Phase I Evaluation of Two Zika Viruses for Use in Controlled Human Infection Models (CHIM)

CIR Protocol Number:	316
JHSPH IRB Protocol Number:	IRB00009756
UVM IRB Number:	
FDA IND Number:	19218
Ionizing Radiation	None
Multi-institution:	Yes
Project Assurance:	FWA# 00000287 (Johns Hopkins Bloomberg School of Public Health) FWA# 00000727 (University of Vermont Medical Center) FWA# 00000723 (University of Vermont)
Protocol Version:	v10.0
Date:	June 7, 2024

Sponsored by:
Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Division of Clinical Research (DCR)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)

Team Roster

Principal Investigator

Anna Durbin, MD
Center for Immunization Research (CIR)
Johns Hopkins School of Public Health
855 N Wolfe St, Baltimore, MD 21205
667-306-9772 (phone)

Co –investigator

Kristen Pierce, MD
University of Vermont, College of Medicine
University of Vermont
89 Beaumont Avenue
Given C205
Burlington, VT 05405
802-847-3819 (phone)

Clinical Investigators:

Kawsar Talaat, MD, MD
Noreen Hynes MD, MPH, DTM&H
Ruby Pritchett, BS
Megan McKnight, MS, PA-C
Lina Moscarella, MSN, FNP, AANP-CB
Sarah Sampson, MSN, FNP
Beulah Sabundayo, PharmD, MPH
Center for Immunization Research
Johns Hopkins School of Public Health
624 N. Broadway, Baltimore, MD 21205
410-502-9627 (phone)
443-287-8043 (phone)
410-502-7451 (phone)

Beth Kirkpatrick, MD
Kristen Pierce, MD
Patricia Lutton, CCRP
University of Vermont, College of Medicine
University of Vermont
89 Beaumont Avenue
Given C205
Burlington, VT 05405
802-656-5822 (phone)

Scientific Investigator:

Stephen S. Whitehead, PhD
Laboratory of Viral Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
301-496-7692 (phone)
240-281-3767 (phone)

OCRPRO Regulatory Affairs:	Mark S. Miller, PharmD, BCPS, RAC CAPT, USPHS Clinical Regulatory and Operations Specialist/IND Manager OCRPRO/DCR/NIAID/NIH 5601 Fishers Lane Room 4B31, MSC-9820 Bethesda, MD 20892 Office: 240-627-3300 Cell: 240-550-7375 Email: mark.miller6@nih.gov
Sponsor Medical Monitor:	Shirley Jankelevich, MD Clinical Monitoring Research Program Directorate Frederick National Laboratory for Cancer Research Leidos Biomedical Research, Inc Support to: Regulatory Compliance and Human Subjects Protection Program National Institute of Allergy and Infectious Diseases National Institutes of Health 5705 Industry Lane Frederick, MD 21704 301-846-7322 (phone) 301-846-6224 (fax) Shirley.Jankelevich@fnlcr.nih.gov
Clinical Study Sites	Center for Immunization Research Johns Hopkins School of Public Health 855 N. Wolfe Street, Suites 600 & 601 Baltimore, MD 21205 ICTR Bayview Clinical Research Unit (Bayview CRU) 301 Building 301 Mason Lord Drive Suite 4200 Baltimore, MD 21224 CIR Isolation Unit 301 Building 301 Mason Lord Drive Suite 4300 Baltimore, MD 21224 410-550-2725 (phone)

	University of Vermont Vaccine Testing Center University of Vermont 89 Beaumont Avenue Given C205 Burlington, VT 05405 University of Vermont Medical Center Clinical Research Center (CRC) Shepardson 2 111 Colchester Avenue Burlington, VT 05401
Research Sites	Center for Immunization Research Johns Hopkins School of Public Health 615 N. Wolfe St Baltimore, MD 21205 410-955-7230 (phone) University of Vermont Vaccine Testing Center University of Vermont 89 Beaumont Avenue Given C205 Burlington, VT 05405
Clinical Laboratories	Quest Diagnostics 1901 Sulphur Spring Road Baltimore, MD 21227 University of Vermont Medical Center 111 Colchester Avenue Burlington, VT 05401
IRB Sites	Johns Hopkins School of Public Health IRB 615 N. Wolfe Street Suite E1100 Baltimore, MD 21205 UVM Research Protections Office 213 Waterman Building University of Vermont Burlington, VT 05401

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b-hCG	beta-human choriogonadotropin
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
CBC	complete blood count
CDC	Center for Disease Control and Prevention
CHIM	controlled human infection model
CI	confidence interval
CIR	Center for Immunization Research
CLIA	Clinical Laboratory Improvement Amendments
CPK	creatine phosphokinase
CRIMSON	Clinical Research Information Management System of the NIAID
CRL	Charles River Laboratories
CSO	Clinical Safety Office
C_T	threshold cycle
CVS	Cervico-vaginal secretion
CZS	congenital Zika syndrome
DENV	dengue virus (serotypes DEN1, DEN2, DEN3, and DEN4)
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GA1	ganglioside asialo GM1
GBS	Guillain-Barré syndrome
GCE	Genome Copy Equivalents
GCP	good clinical practice
HBsAg	hepatitis B surface antigen
HBV/HCV	hepatitis B virus/hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Institutional Review Board
IUD	intrauterine device
JHSPH	Johns Hopkins Bloomberg School of Public Health
LID	Laboratory of Infectious Diseases
LLN	lower limit of normal
N	number of subjects
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OR	odds-ratio
PAHO	Pan American Health Organization
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PFU	plaque-forming units
PHEIC	Public Health Emergency of International Concern
PI	principal investigator
$PRNT_{50}$	50% plaque reduction neutralization titer
PT/PTT	prothrombin time/ partial thromboplastin time
RNA	ribonucleic acid
ROP	retro-orbital pain
RT-PCR	reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SD	standard deviation
SE	standard error
SJRP	São José do Rio Preto, Brazil

SOP	standard operating procedure
SRCP	Safety Review and Communications Plan
SUSAR	serious and unexpected suspected adverse reaction
T_{max}	maximum temperature
TORO	transfer of regulatory obligations
ULN	upper limit of normal
WBC	white blood cell count
WHO	World Health Organization
Wt	wild-type
ZIKV	Zika virus
ZIKV-SJRP	Zika virus- São José do Rio Preto, Brazil

PROTOCOL PRECIS

Protocol Title: Phase I evaluation of two Zika viruses for use in controlled human infection models (CHIM)

Version: 10.0

Version Date: 07 June 2024

Version History: v9.0 01 December 2023
v8.0 01 August 2023
v7.0 20 February 2023
v6.0 24 January 2023
v5.0 14 June 2022
v4.0 17 September 2021
v3.0 13 August 2021
v2.0 23 June 2021
v1.0 31 July 2019

Phase: Phase 1

Principal Investigator Anna Durbin, MD
Center for Immunization Research (CIR)
Johns Hopkins School of Public Health
855 N. Wolfe Street, Suite 600
Baltimore, MD 21205
667-306-9772 (phone)

Co –investigator Kristen Pierce, MD
University of Vermont, College of Medicine
89 Beaumont Avenue
Given C205
Burlington, VT 05405
802-847-3819 (phone)

Subjects: Healthy male and non-pregnant female subjects between 18 - 40 years of age, inclusive, with no history of previous ZIKV or DENV infection.

No. of Subjects: Up to 56

Study Type: Randomized, placebo-controlled, double-blind

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

Dose Escalation Schedule:

Cohort	No. of Subjects[1]	Sex	Dose	Virus
1	14	Female	10^2 PFU	ZIKV-SJRP/2016-184
2	14	Female	10^2 PFU	ZIKV-Nicaragua/2016
3[2]	14	Male	10^2 PFU	ZIKV-SJRP/2016-184
4[2]	14	Male	10^2 PFU	ZIKV-Nicaragua/2016

PFU = plaque-forming units, ZIKV-SJRP = Zika virus-São José do Rio Preto, Brazil

1. Virus:Placebo ratio, 5:2
2. Seven subjects from cohort 3 and 7 subjects from cohort 4 will be selected for mosquito-feeding. They will also be randomized 5:2 (ZIKV:placebo) to ensure the clinical team remains blinded to the treatment assignment.

Product Description: ZIKV-SJRP/2016-184 and ZIKV-Nicaragua/2016 were isolated directly from the serum of subjects who were diagnosed with uncomplicated Zika illness. Each virus will first be evaluated at a dose of 10^2 plaque-forming units (PFU). At each subcutaneous dose, the virus will first be evaluated in 7 subjects (5 will have received ZIKV, 2 will have received placebo). The remaining subjects in each dose cohort will be inoculated with the current dose or dose escalation will occur according to predetermined criteria.

Primary Study Objective: Identify a suitable strain of ZIKV for use in safety-assured CHIM studies by:

1. Determining the proportion of infected subjects who demonstrate clinical signs/symptoms of Zika infection (fever, rash, conjunctivitis, pruritis, myalgia, arthralgia, headache) by ZIKV strain and dose
2. Determining the quantity and duration of ZIKV in blood, urine, cervico-vaginal secretions, semen, and saliva by culture (infectious virus) and by reverse transcriptase – polymerase chain reaction (RT-PCR)
3. Determining if dose correlates with incidence/duration of ZIKV in blood, urine, cervico-vaginal secretions, semen, and saliva

A suitable ZIKV strain for CHIM studies is defined as one in which:

1. ≥ 80% of ZIKV or DENV-naïve inoculated subjects develop detectable viremia, as determined by direct virus culture ***and***
2. Infectious virus is detected on more than 1 day for ≥ 80% of subjects with detectable viremia ***and***
3. The geometric mean peak titer of virus recovered by culture is 2.0 – 3.0 $\log_{10}$ PFU/mL

Ideally, the ZIKV strain would also induce one or more of the following clinical signs in ≥ 50% of infected subjects, however this will not be included in dose escalation criteria.

a) Fever
b) Rash
c) Non-purulent conjunctivitis
d) Myalgia
e) Arthralgia

Secondary Study Objectives:

1. Characterize the clinical presentation of acute ZIKV infection by assessing the frequency of adverse events (AEs), graded by severity, occurring within 28 days of ZIKV administration
2. Longitudinal analysis of virus present in blood, urine, saliva, cervico-vaginal secretions, and semen
3. Time course of the neutralizing antibody response to ZIKV infection

Exploratory Objectives:

1. Evaluate the cellular immune response to primary infection with ZIKV
2. Evaluate the innate immune response to primary infection with ZIKV
3. Evaluate B and T cell memory responses following primary infection with ZIKV
4. Evaluate the antibody repertoire induced by ZIKV infection
5. Evaluate the antibody response in saliva to primary infection with ZIKV as a possible diagnostic tool
6. Determine if ZIKV-SJRP and ZIKV-Nicaragua can be transmitted to mosquitoes from infected volunteers when administered at a dose of 100 PFU

Safety Considerations / Risk Mitigation:

1. The majority of ZIKV infections are asymptomatic. With the noted exception of the fetus, reactogenicity to ZIKV infection is mild or moderate and self-limiting.
2. To reduce the risk of mosquito transmission, study subjects will be housed in an inpatient facility with an active mosquito-control program during the expected period of viremia, Study Days 0 through 9 (discharged on Study Day 9). Data from cohorts 1 & 2 determined that infectious ZIKV was cleared from blood before Study Day 9.
3. To reduce the risk of sexual transmission, volunteers must agree to use barrier contraception through Study Day 56 (women) or Study Day 90 (men) after inoculation with ZIKV.
4. Data from cohorts 1 & 2 demonstrated that infectious ZIKV was rarely detected in saliva or cervico-vaginal secretions following a dose of 10^2 PFU.
5. The inoculation is a low dose of 10^2 PFU.
6. Virus inoculum will be delivered subcutaneously by needle and syringe and is considered less infectious than natural mosquito-borne virus which is augmented by saliva factors.
7. Sexually-active women of child-bearing potential will be required to use hormonal birth control or an intrauterine device to prevent pregnancy while at risk for ZIKV infection (we will follow the Center for Disease Control and Prevention (CDC) guidelines of 8 weeks post-infection (Study day 56); time of infection is defined as inoculation with challenge virus). In addition, they will be required to use barrier contraception through Study Day 56 in accordance with CDC guidance.
8. Men will be required to use barrier contraception for 3 months post-infection (Study Day 90).
9. At the time of subject screening, serum pregnancy testing will be performed and repeated again within 14 days of inoculation, and on the day of inoculation. This will ensure women are not pregnant at the time of ZIKV inoculation.
10. Due to the observed correlation of age and the rare incidence of Guillain-Barré syndrome (GBS) following ZIKV infection, only volunteers age 40 years and younger will be enrolled.

