires more than 1 dose of medication/treatment**	**Event that prevents daily activity and requires medical intervention**	**Event that has life threatening consequences and/or urgent intervention is indicated**
Local Reactogenicity	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Injection Site Tenderness	Tenderness that is easily tolerated	Tenderness that interferes with daily activity	Tenderness that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Injection Site Pain	Pain that is easily tolerated	Pain that interferes with daily activity	Pain that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Injection Site Pruritus	Pruritus that is easily tolerated	Pruritus that interferes with daily activity	Pruritus that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Injection Site Induration	> 0 - 20 mm	> 20 - 50 mm	> 50 mm	Life-threatening consequences; urgent intervention indicated
Injection Site Erythema	> 0 - 20 mm	> 20 - 50 mm	> 50 mm	Life-threatening consequences; urgent intervention indicated
Systemic Reactogenicity	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Fever (Oral)	100.4° F – 101.4°F	101.5°F – 102.4°F	≥ 102.5°F	-

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Headache	Headache that is easily tolerated, may require 1 dose of medication/treatment	Headache that interferes with daily activity or requires > 1 dose of medication	Headache that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Retro-orbital Pain (ROP)	ROP that is easily tolerated, may require 1 dose of medication/treatment	ROP that interferes with daily activity or requires > 1 dose of medication	ROP that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Non-purulent Conjunctivitis	Easily tolerated, may require 1 dose of medication/treatment	Interferes with daily activity or requires > 1 dose of medication/treatment	Prevents daily activity	Life-threatening consequences; urgent intervention indicated
Photophobia	Photophobia that is easily tolerated, may require 1 dose of medication/treatment	Photophobia that interferes with daily activity or requires > 1 dose of medication	Photophobia that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Nausea	Nausea that is easily tolerated	Nausea that interferes with daily activity or requires > 1 dose of medication	Nausea that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Fatigue	Fatigue that is easily tolerated, may require 1 dose of medication/treatment	Fatigue that interferes with daily activity or requires > 1 dose of medication	Fatigue that prevents daily activity	Life-threatening consequences; urgent intervention indicated
Myalgia	Myalgia that is easily tolerated, may require 1 dose of medication/treatment	Myalgia that interferes with daily activity or requires > 1 dose of medication	Myalgia that prevents daily activity	Life-threatening consequences; urgent intervention indicated

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Arthralgia	Arthralgia that is easily tolerated, may require 1 dose of medication/treatment	Arthralgia that interferes with daily activity or requires > 1 dose of medication	Arthralgia that prevents daily activity	Life-threatening consequences; urgent intervention indicated
ZIKV-like Rash	Rash is present but asymptomatic	Rash is symptomatic (pruritus/pain) but does not interfere with function	Rash is symptomatic and interferes with function	Life-threatening consequences; urgent intervention indicated
Solicited Lab AEs	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Leukopenia	2,500 - 3,500/mm^3	1,500 - 2,499/mm^3	1,000 - 1,400/mm^3	Life-threatening consequences; urgent intervention indicated
Neutropenia (Reduced ANC)[2]	≥ 750 - 999/mm3	≥ 500 - 749/mm3	≤500/mm3	Life-threatening consequences; urgent intervention indicated
Thrombocytopenia (Reduced Platelets)	100,000 – 120,000/mm3	75,000 - 99,999/ mm3	≤74,999/mm3	Life-threatening consequences; urgent intervention indicated
Other Laboratory Values	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Leukocytosis (Increased WBCs)	11,500 - 13,000/mm3	13,001 - 15,000/mm3	≥ 15,000 or < 1,000/mm3	Life-threatening consequences; urgent intervention indicated
Hemoglobin (female)	9.5 - 10.7 gm/dL	8.0 - 9.4 gm/dL	≤7.9 gm/dL	Life-threatening consequences; urgent intervention indicated
Hemoglobin (male)	11.0 - 12.5 gm/dL	9.0 – 10.9 gm/dL	≤8.9 gm/dL	Life-threatening consequences; urgent intervention indicated -