Study Design:
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, urine toxicology screening for opiates and serology screening for previous DENV and ZIKV infection. Serum pregnancy testing will be performed on applicable female subjects.
For subjects who are eligible, receipt of inoculation will be scheduled for Study Day 0. Until transmissibility of the two ZIKV strains to mosquitoes is defined by mosquito-feeding on volunteers, volunteers will be admitted to the inpatient unit on Study Day 0 and will be discharged from the unit on Study Day 9 if discharge criteria are met. This represents the period of time when infectious viremia may be present and at a level that could be transmissible to mosquitoes.

Volunteers will be evaluated according to the attached Schedule of Procedures. During the outpatient and inpatient visits through Study Day 28, the subjects will be evaluated by a clinician and will have blood drawn for clinical laboratory studies, virologic assays, and immunologic assays. In addition, urine, vaginal secretions, semen, and saliva will be collected at specific time points for additional virologic assays. Subjects will have their temperature measured and recorded at least twice a day during their inpatient stay, with the exception of the day of discharge (Study Day 9). Subjects will return to the clinic on Study Days 9, 10, 12, 14, 16, 21, 28, 35, 49, 56, 70, 90, 120, and 180 for blood and specimen collection. Study Day 180 will be their final visit.

Cohorts 3 & 4 will evaluate the replication kinetics and shedding in semen of both ZIKV strains in men. It is expected that the replication kinetics of both ZIKV strains will not be significantly different in men compared to women. The transmissibility of both ZIKV strains to mosquitoes will be evaluated in cohorts 3 and 4. Based on the peak titers of ZIKV-SJRP recovered from volunteers in cohort 1, it is expected that this strain will not be transmissible to mosquitoes and future studies utilizing ZIKV-SJRP will be able to be conducted in the outpatient setting. Based on the peak titers of ZIKV-Nicaragua recovered from volunteers in cohort 2, it is expected this virus will be transmissible to mosquitoes..

Table 1: Schedule of Procedures for Cohorts 1, 2, 3 and 4

	Screen		Study Day (inpatient interval marked in gray)																						
Procedure	**-60**	**-14**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**14**	**16**	**21**	**28**	**35**	**56**	**70**	**90**	**120**	**150[4]**	**180**
Informed Consent	X																								
Pregnancy Prevention Review	X		X									X	X	X	X	X	X	X	X	X	X	X	X	X	X
Alk Phos, bilirubin	X																								
AST, ALT, serum creatinine	X										X			X		X								
PT/PTT	X																								
Urinalysis	X																								
HIV test		X																							
HCV test	X																								
HBV test	X																								
Physical exam	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	[X]	[X]	[X]		X
Admit to Unit			X																						
NP swab for COVID-19 testing[2]			[X]																						
Inoculation			X																						
CBC with differential	X		X				X				X			X		X	[X]								
Pregnancy Test (serum)[5]	X	X	X								X		X	X	X	X	X	X	X	X	X	X	X		X
Blood for serology	X		X				X				X				X		X	X		X		X			X
Blood for PBMC			X				X				X				X		X	X		X		X			X
Blood for PAXGene			X	X	X	X	X		X		X		X	X	X	X	X	X							
Urine for virus isolation			X		X		X		X		X		X	X	X	X	X	[X]	[X]	[X]	[X]	[X]	[X]		[X]
Blood for virus isolation			X	X	X	X	X	X	X	X	X	X	X	X	X	X	[X]	[X]	[X]	[X]	[X]	[X]	[X]		[X]
Saliva for virus isolation			X		X		X		X		X		X	X	X	X	X	[X]	[X]	[X]	[X]	[X]	[X]		[X]
Cervico-vaginal secretion (CVS) for virus isolation[5]			X				X				X		X	X	X	X	X	[X]	[X]	[X]	[X]	[X]	[X]		[X]
Semen for virus isolation[6]			X				X				X			X		X	X	[X]	[X]	[X]	[X]	[X]	[X]		[X]
Mosquito feeding[7]								X	X	X															
Oral fluid for serology[3]			X				X		X		X		X	X	X	X	X	X		X	X	X			X

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CBC = complete blood count; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; PBMC = peripheral blood mononuclear cell; PT/PTT = prothrombin time/partial thromboplastin time

1. [ ] indicates that these procedures/assays will be applied only if indicated. Sample collection for urine, CVS, semen and saliva for virus isolation on Study Days 120 and 180 will only be done if indicated. Please see section 6, Study Procedures.
2. 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.

3. This will only be done at the Center for Immunization Research (CIR)
4. Phone Call Visit
5. Female subjects only
6. Male subjects only
7. Only 7 subjects from cohort 3 and 7 subjects from cohort 4 will undergo mosquito-feeding. The 7 subjects will be randomized 5:2 (ZIKV:placebo) to ensure the clinical team remains blinded to the treatment assignment.

1 INTRODUCTION

1.1 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]. Only approximately 19% of subjects found to be seropositive to ZIKV in a serosurvey on Yap island recounted being symptomatic with a Zika-like illness. Of the 21% of infected subjects who were symptomatic, nearly all presented with rash (90%), arthritis/arthralgia (65%), and fever (65%). Unlike dengue virus (DENV), ZIKV does not cause hemorrhagic manifestations, vascular leak syndrome, or liver function abnormalities. 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. In retrospective analyses of the French Polynesian outbreak, an association between ZIKV infection during the first and/or second trimester and microcephaly was reported [7]. Since its introduction into Brazil, ZIKV has spread to more than 69 countries in the Americas, Caribbean, Asia and Africa [8].

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 [9]. 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, 10, 11] and a causal association between ZIKV infection and microcephaly and/or other birth defects was formally established [12]. 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 [13, 14]. Although congenital ZIKV syndrome 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.

ZIKV infection has also been associated with an increased risk of GBS, although a causal relationship has not yet been established. In retrospective analyses of the 2013 French Polynesian outbreak, an association between GBS and ZIKV infection was reported [15]. Forty-two cases of GBS were identified with 88% of those reporting a history of recent viral syndrome a median of 6 days before the onset of neurological symptoms. Serum from the GBS patients was tested by

reverse transcriptase – polymerase chain reaction (RT-PCR) for ZIKV during the hospital admission but was negative in all cases. Although serum from these patients was eventually found to be positive for ZIKV antibody by either immunoglobulin M (IgM), immunoglobulin G (IgG), or neutralization test, the samples were taken up to 3 months post-hospitalization, and it is unclear at what time the sample became positive for ZIKV. In contrast, serum from 98 matched control patients hospitalized with a non-febrile illness was collected at a single time-point within 7 days of the admission date of the matched-GBS case. Forty-one of 42 GBS patients were found to have neutralizing antibody against ZIKV compared with 54/98 (56%) of control patients for an odds-ratio (OR) of 59.7 (non-estimable 95% confidence interval [CI]). However, others suggested that the high OR was an artifact of a small sample size and have calculated an OR of 1.33 (95% CI 0.62 – 2.78) for ZIKV and GBS from this same population when IgG for ZIKV from the first blood sample was used as the only relevant evidence of exposure [16]. The single time-point was believed to be more relevant and comparable to the single time-point used in the control group. Although sera from 6 patients demonstrated high reactivity toward ganglioside asialo GM1 (GA1), further testing did not demonstrate any competition between GA1 and ZIKV proteins suggesting that there was no molecular mimicry between ZIKV antigens and GA1 [15]. The reported incidence of GBS in the French Polynesian outbreak was 0.24/1,000 ZIKV infections which is lower than that observed with *Campylobacter jejuni* infections (0.25 – 0.65/1,000 infections). Section 10.3.6.3 further discusses the risk of GBS following ZIKV infection.

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 [17, 18]. Complicating ZIKV control programs, there have been several reports of sexual transmission of ZIKV [19-24]. 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 5,460 travel-associated ZIKV cases in the United States (<1% of ZIKV cases) as of January 2, 2019, (Zika cases in US, accessed May 28, 2019) and it is not thought to contribute significantly to transmission [25]. All but 2 cases of sexual transmission have been from male to female. ***There has been only one report of female-to-male ZIKV transmission*** and one report of male-to-male ZIKV transmission [26, 27]. In all but one of the cases of sexual transmission, ZIKV has been transmitted from a ***symptomatic person*** to his partner. ***Transmission has occurred during or just after symptomatic illness***. In only one documented case has ZIKV been transmitted from an asymptomatic person to his partner [28]. There have been few longitudinal studies evaluating the sites and duration of ZIKV shed in bodily fluids [29, 30]. ZIKV ribonucleic acid (RNA) has been detected in semen by RT-PCR for 2 - 6 months after symptoms have developed [31-33]. However, to date, the longest interval between onset of symptoms and recovery of infectious ZIKV from semen is 69 days [34]. Studies have demonstrated that a minority of ZIKV-infected men shed ZIKV in semen. Mead *et al.* found ZIKV by RT-PCR in 60/184 men (33%) and found ZIKV by RT-PCR in only

174 of 1,230 (14%) semen samples tested from those men [30]. Of the 174 semen samples that were positive for ZIKV by RT-PCR, infectious virus (as determined by recovery in tissue culture) was found in only 4% of the samples. In addition, infectious virus was found only in samples collected within 30 days of symptom onset [30]. Only 6.5% of the men tested shed infectious ZIKV in semen. In the Mead study, infectious virus was detected in only 3 of 19 semen samples that were obtained within 30 days of illness; infectious virus was not detected in any of the 59 samples that were detected after day 30 of illness onset (Figure 1). The 3 samples that were positive for infectious virus had titers ≥ 7 $\log_{10}$ RNA copies/mL of semen and were the only samples that had titers ≥ 7 $\log_{10}$ RNA copies/mL of semen [30]. A second study collected 700 semen samples from 97 confirmed ZIKV-infected men [35]. Thirty-two of the 97 men (33%) had at least one semen specimen positive for ZIKV by RT-PCR. Infectious virus was isolated from RT-PCR-positive semen samples from 8/32 men (25%) or 8/97 total (8.2%). Few subjects had ZIKV shedding detected in saliva (15/147; 10.2%) and only 1 woman had ZIKV shedding detected in cervico-vaginal secretions (1/50; 2%). Isolation of infectious virus was not performed for saliva or cervico-vaginal secretion specimens. Aside from the Paz-Bailey study above, few studies have evaluated shedding of ZIKV in cervico-vaginal secretions in a longitudinal manner [36-38] and of those studies even fewer have evaluated for infectious virus [39]. Viral RNA was generally detected in vaginal secretions or brush samples by RT-PCR for ~ 2 weeks post-symptom onset [29, 36-38]. There are 2 reports of longer vaginal shedding of ZIKV. The first reported ZIKV vaginal shedding in 5 women, 4 of whom were pregnant women [40]. One pregnant woman shed ZIKV vaginally for 180 days. Testing for infectious virus was not done. A second case reported vaginal shedding at 31 days post-symptom onset [41]. The titer was very low (200 genomic copies/mL) and testing for infectious virus was not done. 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 [24, 26-28, 42]. The primary method of viral detection in these studies was by RT-PCR. When samples were evaluated for infectious virus by tissue culture, infectious virus was found in the minority of samples as noted above. In the Medina study, all but one semen sample containing infectious ZIKV were obtained within 15 days of symptom onset. The mean titer by polymerase chain reaction (PCR) ± standard deviation (SD) in those 8 semen samples that contained infectious virus was 1.8 x10^8 ± 2.5 x 10^8 genome copy equivalents (GCE) per mL. The mean titer ± SD for serum samples that contained infectious virus was 4.8 x 10^7 ± 1.1 x 10^7 GCE/mL. Medina *et al.* concluded that specimens with a titer >1.4 x 10^6 GCE/mL be considered infectious [35]. ***These data suggest that the shedding of infectious ZIKV in semen may occur much less frequently and for a shorter duration than presumed by RT-PCR testing*** and are