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
PT	> 1.0 - 1.25 x ULN	> 1.25 - 1.5 x ULN	> 1.5 x ULN	Life-threatening consequences; urgent intervention indicated
PTT	> 1.0 - 1.66 x ULN	> 1.66 - 2.33 x ULN	> 2.33 x ULN	Life-threatening consequences; urgent intervention indicated -
ALT	> 1.25 - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 x ULN	Life-threatening consequences; urgent intervention indicated -
Creatinine	1.5 - 1.7 mg/dL	> 1.7 – 2.0 mg/dL	> 2.0 mg/dL	Life-threatening consequences; urgent intervention indicated -
Fibrinogen, Decreased	100 mg/dL to < lower limit of normal (LLN)	50 - 99 mg/dL	<50 mg/dL, or associated with gross bleeding, or associated with disseminated coagulation	Life-threatening consequences; urgent intervention indicated -
Fibrinogen, Increased	> ULN to 600 mg/dL	> 600 mg/dL	N/A	Life-threatening consequences; urgent intervention indicated
Creatine phosphokinase (CPK)	≥ 4 x ULN- 6 x ULN	> 6 x ULN- 10 x ULN	> 10 x ULN	Life-threatening consequences; urgent intervention indicated
Sodium: Hyponatremia	130 – 134 mEq/L	123 – 129 mEq/L	< 122 mEq/L	Life-threatening consequences; urgent intervention indicated -
Sodium: Hypernatremia	145 – 150 mEq/L	151 – 157 mEq/L	> 158 mEq/L	Life-threatening consequences; urgent intervention indicated -

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Potassium: Hypokalemia	3.1 – 3.2 mEq/L	2.9 – 3.0 mEq/L	< 2.8 mEq/L	Life-threatening consequences; urgent intervention indicated -
Potassium: Hyperkalemia	5.2 – 5.5 mEq/L	5.6 – 6.0 mEq/L	> 6.1 mEq/L	Life-threatening consequences; urgent intervention indicated
Phosphate: Hypophosphatemia	2.0 – 2.2 mg/dL	1.5 – 1.9 mg/dL	< 1.4 mg/dL	Life-threatening consequences; urgent intervention indicated -
Calcium (Corrected for Albumin): Hypocalcemia	1.95 – 2.04 mmol/L	1.75 – 1.94 mmol/L	< 1.74 mmol/L	Life-threatening consequences; urgent intervention indicated
Calcium (Corrected for Albumin): Hypercalcemia	2.51 – 2.88 mmol/L	2.89 – 3.13 mmol/L	> 3.14 mmol/L	Life-threatening consequences; urgent intervention indicated
Magnesium: Hypomagnesemia	0.60 – 0.74 mmol/L	0.45 – 0.59 mmol/L	< 0.44 mmol/L	Life-threatening consequences; urgent intervention indicated
Bilirubin (hyperbilirubinemia)	> 1.0 – 1.5 x ULN	> 1.5 – 2.5 x ULN	> 2.5 ULN	Life-threatening consequences; urgent intervention indicated -
Glucose: Hypoglycemia (Nonfasting, No Prior Diabetes)	55 – 69 mg/dL	40 – 54 mg/dL	<39 mg/dL	Life-threatening consequences; urgent intervention indicated
Glucose: Hyperglycemia (Nonfasting, No Prior Diabetes)	116 – 160 mg/dL	161 – 250 mg/dL	> 251mg/dL	Life-threatening consequences; urgent intervention indicated