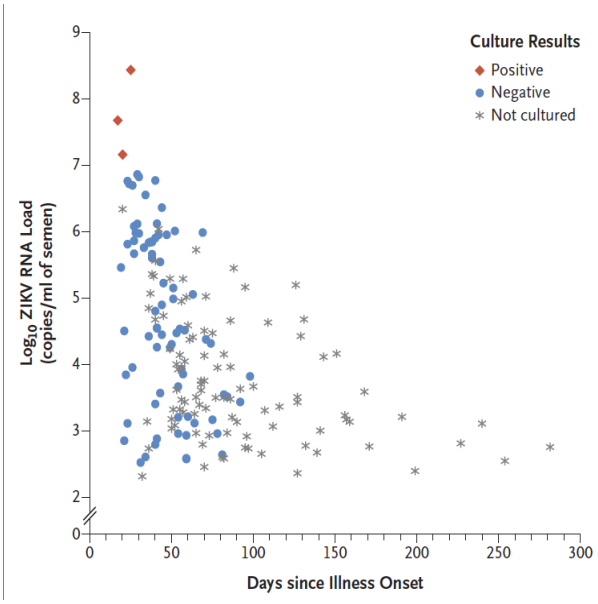

Figure 1: Estimated Viral RNA Load and Culture Results in Semen from 184 Men (Mead et, al 2018)

consistent with epidemiologic data reporting that all but one case of sexual transmission thus far have occurred within 3 weeks of return from an endemic area with all but one of those cases occurring within 2 weeks. Data from these studies suggest ***that detection of ZIKV RNA by RT-PCR may overstate the duration and magnitude of the risk of sexual transmission***. In summary, these studies determined that:

1. Infectious ZIKV will likely be cleared from the blood well before Study Day 14 post-infection.
2. Although ZIKV can be detected by RT-PCR in semen as late as 6 months following infection, the shedding of infectious virus is rare, is associated with high titer by RT-PCR (≥ 7.0 $\log_{10}$ GCE copies/mL semen or threshold cycle [C_T] ≤ 27) and occurs within 30 days of illness.
3. Samples with a titer > 1.4×10^6 GCE/mL should be considered infectious.
4. ZIKV was rarely detected in saliva or cervico-vaginal secretions (minimizing the risk of transmission from women)

The purpose of this protocol is to develop a ZIKV controlled human infection model (CHIM) for use in ZIKV vaccine development and possibly licensure. However, there are several issues to consider in the development of a safe and useful ZIKV CHIM. To this end, in December of 2016, an ethics consultation was convened by the U.S. National Institutes of Health (NIH) in collaboration with the Walter Reed Army Institute of Research (WRAIR) with the purpose of identifying if and under what circumstances a ZIKV CHIM could be ethically conducted. The report of the consultation was released in February 2017 and can be found at: https://www.niaid.nih.gov/sites/default/files/EthicsZikaHumanChallengeStudiesReport2017.pdf. The committee identified 2 major risks of a ZIKV CHIM:

1.) the risk of a ZIKV CHIM to the fetus should a pregnant woman become infected and
2.) the risk to third parties, through mosquito and sexual transmission.

Because of ongoing transmission of ZIKV in endemic areas at that time, the committee concluded that these risks did not outweigh the benefits of a ZIKV CHIM as the ongoing ZIKV circulation would allow for natural history studies of ZIKV infection to fill knowledge gaps related to the kinetics of ZIKV infection, transmissibility, shedding, and clinical illness, and would also allow for the conduct of traditional Phase 3 efficacy studies of vaccines and therapeutics. The committee did state that if circumstances changed or if the protocol was designed to mitigate the risks raised by the committee a specific proposal for a Zika virus human challenge study is ethically sound.

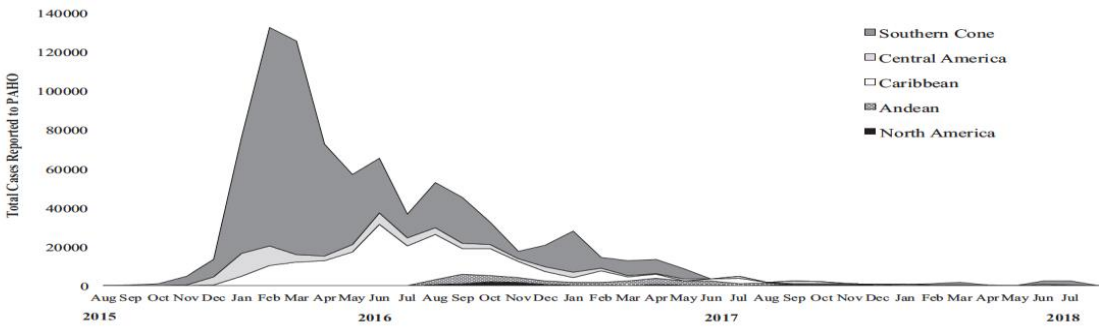

Figure 2: Monthly ZIKV Illness Case Counts Reported to PAHO (Vannice et al, 2019)

At the time this report was released, ZIKV transmission was already waning. Currently, only sporadic cases of ZIKV have been reported to Pan American Health Organization (PAHO) (Figure 2) [43]. For this reason, in March of 2018 an NIH/WHO meeting was convened to report on pathways to licensure for ZIKV vaccines given the low transmission of ZIKV. A ZIKV CHIM was considered as part of a pathway to licensure of a ZIKV vaccine and the report concluded that the circumstances and calculus have changed substantially and that a ZIKV CHIM could move forward [43]. In designing this protocol, we have addressed the risks raised during the 2016 ethics consultation and have provided a detailed risk mitigation strategy. We have adopted many of the recommendations of the ethical committee, as outlined in the 2017 publication.

The primary objective of this study is to identify and characterize a suitable ZIKV strain for use in future ZIKV controlled human infection models (CHIM). The use of a ZIKV CHIM will be critical for evaluation and down-selection of candidate ZIKV vaccines. Because ZIKV congenital syndrome can occur with asymptomatic infection and infection during any trimester, it is likely that a ZIKV vaccine will need to induce protection against ZIKV viremia to prevent congenital infection. Prevention of symptoms in the mother may not be sufficient to prevent congenital Zika syndrome. The ability of a vaccine to prevent ZIKV viremia (sterilizing immunity) can best be evaluated in a CHIM, prior to Phase 2 or Phase 3 studies. In addition, the ZIKV epidemic has waned over the past 2 years and modelling exercises predict that, in the future, outbreaks may occur only once every 10 years [44]. For this reason, it is not likely that there is sufficient predictable ZIKV transmission for a Phase 3 efficacy study to be performed and the efficacy of candidate ZIKV vaccines may only be able to be determined by CHIM studies. As stated above, the value of a ZIKV CHIM was affirmed at a recent WHO/NIH consultation on immune correlates of protection for ZIKV [43]. Because of the current lack of ZIKV transmission and the important role that a ZIKV CHIM can play in the pathway to licensure of a ZIKV vaccine, the consultation concluded that a ZIKV CHIM was ethically justified at this time should the studies sufficiently mitigate the risk of CZS and the risk of third-party transmission [43]. A ZIKV CHIM could also be critical to the development and licensure of diagnostics and therapeutics for ZIKV.

A secondary objective of the protocol is to further define the clinical presentation, viral replication, and sites and duration of ZIKV shedding following infection in healthy adult women and men. This study will contribute to the body of knowledge related to ZIKV infection and transmission. Although the data from natural ZIKV cases suggest that the risk of sexual transmission from men to women does not appear to be high, there are gaps in that knowledge.

We will enroll men to evaluate the shedding of infectious ZIKV in semen to advance knowledge of the risk of sexual transmission from men to women.

The 2 greatest risks of a ZIKV CHIM, as articulated by the ethics consultation, are the risk to the fetus should a pregnant woman become infected, and the risk of third-party transmission, either by mosquito or by sexual transmission. As recommended by the ethics review, cohorts 1 and 2 only enrolled non-pregnant and non-lactating woman. Enrolled women were required to use hormonal birth control, an intrauterine device (IUD), or be incapable of conceiving (either surgically or biologically) to meet enrollment criteria (Section 4.1). In addition, women were tested using a serum pregnancy test at initial screening, within 14 days prior to inoculation, and the day of inoculation (prior to receipt of test article) (Section 6). Serum pregnancy tests can detect a pregnancy as early as 7 – 10 days post-conception. The embryo attaches to the uterine wall during the first 2 weeks of development. Problems with uterine attachment and severe cell damage can result in miscarriage. Because the cells of the embryo have a greater ability to recover at this stage, exposures during the 3 – 5 weeks post-conception are not likely to cause a birth defect [45]. Requiring the use of highly effective birth control and serially performing serum pregnancy tests will ensure to the greatest degree possible that only non-pregnant women are enrolled in the study. Should a woman have conceived within 7 days of ZIKV inoculation, the inoculation would have occurred extremely early post-conception, at a time when the expected outcome would either be a spontaneous abortion or a normal pregnancy without any resultant birth defect. This strategy minimizes the risk of CZS to the greatest possible extent. None of the women enrolled in cohorts 1 or 2 became pregnant during the study.

As there has been only one documented case of sexual transmission from a woman to a man, and data from the Paz-Bailey paper indicated that ZIKV was recovered only infrequently from cervico-vaginal secretions. Infectious ZIKV was recovered very infrequently from saliva, CVS, and urine following inoculation with ZIKV-SJRP or ZIKV-Nicaragua (Table 5 and Table 7). Shedding of ZIKV in semen will be evaluated in cohorts 3 & 4, which will enroll men. Transmission of both ZIKV strains to mosquitoes will be evaluated in a subset of volunteers in cohorts 3 and 4 to assess the possibility of conducting future ZIKV CHIM studies in the outpatient setting. The peak infectious titers of ZIKV-SJRP recovered from volunteers in cohort 1 were well below 3 $\log_{10}$ PFU/mL. Given that mosquitoes ingest only ~2 µL per blood meal, it is unlikely that virus at this low titer will be transmitted to mosquitoes. Peak titers of infectious ZIKV-Nicaragua were significantly higher than those of ZIKV-SJRP and it is likely that this virus will be transmitted to mosquitoes from volunteers enrolled in cohort 4. Volunteers in cohorts 3 and 4 will be housed in an inpatient unit from inoculation day through Study Day 8 to reduce the risk of mosquito transmission, as was recommended by the ethics consultation. Volunteers will not be discharged from the unit until ZIKV titer in serum, urine, saliva, semen, and cervico-vaginal secretions is $< 1.4 \times 10^6$ genomic copies/mL. Additional components of the risk mitigation strategy can be found in Section 10.3.7.