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Triglycerides	—	400 – 750 mg/dL	> 751 mg/dL	Life-threatening consequences; urgent intervention indicated -
Liver Transaminase (LFTs) GGT AST Alk Phos	 > 1.25 – 2.5 x ULN > 1.25 – 2.5 x ULN > 1.25 – 2.5 x ULN	 > 2.5 – 5.0 x ULN > 2.5 – 5.0 x ULN > 2.5 – 5.0 x ULN	 > 5.0 x ULN > 5.0 x ULN > 5.0 x ULN	Life-threatening consequences; urgent intervention indicated
Pancreatic Amylase Lipase	> 1.0 – 1.5 x ULN > 1.0 – 1.5 x ULN	> 1.5 – 2.0 x ULN > 1.5 – 2.0 x ULN	> 2.0 x ULN > 2.0 x ULN	Life-threatening consequences; urgent intervention indicated -
Other Cardiovascular	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Cardiac Arrhythmia	Asymptomatic; transient dysrhythmia, no therapy required	Recurrent/persistent dysrhythmia; symptomatic therapy required	unstable dysrhythmia, hospitalization and therapy required	Life-threatening consequences; urgent intervention indicated
Hypertension	Transient, increase > 20 mm Hg diastolic BP; no therapy required	Recurrent; chronic increase > 20 mm Hg diastolic BP; therapy req.	acute therapy required outpatient or hospitalization possible	Life-threatening consequences; urgent intervention indicated
Hypotension	Transient orthostatic hypotension with heart rate increased by 20 beats/min or decreased by < 10 mm Hg systolic BP, no therapy required	Symptoms or BP decreased by < 20 mm Hg systolic, correctable with oral fluid therapy	Mean arterial pressure < 60 mm Hg, IV fluids required, or hospitalization	Life-threatening consequences; urgent intervention indicated
Pericarditis	Mild/moderate asymptomatic effusion, no therapy	Symptomatic effusion, pain, EKG changes	Tamponade or pericardiocentesis or surgery required	Life-threatening consequences; urgent intervention indicated

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Hemorrhage, Blood Loss	Minimal blood loss, asymptomatic, no therapy required	Symptomatic blood loss and no transfusion required	Massive blood loss or > 2 units transfused Symptomatic AND transfusion of 1-2 units of blood or packed red cells	Life-threatening consequences; urgent intervention indicated
Other Gastrointestinal	Grade I – Mild	Grade II – Moderate	Grade III - Severe	
Vomiting	Mild or transient; 2 – 3 episodes per day or mild vomiting lasting < 1 week	Moderate or persistent; 4 – 5 episodes per day; or vomiting lasting ≥ 1 week, therapy required	Severe vomiting of all food/fluids in 24 hours or orthostatic hypotension or IV therapy required	Life-threatening consequences; urgent intervention indicated
Diarrhea	Mild or transient; 3 - 4 loose stools per day or mild diarrhea lasting less than 1 week	Moderate or persistent; 5 - 10 loose stools per day or diarrhea lasting ≥ 1 week, therapy required	> 10 loose stools/day bloody diarrhea; or orthostatic hypotension or electrolyte imbalance, > 2 L IV fluid required	Life-threatening consequences; urgent intervention indicated
Oral Discomfort/Dysphagia	Difficulty swallowing but able to eat and drink	Unable to swallow solids	Unable to drink fluids; IV fluids required	Life-threatening consequences; urgent intervention indicated
Constipation	Constipation less than 78 hours and requires medication for relief	Moderate abdominal pain 78 hours with impaction, requiring therapy	Requiring disimpaction or hospital treatment	Life-threatening consequences; urgent intervention indicated
Other Respiratory	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Bronchospasm Acute	Transient; no therapy; FEV1 or peak flow reduced to 70- < 80%	Therapy required; normalizes with bronchodilator; FEV1 or peak flow 50 - 69%	No normalization with bronchodilator; FEV1 or peak flow 25 – 49%, retractions	Life-threatening consequences; urgent intervention indicated

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Dyspnea	Dyspnea on exertion	Dyspnea with normal activity	Dyspnea at rest	Life-threatening consequences; urgent intervention indicated
Other Neurologic	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Nuchal Rigidity	--	--	Presence of Nuchal rigidity	Life-threatening consequences; urgent intervention indicated
Neuropsychological	Mild confusion or cognitive impairment	Moderate confusion or cognitive impairment	Severe confusion or cognitive impairment	Life-threatening consequences; urgent intervention indicated
Neurocerebellar	Slight incoordination or dysdiadochokinesia	Intention tremor, dysmetria, slurred speech, or nystagmus	Ataxia requiring assistance to walk or arm incoordination interfering with ADLs	Life-threatening consequences; urgent intervention indicated
Neuromotor	Mild weakness in muscle of feet, but able to walk; and/or mild increase in reflexes	Moderate weakness in feet or legs, e.g. unable to perform deep knee bend, mild weakness in hands, loss of previously present reflex or development of hyperreflexia.	Marked distal weakness	Life-threatening consequences; urgent intervention indicated
Neurosensory	Mild impairment (decreased sensation in focal area or symmetrical distribution)	Moderate symmetrical impairment, mild impairment that is not symmetrical	Severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least moderate degree in multiple areas or functions	Life-threatening consequences; urgent intervention indicated

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Paresthesia (Burning, Tingling, etc.)	Mild discomfort; no therapy required	Moderate discomfort; non-narcotic analgesia required	Severe discomfort; or narcotic analgesia required with symptomatic improvement	Life-threatening consequences; urgent intervention indicated
Other Dermatologic	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Dermatitis	Rash is present but asymptomatic	Rash is symptomatic (pruritus/pain) but does not interfere with function	Rash is symptomatic and interferes with function	Life-threatening consequences; urgent intervention indicated
Other Urinalysis	Grade I – Mild	Grade II – Moderate	Grade III – Severe	Grade IV – Life Threatening
Proteinuria: Random Urine	1+	2+ - 3+	4+	-
Proteinuria: 24 Hour Urine	200 mg – 1 g loss/day or < 0.3% or < 3 g/L	> 1 – 2 g loss/day or 0.3% - 1.0% or 3 – 10 g/L	> 2 g loss/day or > 1.0% or > 10 g/L	-
Hematuria (In the Absence of Vaginal Bleeding)	Microscopic only, 6-10 rbc/hpf	> 10 rbc/hpf	Gross, with or without clots; or RBC casts	Life-threatening consequences; urgent intervention indicated
Other Miscellaneous	Grade I – Mild	Grade II – Moderate	Grade III - Severe	Grade IV – Life Threatening
Malaise	Malaise that is easily tolerated	Malaise that interferes with daily activity	Malaise that prevents daily activity	Life-threatening consequences; urgent intervention indicated

[1]Grade 4 will be assigned to any AE that is determined to be potentially life-threatening. Grade 5 will be assigned to any AE that results in death

14.2 Appendix 2: Brighton Criteria for Guillain-Barré Syndrome

Level 1 of diagnostic certainty	Level 2 of diagnostic certainty	Level 3 of diagnostic certainty
☐ Bilateral and flaccid weakness of the limbs	☐ Bilateral and flaccid weakness of the limbs	☐ Bilateral and flaccid weakness of the limbs
☐ Decreased or absent deep tendon reflexes in weak limbs	☐ Decreased or absent deep tendon reflexes in weak limbs	☐ Decreased or absent deep tendon reflexes in weak limbs
☐ Monophasic illness pattern; and interval between onset and nadir of weakness between 12 hours and 28 days; and subsequent clinical plateau	☐ Monophasic illness pattern; and interval between onset and nadir of weakness between 12 hours and 28 days; and subsequent clinical plateau	☐ Monophasic illness pattern; and interval between onset and nadir of weakness between 12 hours and 28 days; and subsequent clinical plateau
☐ Cytoalbuminologic dissociation (i.e. laboratory normal value and CSF total laboratory normal value and CSF total white cell count < 50 cells/µL	☐ Absence of identified alternative diagnosis for weakness	☐ Absence of identified alternative diagnosis for weakness
☐ Electrophysiological findings consistent with GBS	☐ CSF total white cell count < 50 cells/µL (with or without CSF protein elevation above laboratory normal value); **OR** electrophysiological studies consistent with GBS if CSF not collected or results not available	