In characterizing the 2 ZIKV strains that will be studied in this protocol, we will conduct sampling of blood, urine, cervico-vaginal secretions, semen, and saliva at multiple time-points throughout the 6-month duration of subject participation to determine the quantity and duration of shedding of ZIKV in these samples. Detection of ZIKV in these samples will be performed by RT-PCR as well as by culture of the virus to determine the shedding of infectious virus. The incidence of symptomatic illness will be determined, and the clinical characteristics of illness

will be described. The data obtained from this study will inform the scientific community regarding the sites, magnitude, and duration of infectious ZIKV in women and men. Should a safe and effective ZIKV CHIM be developed, it would be extremely valuable in the down-selection of candidate vaccines and therapeutics and could be used as a critical component of the pathway to licensure.

1.2 Background: ZIKV Strains

1.2.1 ZIKV-SJRP/2016

ZIKV-SJRP/2016 was isolated from the serum of a previously healthy non-pregnant adult female in São Jose do Rio Preto, (São Paulo State), Brazil who presented with uncomplicated ZIKV infection (fever, rash, and conjunctivitis). The virus was isolated directly from serum, expanded, and biologically cloned at the Laboratory of Infectious Diseases (LID), National Institute of Allergy and Infectious Diseases (NIAID), NIH and the clinical lot was manufactured at Charles River Laboratories (CRL) Biopharmaceutical Services facility in Malvern, PA. The final drug product, ZIKV-SJRP/2016 (Lot ZIKV#117A) was manufactured in qualified Vero cells at CRL using a method developed by LID/NIAID/NIH and CRL.

1.2.1.1 Final Container of ZIKV-SJRP/2016

The Final Drug Product was dispensed as 1.0 mL aliquots of live, Vero Grown Zika virus into 1.8 mL sterile cryovials and stored at -75C°± 15°C. The titer of ZIKV-SJRP/2016 Lot ZIKV#117A Final Drug Product determined on December 5, 2016 was approximately $10^{5.7}$ PFU/mL.

1.2.1.2 Composition of ZIKV-SJRP/2016

The Final Drug Product composition is a concentration of live, Vero Grown Zika virus, Lot ZIKV#117A in L-15 medium containing 1X SPG (sucrose 0.128M; KH_2PO_4, 0.0038M; K_2HPO_4, 0.0072M; mono-sodium glutamate, 0.0054M). The potency of ZIKV-SJRP/2016 (Lot ZIKV#117A) is $10^{5.7}$ PFU/mL.

1.2.1.3 Investigational Product Label of ZIKV-SJRP/2016

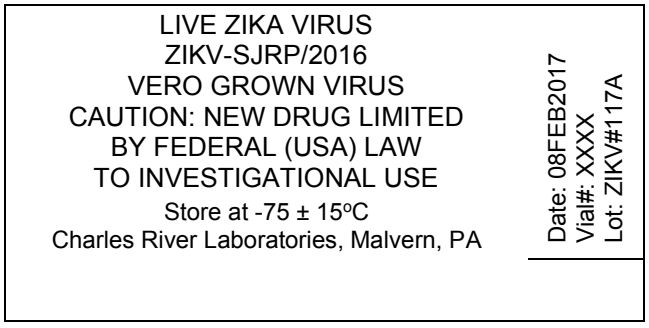

Figure 3: Label for Final Vial of ZIKV-SJRP/2016 (Enlarged Sample)

1.2.2 ZIKV-Nicaragua/2016

ZIKV-Nicaragua/2016 was isolated from the serum of a previously healthy 9-year-old female patient from Nicaragua who presented with uncomplicated ZIKV infection (fever

only). The virus was isolated from serum, expanded, and biologically cloned at the LID, NIAID, NIH and the clinical lot was manufactured at CRL Biopharmaceutical Services facility in Malvern, PA. The final drug product, ZIKV-Nicaragua/2016 (Lot ZIKV#116A) was manufactured in qualified Vero cells at CRL using a method developed by LID/NIAID/NIH and CRL.

1.2.2.1 Final Container of ZIKV-Nicaragua/2016

The Final Drug Product was dispensed as 1.0 mL aliquots of live, Vero Grown Zika virus into 1.8 mL sterile cryovials and stored at -75C°± 15°C. The titer of ZIKV-Nicaragua/2016 Lot ZIKV#116A Final Drug Product determined on December 5, 2016 was approximately $10^{6.6}$ PFU/mL.

1.2.2.2 Composition ZIKV-Nicaragua/2016

The Final Drug Product composition is a concentration of live, Vero Grown Zika virus, Lot ZIKV#116A in L-15 medium containing 1X SPG (sucrose 0.128M; KH_2PO_4, 0.0038M; K_2HPO_4, 0.0072M; mono-sodium glutamate, 0.0054M). The potency of ZIKV-Nicaragua/2016 (Lot ZIKV#116A) is $10^{6.6}$ PFU/mL.

1.2.2.3 Investigational Product Label of ZIKV-Nicaragua/2016

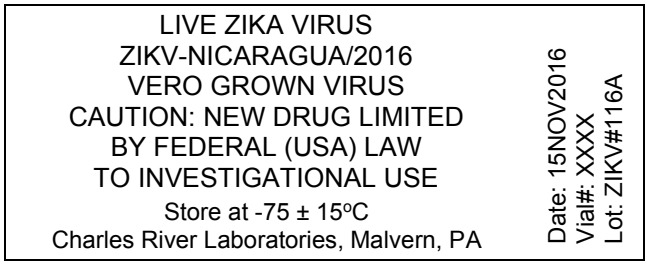

Figure 4: Label for Final Vial of ZIKV-Nicaragua/2016 (Enlarged Sample)

1.3 Rationale

1.3.1 Animal Experience

1.3.1.1 Non-human Primate Study

Four rhesus macaques were infected with $2x10^4$ PFU of the Current Good Manufacturing Practice (cGMP) material of either ZIKV-SJRP/2016 or ZIKV-Nicaragua/2016 by subcutaneous injection. The macaques were examined daily and bled daily from Day 2 post-infection through Day 8 post-infection for detection of virus. All 4 animals from each group had ZIKV detectable by viral culture on multiple days from Day 2 through Day 5 post-infection. Peak virus was recovered on Day 3 or Day 4 post-infection (Table 2). It has been our experience from our dengue models that NHP develop viremia earlier than do humans. Of note, the ZIKV that will be administered in this protocol will be first given at a dose 200-fold lower than that which was administered to rhesus monkeys.

Table 2: ZIKV Viremia in Rhesus macaques

ZIKV strain	Dose	Mean Peak Titer (log_{10} PFU/mL)	Peak Titer (log_{10} PFU/mL)	Mean Day of Onset	Mean Duration of

					viremia, days (range)
SJRP/2016	20,000 PFU	1.8 ± 0.1	1.9	2.3	3.5 (3 – 4)
Nicaragua/2016	20,000 PFU	2.3 ± 0.1[1]	2.5	2.0 (all)	3.3 (3 – 4)

PFU = plaque-forming units, ZIKV = Zika virus

[1]The mean peak titer of ZIKV SJRP/2016 was significantly lower than that of ZIKV Nicaragua/2016, $p < 0.05$.

1.3.2 Previous Human Experience

1.3.2.1 DENV-2 Controlled Human Infection Model (CHIM)

We have utilized a DENV-2 and a DENV-3 CHIM to evaluate the protective efficacy of the live attenuated tetravalent dengue vaccine candidates TV003 and TV005. The DENV-2 CHIM virus was derived from DENV-2 Tonga/74, which caused only mild disease during an outbreak in Tonga [46]. Although originally developed as a DENV-2 candidate vaccine, the recombinant virus (rDEN2Δ30) was not attenuated when compared to its wild-type (Wt) parent in rhesus macaques and was not considered for evaluation as a vaccine candidate. However, it was repurposed for use as a DENV-2 challenge virus in the dengue CHIM. rDEN2Δ30 has been administered to more than 50 subjects in 3 different challenge studies. A dose of 1,000 PFU induced viremia in 100% of subjects at a mean peak titer of 2.3 $\log_{10}$ PFU/mL, approximately 100-fold higher than that observed with the attenuated DENV included in TV003 and TV005 [47, 48]. The mean day of onset of viremia was nearly day 5 post-infection and the mean day of peak viremia was nearly 7 days post-infection (Table 3**)**. The level of viremia observed in humans was similar to that observed in rhesus macaques (2.3 $\log_{10}$/mL PFU vs 1.7 $\log_{10}$ PFU/mL) but the duration of viremia was longer in humans (5.2 days vs 2.8 days) [49]. It also induced a mild to moderate rash in 88% of subjects to whom it was administered. Fever was not observed in any subject. Both TV003 and TV005 were demonstrated to protect 100% of vaccinated volunteers against challenge with DEN2Δ30 administered 6 months after vaccination [48].

1.3.2.2 DENV-3 CHIM

The DENV-3 CHIM virus rDEN3Δ30 was derived from a Wt DENV-3 (Sleman/78) that was noted to cause mild illness during its outbreak in Indonesia [50]. It was originally developed as a candidate DENV-3 vaccine virus but was not attenuated in rhesus macaques compared with its Wt parent. It was first evaluated in 10 healthy, flavivirus-naïve subjects at a dose of 1,000 PFU and induced detectable infectious virus in all 10 subjects. The mean peak titer was 0.5 $\log_{10}$ lower than that observed with the DEN2Δ30 and the absolute peak titer was more than a log lower (Table 3). The level of viremia observed in humans was similar to that observed in rhesus macaques (1.77 $\log_{10}$/mL PFU vs 1.5 $\log_{10}$ PFU/mL) but the duration of viremia was slightly longer in humans (3.4 days vs 2.0 days) [51]. A mild to moderate rash was observed in 80% of DEN3Δ30 recipients; fever was not observed in any recipient. The DENV-3 CHIM was utilized at a dose of 10,000 PFU to evaluate the protective efficacy of TV005 against DENV-3. TV005 elicited 100% protection against challenge with DENV-3 at 6 months.

Table 3: Viremia Induced by the DENV-2 and DENV-3 CHIM Viruses in Normal Healthy Volunteers

Virus	N	Dose	Mean peak titer ± SE (log_{10} PFU/mL)	Peak titer (log_{10} PFU/mL)	Mean day of peak titer ± SE	Mean Day of onset ± SE	Mean duration of viremia, days ± SE
rDEN2Δ30	57	1,000 PFU	2.31 ± 0.07	3.3	6.9 ± 0.2	4.9 ± 0.3	5.2 ± 0.3
rDEN3Δ30	10	1,000 PFU	1.77 ± 0.15	2.1	5.3 ± 0.3	4.6 ± 0.4	3.4 ± 0.6

N = number of subjects, PFU = plaque-forming units, SE = standard error

1.3.2.3 ZIKV-SJRP (Cohort 1)

ZIKV-SJRP or placebo was administered to 14 women in cohort 1 randomized 5:2 in 2 blocks of volunteers. Ten women received ZIKV-SJRP and 4 women received placebo. Adverse events following administration of ZIKV-SJRP or placebo are shown in Table 4. ZIKV-like rash was observed in 9/10 volunteers who received ZIKV-SJRP and was the most common adverse event related to ZIKV-administration. Non-purulent conjunctivitis occurred in 50% of ZIKV-SJRP recipients, arthralgia in 70% and myalgia in 50%. Fever was not observed nor were any signs or symptoms consistent with GBS.

ZIKV-SJRP was recovered from 100% of those who received the virus. Both infectious virus and virus detected by PCR were recovered from the serum of all 10 infected volunteers (Figure 5). The virus detected by PCR was below the cut-off for discharge from the inpatient unit at all times post-administration. Infectious virus titer peaked at day 5 in 6/10 volunteers. The mean peak titer was 46.8 PFU/mL (1.67 log_{10} PFU/mL). Peak virus titer was below 100 PFU/mL (2 log_{10} PFU/mL) in 8/10 volunteers and below 1,000 PFU/mL (3 log_{10} PFU/mL in the remaining 2 volunteers. Infectious ZIKV-SJRP was found infrequently in CVS, saliva, and urine (Table 5). ZIKV-SJRP was found by qPCR in urine, saliva, and CVS in 80%, 70%, and 50% of women, although the titers were low and the duration of shedding short (Table 5). ZIKV-SJRP was not recovered from any specimen taken from placebo recipients. The low peak titer following inoculation with 100 PFU of ZIKV-SJRP suggests that challenge studies may be safely performed in an outpatient setting.

Table 4: All Adverse Events Following One Dose of Cohort 1 (ZIKV-SJRP/placebo) (CIR 316)

	ZIKV-SJRP (n=10) N (%)	Placebo (n=4) N (%)	P value (1-sided)[1]
Local			
Erythema	1 (10.0%)	0 (0.0%)	0.7143
Tenderness	1 (10.0%)	0 (0.0%)	0.7143
Pain	0 (0.0%)	0 (0.0%)	n/a
Pruritus	0 (0.0%)	0 (0.0%)	n/a
Induration	0 (0.0%)	0 (0.0%)	n/a
Systemic			
Fever	0 (0.0%)	0 (0.0%)	n/a
Headache	9 (90.0%)	3 (75.0%)	0.9341
Zika-like Rash	9 (90.0%)	0 (0.0%)	0.0050
Neutropenia[2]	1 (10.0%)	0 (0.0%)	0.7143

	ZIKV-SJRP (n=10) N (%)	Placebo (n=4) N (%)	P value (1-sided)[1]
Nonpurulent conjunctivitis	5 (50.0%)	0 (0.0%)	0.1259
Elevated ALT	0 (0.0%)	1 (25.0%)	1.0000
Myalgia	5 (50.0%)	0 (0.0%)	0.1259
Arthralgia	7 (70.0%)	0 (0.0%)	0.0350
Retro-orbital Pain	4 (40.0%)	0 (0.0%)	0.7203
Fatigue	5 (50.0%)	2 (50.0%)	0.4286
Muscle weakness	1[3] (10.0%)	0 (0.0%)	0.7143
Prolonged PT	0 (0.0%)	0 (0.0%)	n/a
Hyporeflexia	0 (0.0%)	0 (0.0%)	n/a
Prolonged PTT	0 (0.0%)	0 (0.0%)	n/a
Thrombocytopenia	0 (0.0%)	0 (0.0%)	n/a

1. Greater for ZIKV-SJRP
2. Neutropenia was defined as an ANC ≤ 1,000/mm^3.
3. Muscle weakness was reported by the volunteer but was not detected on physical examination. It lasted 1 day.

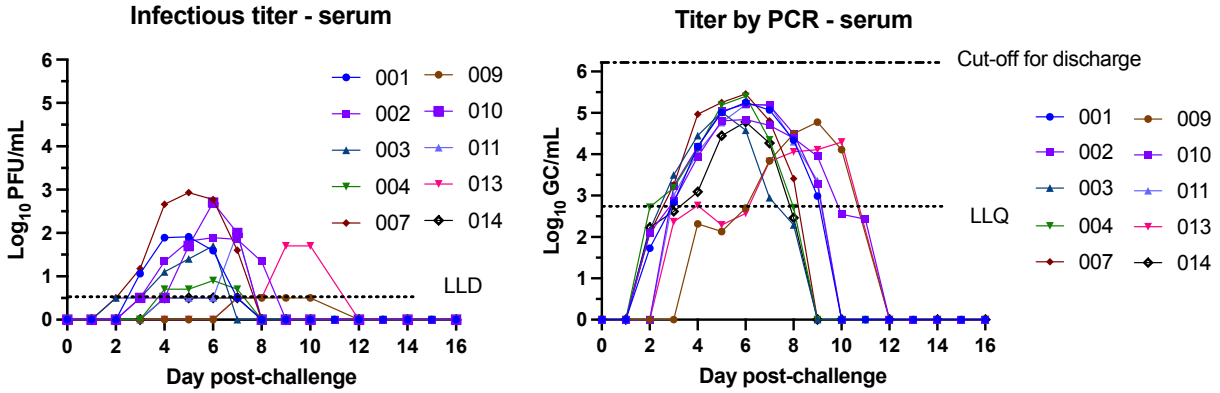

Figure 5: Kinetics of ZIKV-SJRP in Serum by Culture and Quantitative PCR

Table 5: Recovery of ZIKV-SJRP from Serum, Whole Blood, CVS, Saliva, and Urine

Sample	Frequency (N=10)	Mean peak titer (log_{10}PFU/mL)	Mean day of onset	Mean duration (days)	Duration range (days)
ZIKV-SJRP recovered by virus culture (infectious virus) (Day 0 – 28)					
Serum	100%	1.67	4.0	4.7	4 - 6
Whole blood	40%	1.7	5.3	2.3	1 - 3
CVS	10%	2.30	10.0	1.0	1 - 1
Saliva	30%	0.50	8.7	1.0	1 - 1
Urine	10%	0.7	10.0	3.0	3 - 3
ZIKV-SJRP recovered by quantitative PCR (Day 0 – 28)					
Serum	100%	5.11	2.5	7.5	7 - 10
Whole blood	100%	4.19	4.2	7.8	4 - 15
CVS	50%	2.72	10.7	3.8	1 - 7
Saliva	70%	2.69	10.3	1.9	1 – 5
Urine	80%	3.00	11.5	3.4	1 - 10

1.3.2.4 ZIKV-Nicaragua (Cohort 2)

ZIKV-Nicaragua or placebo has been administered to 14 women in cohort 2 randomized 5:2 in 2 blocks of volunteers. Ten women received ZIKV-Nicaragua and 4 women received placebo, however the treatment assignment has been unblinded for only 7 of the 14 volunteers at this time. Adverse events following administration of ZIKV-Nicaragua or placebo are shown in Table 6. ZIKV-like rash was observed in 5/5 volunteers who received ZIKV-Nicaragua and was the most common adverse event related to ZIKV-administration. Myalgia in 80% of ZIKV-Nicaragua recipients and arthralgia was observed in 60%. Fever was not observed nor were any signs or symptoms consistent with GBS. Non-purulent conjunctivitis was not observed in recipients of ZIKV-Nicaragua.

ZIKV-Nicaragua was recovered from the serum and whole blood of 5/5 volunteers who received the virus (100%). Both infectious virus and virus detected by quantitative PCR were recovered from the serum of all 5 infected volunteers (Figure 6). The virus detected by PCR was above the cut-off for discharge from the inpatient unit (> 6.4 log_{10} GEQ/mL) for only 1 time-point for only 2 volunteers (both day 6). The titer was below the cut-off for discharge for all other time-points and all other volunteers. The mean peak titer of infectious ZIKV-Nicaragua in the serum was 4,266 PFU/mL (3.63 log_{10} PFU/mL) which was significantly higher than that of ZIKV-SJRP (46.8 PFU/mL; 1.67 log_{10} PFU/mL), $\alpha = 0.01$. The higher infectious peak titer following inoculation with 100 PFU of ZIKV-Nicaragua suggests that this virus is likely to be transmitted to mosquitoes. The mean peak titer in serum by quantitative PCR was also significantly higher for ZIKV-Nicaragua compared with ZIKV-SJRP (6.29 log_{10} GEQ/mL vs 5.11 log_{10} GEQ/mL, respectively, α=0.01).

Table 6: All Adverse Events Following One Dose of Cohort 2a (ZIKV-Nicaragua/Placebo) (CIR 316)

	ZIKV-Nicaragua (n=5) N (%)	Placebo (n=2) N (%)	P value (1-sided)[1]
Local			
Erythema	0 (0.0%)	0 (0.0%)	n/a
Tenderness	0 (0.0%)	0 (0.0%)	n/a
Pain	0 (0.0%)	0 (0.0%)	n/a
Pruritus	0 (0.0%)	0 (0.0%)	n/a
Induration	0 (0.0%)	0 (0.0%)	n/a
Systemic			
Fever	0 (0.0%)	0 (0.0%)	n/a
Headache	4 (80.0%)	2 (100.0%)	1.0000
Zika-like Rash	5 (100.0%)	0 (0.0%)	0.0476
Neutropenia[2]	0 (0.0%)	0 (0.0%)	n/a
Nonpurulent conjunctivitis	0 (0.0%)	1 (50.0%)	1.0000
Elevated ALT	2 (40.0%)	0 (0.0%)	0.4762
Myalgia	5 (100.0%)	1 (50.0%)	0.2857
Arthralgia	3 (60.0%)	0 (0.0%)	0.2857
Retro-orbital Pain	4 (80.0%)	1 (50.0%)	0.9524
Fatigue	5 (100.0%)	2 (100.0%)	n/a
Muscle weakness	0 (0.0%)	0 (0.0%)	n/a
Prolonged PT	0 (0.0%)	0 (0.0%)	n/a
Hyporeflexia	0 (0.0%)	0 (0.0%)	n/a
Prolonged PTT	0 (0.0%)	0 (0.0%)	n/a
Thrombocytopenia	0 (0.0%)	0 (0.0%)	n/a

1. Greater for ZIKV-Nicaragua
2. Neutropenia was defined as an ANC $\leq$ 1,000/mm^3.

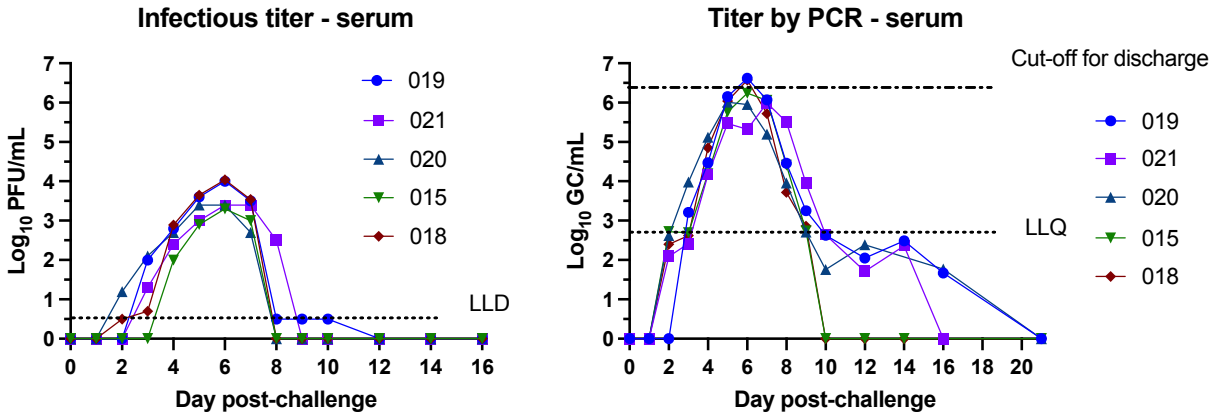

Figure 6: Kinetics of ZIKV-Nicaragua in Serum by Culture and Quantitative PCR

Table 7: Recovery of ZIKV-Nicaragua from Serum, Whole Blood, CVS, Saliva, and Urine