14.3 Appendix 3: Guillain-Barré Syndrome disability scale

0. Healthy
1. Minor symptoms or signs of neuropathy but capable of manual work / capable of running
2. Able to walk without support of as stick (5 meters across an open space) but incapable of manual work / running
3. Able to walk with a stick, appliance of support (5 meters across an open space)
4. Confined to bed or chair bound
5. Requiring assisted ventilation (for any part of the day or night)
6. Death

From: Assessment and management of Guillain-Barré syndrome in the context of Zika virus infection
Interim guidance update; 18 August 2016, WHO/ZIKV/MOC/16.4 Rev.1

14.4 Appendix 4: Schedule of Procedures Post-inoculation with ZIKV Vaccine

		Study Day													
Procedure	**Screen**	**0**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**21**	**28**	**56**	**90**	**150**	**180**
Informed Consent	X														
Alk.Phos, bilirubin, AST	X														
PT/PTT	X														
Urinalysis	X														
HIV test	X														
HCV test	X														
HBV test	X														
Flavivirus antibody	X														
SARS-CoV-2 PCR (NP or mid-turbinate swab)	X[7]	X[7]			X[7]			X[7]							
Physical examination	X	X	X	X	X	X	X	X	X	X	X	[X][1]	[X][1]	[X][1]	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Inoculation		X													
CBC with differential	X	X	X	X	X	X	X	X	X	[X][4]					
ALT / Creatinine	X	X		X[6]				X		[X][4]					
Pregnancy Test	X	X								X	X	X	X	X	X
Serology (blood)		X								X	X	X	X	X	X
PBMC collection		X	X		X			X		X	X	X	X	X	X
PAXGene collection		X	X	X	X	X	X	X	X	X	X				
Urine for virus isolation		X	X	X	X	X	X	X	X	[X][2]	[X][2]	[X][2]	[X][2]	[X][3]	[X][3]
Blood for virus isolation		X	X	X	X	X	X	X	X	[X][2]	[X][2]	[X][2]	[X][2]	[X][2]	
Vaginal secretion for virus isolation		X			X				X		[X][2]	[X][2]	[X][2]	[X][3]	[X][3]
Semen for virus isolation		X			X				X		[X][2]	[X][2]	[X][2]	[X][3]	[X][3]
Oral fluid for serology[5]		X			X		X		X		X	X	X	X	X

1. [X][1] indicates that the physical exam is optional and will be performed if the subject has had a medically attended adverse event since the last visit or at the discretion of the investigator
2. [X][2] indicates that samples will be collected. Viral culture and PCR is optional and will be done only if virus was recovered from the sample drawn at the previous time-point
3. [X][3] indicates that samples for virology will be collected on study day if virus was recovered from the sample drawn at the previous time-point or virology results are not available
4. [X][4] indicates that the lab is optional and will be done if the previous sample was a grade 1 or higher
5. This will only be done at JHU CIR
6. ALT testing only
7. Nasopharyngeal (NP) or mid-turbinate swab to test for SARS-CoV 2 – the virus that causes COVID-19 – will be collected from all volunteers regardless of vaccination status based on guidance from federal, state, local, and/or institutional health officials* or at the discretion of the clinician (may be collected up to 72 hours in advance of stated collection time).
8. *This guidance may be informed by the local COVID infection rate, severity of infections, vaccination rates, and other factors as the COVID-19 pandemic continues to evolve and change.. NP or mid-turbinate swabs may be collected at other time-points if volunteer has COVID-like symptoms.