Sample	Frequency (N=5)	Mean peak titer (log_{10}PFU/mL)	Mean day of onset	Mean duration (days)	Duration range (days)
ZIKV-Nicaragua recovered by virus culture (infectious virus) (Day 0 – 28)					
Serum	100%	3.63	4.04	3.0	5.4
Whole blood	100%	3.52	3.90	4.4	4.2
CVS	0%	n/a	n/a	n/a	n/a
Saliva	100%	0.58	0.70	8.0	3.4
Urine	20%	0.50	0.50	6.0	1.0
ZIKV-Nicaragua recovered by quantitative PCR (Day 0 – 28)					
Serum	100%	6.29	6.57	2.4	11.4
Whole blood	100%	5.62	6.21	3.2	8.8
CVS	80%	2.43	2.89	8.5	4.0
Saliva	100%	2.71	3.80	10.8	2.2
Urine	80%	2.86	4.23	8.0	5.5

2 OBJECTIVES

2.1 Primary Objectives

- Determine the suitability of both ZIKV strains for use in a safety-assured Zika CHIM. Suitability will be assessed by:
  - The frequency and severity of clinical signs and symptoms of infection such as fever, rash, conjunctivitis, myalgia, arthralgia, and headache following ZIKV administration compared with placebo.
  - The infection frequency of ZIKV by strain and by dose. Infection is defined as recovery of ZIKV (determined by RT-PCR or culture) from blood, urine, cervico-vaginal secretions, semen, or saliva and/or by seroconversion to ZIKV (50% plaque reduction neutralization titer [$PRNT_{50}$]≥ 1:10) by 90 days post-administration of the ZIKV strain.

  - The frequency, magnitude, and duration of ZIKV presence in the blood, urine, cervico-vaginal secretions, semen, and saliva by both RT-PCR and virus titration in tissue culture by ZIKV strain and dose.
- A suitable ZIKV CHIM strain is defined as one that:
  a. ≥ 80% of ZIKV and DENV-naïve inoculated subjects develop detectable viremia by virus culture (infectious virus) ***and***
  b. Infectious virus is detected on more than 1 day for ≥ 80% of subjects with detectable viremia ***and***
  c. The mean peak titer of infectious virus recovered from subjects is 2 - 3.0 $\log_{10}$ PFU/mL

  Ideally ≥ 50% of ZIKV and DENV-naïve inoculated subjects will develop ≥ 1 of the following clinical signs thought to be definitely, probably, or possibly due to the ZIKV strain, however this will not be required for dose expansion or dose escalation:

  i. Fever
  ii. Rash
  iii. Non-purulent conjunctivitis
  iv. Myalgia
  v. Arthralgia

A mean peak titer of 2.0 – 3.0 $\log_{10}$ PFU/mL should ensure that virus is readily detected but at a level that reduces the risk of symptomatic illness and virus transmissibility.

2.2 Secondary Objectives

- Characterize the clinical presentation of acute ZIKV infection by assessing the frequency of adverse events (AEs), graded by severity, occurring within 28 days of ZIKV administration.
- 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 ZIKV strain at each tested dose as determined by RT-PCR and virus culture
  - The quantity and duration of ZIKV shedding in saliva determined by RT-PCR and virus culture
  - The quantity and duration of ZIKV shedding in urine determined by RT-PCR and virus culture
  - The quantity and duration of ZIKV shedding in cervico-vaginal secretions determined by RT-PCR and virus culture
  - The quantity and duration of ZIKV shedding in semen determined by RT-PCR and virus culture
- Evaluate the kinetics of the serum neutralizing antibody response following primary ZIKV infection and determine the peak neutralizing antibody response to ZIKV through Study Day 90.

2.3 Exploratory Objectives

The exploratory objectives of this study are the following:

- Evaluate the cellular immune response to primary infection with ZIKV.
- Evaluate the innate immune response to primary infection with ZIKV.

- Evaluate B and T cell memory responses following primary infection with ZIKV.
- Evaluate the antibody repertoire induced by ZIKV infection.
- Evaluate the antibody response in saliva to primary infection with ZIKV as a possible diagnostic tool. This will be done in a subset of subjects (those enrolled at the Center for Immunization Research [CIR]).
- Evaluate the transmissibility of ZIKV-SJRP and ZIKV-Nicaragua from infected volunteers to mosquitoes.

3 STUDY DESIGN

3.1 Receipt of Test Article

3.1.1 Overall Design

This study is a placebo-controlled, double-blind study in normal healthy adult male and non-pregnant female subjects 18 - 40 years of age, inclusive, recruited from the metropolitan Baltimore/Washington, DC and Burlington, VT areas. The purpose of this study is to evaluate the clinical and virologic response to escalating doses of 2 different ZIKV strains administered subcutaneously in healthy, ZIKV and DENV-naïve, male and non-pregnant, female adult volunteers to identify the most suitable ZIKV strain and dose for use in a ZIKV CHIM. The ZIKV CHIM will then be used to evaluate the protective efficacy of candidate ZIKV vaccines prior to evaluation of these candidates in Phase 2 clinical trials. Placebo recipients are included in the study as a control to better assess ZIKV-associated versus non-ZIKV-associated AEs.
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, urine toxicology screening for opiates and serology screening for previous ZIKV or DENV infection. Serum pregnancy testing will be performed on applicable female subjects. All screening tests must be performed within 60 days of inoculation. HIV screening must be performed within 2 weeks of inoculation. Pregnancy screening will be repeated within 14 days prior to inoculation and on the day 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 4 of this protocol. For subjects who are eligible, the Day 0 visit will be scheduled for receipt of inoculation. Serum pregnancy testing will be repeated on 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 (ZIKV or placebo). Subjects will present to the unit on Study Day 0. After eligibility criteria have been reviewed and confirmed, they will be inoculated with test article on Study Day 0. Until mosquito-transmissibility data are available, subjects will be admitted to the inpatient unit and will be kept in the inpatient unit through Study Day 9 (discharged on Study Day 9, for a total of 9 nights), the period of time when viremia is expected to be present and at a level that would be transmissible to mosquitoes. If mosquito-transmissibility data from cohorts 3 and 4 demonstrate that ZIKV-SJRP or ZIKV-Nicaragua cannot escape the mid-gut barrier of the mosquito, additional studies of these ZIKV may be considered for outpatient evaluation for the duration the subjects' enrollment. The protocol will be revised to include an outpatient enrollment if the transmissibility studies support this.
For the inpatient portion of the study, subjects will remain in the inpatient unit from Study Day 0 through Study Day 9 (9 nights, discharged on Study Day 9). If they meet discharge criteria (See

Section 6.4.2), they will be discharged from the inpatient unit on Study Day 9. They will be evaluated every day during the inpatient stay and will be seen again on Study Day 9, Study Day 10, Study Day 12, Study Day 14, and Study Day 16. During the outpatient and inpatient visits through Study Day 28, the subjects will be evaluated by a clinician and will have blood drawn for clinical laboratory studies, virologic assays, and immunologic assays. In addition, urine, cervico-vaginal secretions, semen, and saliva will be collected at specific time points for additional virologic assays (see Section 6 for a detailed description of study procedures). Subjects will return to the clinic on Study Days 21, 28, 35, 56, 70, 90, 120, and 180 for evaluation, blood and specimen collection. Study Day 180 will be their final visit. Subjects will have their temperature measured and recorded at least twice a day during their inpatient stay, with the exception of day of discharge. Temperature will be recorded once by study staff prior to discharge.

3.1.2 Study Design

This study will include 4 cohorts of 14 ZIKV and DENV-naïve female and male subjects, 18 - 40 years of age (total: up to 56 subjects). Within each cohort, 10 subjects will receive ZIKV and 4 subjects will receive a placebo on Study Day 0. Cohorts 1 and 2 (Dose = 10^2 PFU) will be enrolled first and will enroll only women. Cohorts 3 and 4 (Dose = 10^2 PFU) will enroll men.

3.1.2.1 Initial Enrollment of Each Cohort

For each cohort, only the first 7 subjects (5 ZIKV recipients; 2 placebo recipients) will be enrolled until all 7 subjects have completed Study Day 28 and the criteria listed in Section 3.1.2.2 have been met. If the criteria to enroll the remaining 7 subjects from a dose cohort are met, enrollment will proceed. For cohorts 3 and 4, mosquito-feeding will be performed on a subset of enrolled volunteers. A total of 7 volunteers (randomized 5:2 ZIKV:placebo) from cohort 3 and 7 volunteers (randomized 5:2 ZIKV:placebo) from cohort 4 will undergo mosquito-feeding.

3.1.2.2 Enrollment of Remaining 7 Subjects in a Dose Cohort

The remaining 7 subjects in a dose cohort may be enrolled if the following criteria are met:

1. Halting criteria have not been met ***and***
2. ≥ 4 subjects in a cohort have detectable infectious virus with a mean peak titer ≥ 1.0 $\log_{10}$ PFU/mL ***and***
3. ≥ 4 subjects in a cohort have detectable infectious virus for at least 2 days

3.1.3 Sample Size and Placebo Ratio Primary Study

Fourteen subjects (10 ZIKV and 4 placebo recipients) will be enrolled into each dose cohort. This sample size was chosen based on our experience in developing the DENV-2 CHIM (CIR286, clinical trials.gov NCT01931176, [47]) and the DENV-3 CHIM (CIR304, clinical trials.gov NCT02684383).

Ten subjects are chosen to receive each ZIKV strain at each dose for the following reasons:

- A sample size of 10 ZIKV recipients and 4 placebo recipients in each cohort will be able to detect a statistically significant difference ($\alpha = 0.05$) in recovery of infectious ZIKV from the blood between the 10 ZIKV recipients and 4 placebo recipients at a power of 0.99 with ≥80% of ZIKV-inoculated subjects having detectable ZIKV viremia and none of the placebo recipients having detectable viremia.

- A sample size of 10 ZIKV recipients and 4 placebo recipients in each cohort will be able to detect a statistically significant difference ($\alpha = 0.05$) in any clinical sign between the 2 groups at a power of 0.71 if the clinical sign occurs in ≥80% of ZIKV recipients and occurs in ≤ 1 placebo recipients. We have included placebo-recipients so that we can determine if common AEs are ZIKV-related.

3.1.4 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.

3.1.5 Estimated Duration of the Study

The entire duration of the study is approximately 52 weeks. 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).

3.1.6 Treatment Assignment

Subjects will be randomly assigned to receive ZIKV or placebo in a 5:2 ratio within each cohort. Inoculation assignment will be done using a random number generator to prepare the sequence in which subjects are assigned to receive ZIKV or placebo. Subjects will be centrally randomized by the test article preparation study staff at Johns Hopkins Bloomberg School of Public Health (JHSPH) in blocks of 7. There will be 2 blocks of 7 subjects for each cohort and there will be a total of 4 cohorts enrolled. Each block of 7 will include 5 ZIKV recipients and 2 placebo recipients. As described above, each cohort will initially enroll one block of 7 subjects. The second block of 7 subjects will be enrolled if criteria in Section 3.1.2.2 are met. Subjects will be enrolled sequentially. Cohorts of both ZIKV strains may be enrolled simultaneously. Once all 7 subjects in a block have reached Study Day 90, the inoculation assignment for that block may be unblinded to all staff.

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 are inoculated. Clinical staff will remain blinded to inoculation assignment until all subjects within a block have reached Study Day 90.

A master log of inoculation assignments will be maintained in a record separate from other study records. This log will be maintained in a locked room with limited access. A sealed envelope containing a copy of the inoculation assignment will also be kept by the Data and Safety Monitoring Board (DSMB) Executive Secretary.

The subjects will be informed of their inoculation assignment after their block has completed Study Day 90.

3.1.7 Blinding

This study will be conducted as a double-blind study to avoid biased assessment of AEs. ZIKV or placebo will be prepared and drawn up into syringes by pharmacy/laboratory personnel who are not involved with the clinical assessment of subjects or performing research assays. Syringes are labeled according to standard operating procedures (SOPs)/recommendations. Because the

ZIKV diluent, PlasmaLyte, is used as placebo, there will be no difference in the appearance of the syringes.

The subject, investigator, and clinical staff will not know to which inoculation group the subject has been assigned. In addition, other personnel assigned to monitor the study will not know the inoculation assignment of the subject. If all volunteers within a block of 7 are able to be discharged from the inpatient unit on Study Day 9, the clinical staff will remain blinded to inoculation 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 inoculation assignments in writing from the unblinded study staff.

If one or more subjects within a block of 7 does not meet discharge criteria by Study Day 9, those subjects must remain in the unit. To avoid undue burden on the subjects who have met discharge criteria, only those subjects who did not meet discharge criteria will remain in the unit even though this will be unblinding. If the need arises to unblind a specific subject's assignment for emergency medical management prior to all subjects within a block completing Study Day 90 post-inoculation, the PI will contact the CIR unblinded staff to obtain the inoculation assignment of the subject in question. Only that specific subject's assignment will be unblinded. The Sponsor 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.

4 SELECTION AND ENROLLMENT OF SUBJECTS

Subjects must meet all eligibility criteria to be enrolled in the study. Because male-to-female sexual transmission of ZIKV is by far the most common pattern of sexual transmission, all male volunteers enrolled in the study must agree to use barrier contraception through Study Day 90 (per CDC guidance) post-enrollment. Female volunteers must use barrier contraception through Study Day 56 (per CDC guidance). Men will be enrolled to quantify the shedding of infectious ZIKV in semen. These data will better assess the risk of male-to-female transmission. Because ZIKV infection during pregnancy can be catastrophic to the fetus, female volunteers of childbearing potential will be required to use reliable methods of contraception, which include hormonal birth control (implantable, hormonal patch, NuvaRing®, oral contraception, Depo-Provera injection, etc.), IUD, or surgical sterilization (documented hysterectomy, tubal ligation or tubal coil; surgical sterilization procedures must be at least 3 months prior to inoculation) following CDC recommendations. Barrier contraception alone will not be considered a reliable method of birth control. Females who are postmenopausal (as defined as at least 1 year since last menstrual period); and females who exclusively have sex with females (and have no intention of conceiving a child during the study) are not considered to be of childbearing potential. Female volunteers on hormonal birth control must not be on medications or other agents that decrease the effectiveness of hormonal birth control.

To reduce the potential risk of transmission, ALL male subjects will be required to use barrier contraception through Study Day 90 and all female subjects will be required to use barrier contraception through Study Day 56 in accordance with CDC guidance. This includes females who exclusively have sex with females, females who are post-menopausal, and subjects who have undergone surgical sterilization.

Should a potential volunteer wish to use hormonal contraception or an IUD, study staff will refer her to a provider who can discuss the available contraception options with the subject. The study will pay for any costs of hormonal contraception not covered by insurance for the duration of the study and for the cost of placement of an IUD not covered by insurance if the subject decides she wants to enroll in the study and would like to use one of these methods of contraception.

4.1 Inclusion Criteria

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

1. Adult ZIKV or DENV-naïve male and non-pregnant females 18 – 40 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, approximately 26 weeks post-inoculation.
4. Must be able to complete the informed consent process and comprehension assessment independently and without assistance.
5. Willingness to participate in the study as evidenced by signing the informed consent document.
6. Willingness to reside in the inpatient unit for 9 days (or longer for safety if necessary) following receipt of ZIKV or placebo.
7. Male subjects: Willingness to use barrier contraception during cervico-vaginal, anal, and oral intercourse through Study Day 90 (in accordance with CDC guidance).
8. Female subjects: Willingness to use barrier contraception during cervico-vaginal, anal, and oral intercourse through Study Day 56 (in accordance with CDC guidance).
9. Female subjects of childbearing potential must be willing to use effective contraception while at risk of Zika infection. We will follow CDC guidelines for the use of effective contraception of 8 weeks post-infection; time of infection is defined as inoculation with challenge virus. Reliable methods of contraception include: hormonal birth control* (implantable, hormonal patch, hormonal vaginal ring, oral contraception, Depo-Provera injection, etc.), surgical sterilization (hysterectomy, tubal ligation, or tubal coil at least 3 months prior to inoculation), and intrauterine device. All female subjects will be considered having child-bearing potential except for those with post-menopausal status documented as at least 1 year since last menstrual period and females who have sex with females (exclusively) and have no intention of conceiving a child during the study. Females who are not considered to be of childbearing potential will not be required to use contraception other than barrier contraception for the purpose of reducing potential transmission.

   *Volunteers on hormonal birth control must not be on medications or other agents that decrease the effectiveness of hormonal birth control.

4.2 Exclusion Criteria

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

1. Currently pregnant, as determined by positive beta-human choriogonadotropin (β-hCG) test, breast-feeding or planning to become pregnant during the 6-month duration of the study.
2. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, rheumatologic, autoimmune, or renal disease by history, physical examination, and/or laboratory studies.
3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and cooperate with the requirements of the study protocol.
4. Evidence of recent opiate use based on urine toxicology screen.
5. Screening laboratory values of Grade 1 or above for absolute neutrophil count (ANC), 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 which 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, by screening and confirmatory assays.
11. Hepatitis C virus (HCV) infection, by screening and confirmatory assays.
12. Hepatitis B virus (HBV) infection, by hepatitis B surface antigen (HBsAg) screening.
13. History of Guillain-Barré syndrome (GBS).
14. History of seizure disease or peripheral neuropathy
15. History of any neuroinflammatory disorder i.e. Bell's Palsy, transverse myelitis
16. Any known immunodeficiency syndrome, including that caused by malignancy.
17. Use of anticoagulant medications (use of antiplatelet medication such as aspirin or non-steroidal anti-inflammatory medication is permitted and will not exclude a subject from enrollment).
18. Use of corticosteroids (excluding topical or nasal) or immunosuppressive drugs within 28 days prior to or following inoculation. Immunosuppressive dose of corticosteroids is defined as ≥10 mg prednisone equivalent per day for ≥14 days.
19. Receipt of a live vaccine within 21 days or a killed vaccine within the 14 days prior to inoculation or anticipated receipt of any vaccine during the 21 days following inoculation with the exception of the killed influenza vaccine and COVID-19 vaccines either licensed or under EUA which can be given at any time, however all effort will be made to avoid giving influenza and COVID-19 vaccines within the above windows.
20. Asplenia.
21. 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.
22. History or serologic evidence of previous ZIKV infection or DENV infection
23. Previous receipt of a ZIKV or DENV vaccine (licensed or investigational).
24. Anticipated receipt of any investigational agent in the 28 days before or after inoculation.
25. Subject has definite plans to travel to a ZIKV-endemic or dengue-endemic area during the study.
26. Previous hypersensitivity to any study product component.

27. Anaphylactic reaction to mosquito bites.
28. Refusal to allow storage of specimens for future research.

4.3 Subject Withdrawal and Termination Criteria

A subject will not be considered to have completed the trial if any of the following apply. However, any subject who has received ZIKV 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 withdrawn will not be replaced.

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 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 integrity of the study data 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*** – is a category used when previous categories do not apply and requires an explanation.

Special Situations

Pregnancy:

To avoid the possibility of becoming infected with ZIKV while pregnant, all female subjects will be screened for pregnancy during screening and again within 14 days prior to enrollment and on Day 0 prior to the administration of the test article. All female subjects, with the exception of females who exclusively have sex with females (and have no intention of conceiving a child during the study) and women who are postmenopausal, must agree to use reliable birth control while at risk of Zika infection. We will follow CDC guidelines for the use of effective contraception of 8 weeks post-infection; time of infection is defined as inoculation with challenge virus. Female subjects may be asked to sign a release of medical information form so that records can be obtained to confirm the method of birth control. All subjects will receive pregnancy prevention counseling from study staff prior to enrollment, during their inpatient stay, and at outpatient in-person visits at Days 9, 10, 12, 14, 16, 21 and at each in-person visit after Study Day 21. If outpatient in-person visits are greater than 30 days apart, we will perform pregnancy prevention counseling by phone. If a subject becomes pregnant at any time during the study, she will be followed for the duration of her pregnancy and will be seen in the clinic for periodic safety evaluations. She 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. In addition, after she has completed her pregnancy, she may be asked to return to the clinic for an in-person visit to review the outcome of her pregnancy and the health of the baby.

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 3 attempts have been made to contact the subject, followed by nonresponse to a certified letter to the last known address requesting that the subject contact the clinical site.

Incarceration:

In the event a subject becomes incarcerated during the study, they may be terminated from the study if the period of incarceration will prevent the subject from making the scheduled visits.

4.4 Access to Medical Records

The medical history of a subject will be obtained from the subject and will be documented. Medical records from an outside institution may be requested to verify method of contraception and may also be requested to clarify the subject's medical history or clarification of Adverse Events as needed. Medical records from an outside institution will not be requested without the medical release form signed by the subject.

5 TEST ARTICLE PREPARATION

5.1 Pre-inoculation Preparation

ZIKV strains for this protocol will be stored at a NIAID-contracted repository until requested by the clinical site. Vials of frozen ZIKV for administration will be formally requested to be transferred 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. ZIKV may be transferred to the study site prior to IRB approval and FDA review for the purpose of determining the titer of the test article only.

After transfer to the clinical site, each ZIKV strain will be stored in a locked freezer at -75°C ± 15°C until time of use. Each ZIKV strain is supplied as a concentrate that must be diluted with PlasmaLyte to the proper dose prior to administration.

After the ZIKV strain(s) has been prepared at the clinical site, an aliquot of thawed, undiluted (if available), and diluted ZIKV will be titrated. Additionally, the ZIKV strain(s) may be titrated periodically to ensure potency. Clinical site pharmacy/lab personnel who are not blinded to inoculation assignment will be responsible for preparing the ZIKV strain(s) and placebo. The ZIKV strain(s) 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 ZIKV strain(s) preparation that will include the protocol number, date of inoculation, dose, route of administration, the ZIKV virus name, ZIKV lot number, the titer (concentration), the investigational new drug (IND) number, and the number of doses to be administered.

The ZIKV strain(s) will be prepared according to the site's SOPs. Study staff will prepare the correct dose of ZIKV (or placebo) for each subject in a biosafety cabinet using aseptic technique. The ZIKV strains will be diluted with PlasmaLyte, which is a commercially available crystalloid solution of intravenous administration. The diluted ZIKV strain(s) (or placebo) will be drawn up to a volume of 0.5 mL in a 1 mL syringe and labeled according to SOP. The labeled syringes will be transported in a cold container to the clinic for administration. The test article must be used within 4 hours of being removed from the freezer.

Placebo for this study will be the ZIKV diluent, Plasmalyte, which is commercially available. The physical appearance of the placebo and diluted ZIKV strains is colorless and identical.

5.2 ZIKV and Diluent Storage

Each ZIKV strain should remain frozen at -75°C ± 15°C until just prior to use. ZIKV strains should never be refrozen for reuse in test article preparation. Diluent components will be stored per the manufacturer's recommendation. ZIKV and diluent components should be opened from new containers for each use. No component should be reused for future ZIKV strain or placebo preparation.

Neither ZIKV strain can be used until it is verified by the Sponsor as acceptable for use. If the Sponsor deems the ZIKV unacceptable for use, it will be quarantined until further directions from the Sponsor are received. If ZIKV is deemed unacceptable for use, it will be disposed of per the Sponsor's instruction and a new shipment will be requested.

5.3 ZIKV Strain and Diluent Accountability

Laboratory/pharmacy test article personnel will maintain an accurate inventory and accountability record of each ZIKV strain. Partially used vials of ZIKV or placebo components will not be refrozen or reused for future inoculations.

5.4 Storage Disposition of Used/Unused Supplies

After laboratory/pharmacy personnel have diluted the test article and drawn up the syringes for administration, they will remove the label from the ZIKV vials and place it on the test article preparation form. In this manner, monitoring personnel will be able to verify the accountability of all ZIKV vials used for the study. In addition, the number of ZIKV vials used will be accounted for in the study specific drug accountability log.

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

At least 1 aliquot (if available) of diluted ZIKV 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 ZIKV will be destroyed by the laboratory personnel per SOP. Any unused diluent/placebo from opened vials/bottles will also be destroyed.
After study completion or termination, all unused study product will be disposed of per sponsor's instructions.

6 STUDY PROCEDURES

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

6.1 Recruitment and General Screening

Subjects may be recruited from a variety of sources including, but not limited to, subjects previously enrolled in trials at the clinical sites, the use of a center-wide IRB-approved screening

protocol, and/or using study-specific IRB-approved print, electronic and/or other media advertising.

After an initial phone screen (using an IRB-approved Phone Screen/Initial Contact form) by clinic staff focused on providing background information of the trial and a review of basic inclusion and exclusion criteria, a screening visit may be scheduled.

6.2 Consenting Process

During the screening process, which may require more than 1 visit, the subject will undergo a robust informed consent process. At the first screening visit, the subjects may be provided with a read only copy of the informed consent form to take home with them. Additional materials including a fact sheet (with answers to frequently asked questions and URL for ethics documentation) will also be provided. We encourage our subjects to read the consent form at home prior to coming to their final screening visit. At this visit, the subject will read the consent form, be encouraged to ask questions, and then complete a comprehension assessment. The comprehension assessment is designed to test the subject's knowledge of the most pertinent and important details about the study. The subject must be able to complete the informed consent process and comprehension assessment independently and without assistance. Site specific consenting documents will be used. 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 robust 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 be encouraged to ask questions to family, friends or anyone else at home if desired. The subject will also be provided with a signed copy of the informed consent. All study-related procedures will occur only after the informed consent is signed.

6.3 Screening Procedures

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

1. Explain the study and Informed Consent process to the subject.
2. Ensure that the subject has successfully completed the informed consent comprehension assessment, has signed the informed consent, and received a copy of the signed informed consent. The comprehension assessment must be completed independently and without assistance.
3. Ensure that HIV pre-test counseling has been performed and documented (within 14 days of inoculation).
4. Elicit a complete medical history, including menstrual and contraceptive history, history of surgical sterilization, sexual history, and current sexual practices and method of contraception for all subjects.
5. Obtain a nasopharyngeal (NP) or mid-turbinate swab to test for SARS-CoV 2 – the virus that causes COVID-19 – 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.

6. Perform a complete physical examination, including vital signs (height, weight, blood pressure, respiratory rate, temperature, and pulse).
7. Obtain approximately 30 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 ZIKV or DENV infection
    - creatinine
    - alkaline phosphatase
    - HBV
    - HCV
8. Obtain approximately 5mL 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. Obtain urine for urine dipstick testing (glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, leukocyte esterase) and urine toxicology screen for opiates.
10. Obtain serum for β-HCG testing in females. Serum pregnancy will be performed at the initial screening visit, within 14 days of inoculation, and on Study Day 0 prior to inoculation. A positive β-HCG will exclude the subject from the trial.
11. Obtain medical release form to confirm method of birth control (for applicable female subjects).
12. Counsel all subjects on pregnancy prevention during the entire study period.
13. Counsel all female subjects to use barrier contraception through Study Day 56.
14. Counsel all male subjects to use barrier contraception through Study Day 90.
15. Provide education about residing in the inpatient unit until discharge on Study Day 9 (or longer as needed).
16. Provide education on the use of Cervico-vaginal cup for collection of cervico-vaginal secretions.
17. Provide education on the collection of semen.
18. Provide education on mosquito feeding (to the subset of volunteers enrolled in the mosquito-feeding study).

6.4 Detailed Study Procedures

The study procedures to be performed at each visit are listed below and in Table 1: Schedule of Procedures. Additional tests may be done at the discretion of the PI/Provider to evaluate concomitant illness or to further evaluate an adverse event 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. To maintain confidentiality, possible identifiers, such as tattoos, jewelry, and clothing will not be included in the photographs. The total volume of blood to be drawn over the 26-week post-inoculation period is approximately 700 mL, which is like donating 1 1/2 units of blood. This amount is within NIH guidelines (Medical Administrative Policy 95-9) of 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. Additional serum or urine pregnancy tests may be performed, as determined necessary by the clinician, to establish the absence of pregnancy at any time during the participation in the study.

6.4.1 Admission to the Inpatient Unit, Inoculation, and Follow-up

Subjects will be admitted to the inpatient unit on Study Day 0 and inoculated with ZIKV or placebo on Study Day 0. On the day of admission to the inpatient unit, 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. Subjects who may be alternates will be informed that they may be alternates when they are invited to the clinic.

The ZIKV will be kept frozen at -75°C ± 15°C until just before use, whereupon they will be thawed, diluted to the appropriate dose, and drawn up for administration (see Section 5 for test article preparation). The ZIKV strains and placebo will be kept in a cold container from the time they are thawed or removed from the refrigerator until they are delivered 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. For outpatient study visits following discharge, 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.

6.4.1.1 Inpatient Stay Procedures

During the inpatient stay, the subject will have vital signs (blood pressure, heart rate, respiratory rate, and temperature) taken twice a day, except for day of discharge (vital signs will be collected prior to discharge). Should a subject develop a temperature ≥ 100.4°F (38°C), the frequency of vital signs monitoring will increase to at least 3 times a day and the subject will be evaluated by a clinician at least twice a day until the subject is afebrile for 48 hours. A focused physical examination will be performed each day while inpatient and at designated outpatient visits. A focused physical exam may include, but is not limited to, blood pressure, heart rate, temperature, respiratory rate, skin examination, eyes, pertinent lymph nodes, throat, mental status, pertinent cranial nerves, gait coordination (observed gait), muscle strength, and biceps and patellar deep tendon reflexes. Additional physical exam components may be done if clinically indicated at the provider's discretion.

Laboratory studies will be done according to the schedule below. β-HCG testing will be performed on specimens collected from female volunteers only. Should a subject develop a fever ≥ 100.4°F (38°C), laboratory studies may be done more frequently at the discretion of the clinician. The unit will be staffed 24 hours a day for the duration of the inpatient stay. A clinician will evaluate all subjects at least once per day for the duration of the inpatient stay and will be available 24 hours a day for the duration of the inpatient stay should he/she be needed. During the inpatient stay, additional clinical laboratory studies may be performed to follow-up on any clinical laboratory abnormalities that may have developed during the inpatient stay. Additional diagnostic studies may be performed should the subject's clinical condition warrant (e.g., CXR, ultrasound, and ECG). Should a subject leave the inpatient unit before the 9-day stay has been completed, every effort will be made to follow-up with the subject daily to monitor for signs/symptoms of illness.

6.4.1.2 Detailed Study Procedures

Study Day 0 (Day of Inoculation with Test Article)

1. Verify that Informed Consent was obtained and that the consent form was signed by both the subject and by the study staff.
2. Verify that all applicable eligibility criteria have been met.
3. Collect 3.5mL to perform serum β-HCG testing (females only). Ensure the test is negative before proceeding; a positive test will exclude the subject from the study as per protocol, Section 4.2.
4. Obtain a nasopharyngeal (NP) or mid-turbinate swab to test for SARS-CoV 2 – the virus that causes COVID-19 – 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.
5. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints. Physical exam may extend beyond focused if indicated by complaint or physical finding.
6. Admit the volunteer to the inpatient unit.
7. Record vital signs (blood pressure, temperature, heart rate, and respiratory rate) twice a day.
8. Review and document concomitant medications.
9. Obtain approximately 57 mL of blood for CBC with differential, virus isolation and culture from serum and whole blood (PCR/culture), and immunology (serum, plasma, peripheral blood mononuclear cells [PBMCs]), and PAXGene. These laboratory studies are drawn as baseline values and will not determine eligibility.
10. Obtain 5 mL of clean-catch urine for virus isolation & culture.

11. Obtain cervico-vaginal secretion or semen sample for virus isolation & culture.
12. Obtain saliva sample for virus isolation and culture.
13. Review pregnancy prevention with the subject.
14. Obtain oral fluid for serology (CIR site only).
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 the signs and symptoms of potential AEs, and how and when to contact study staff.

Study Day 1

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.
3. Obtain approximately 15 mL of blood for virus isolation from serum and whole blood (PCR and culture) and PAXGene.

Study Day 2

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.
3. Obtain approximately 15 mL of blood for virus isolation from serum and whole blood (PCR and culture).
4. Obtain clean-catch urine for virus isolation (PCR/culture).
5. Obtain saliva sample for virus isolation (PCR/culture).
6. Obtain 2 mL of blood for PAXGene.

Study Day 3

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.
3. Obtain approximately 15 mL of blood for virus isolation from serum and whole blood (PCR and culture) and PAXGene.

Study Day 4

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.

3. Obtain approximately 47 mL of blood for CBC with differential, virus isolation from serum and whole blood (PCR/culture), immunology (serum, plasma, PBMCs), and PAXGene.
4. Obtain clean-catch urine for virus isolation (PCR/culture).
5. Obtain cervico-vaginal secretion or semen sample for virus isolation (PCR/culture).
6. Obtain saliva sample for virus isolation (culture/PCR).
7. Obtain oral fluid for serology (CIR site only).

Study Day 5

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.
3. Obtain 13 mL of blood for virus isolation from serum and whole blood (PCR/culture).
4. Cohorts 3 and 4: Perform mosquito feeding on subset of volunteers.

Study Day 6

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.
3. Obtain approximately 15 mL of blood for virus isolation from serum and whole blood (culture/PCR) and PAXGene.
4. Obtain clean-catch urine for virus isolation (PCR/culture).
5. Obtain saliva sample for virus isolation (PCR/culture).
6. Obtain oral fluid for serology (CIR site only).
7. Cohorts 3 and 4: Perform mosquito feeding on subset of volunteers.

Study Day 7

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.
3. Obtain 13 mL of blood for virus isolation from serum and whole blood (PCR/culture).
4. Cohorts 3 and 4: Perform mosquito feeding on subset of volunteers.

Study Day 8

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs twice a day.
3. Obtain approximately 52 mL of blood for CBC with differential, AST, ALT, serum creatinine, virus isolation from serum and whole blood (culture/PCR), immunology (serum, plasma, PBMCs), and PAXGene.
4. Perform serum β-HCG testing (females only).

5. Obtain clean-catch urine for virus isolation (PCR/culture).
6. Obtain cervico-vaginal secretion or semen sample for virus isolation (PCR/culture).
7. Obtain saliva sample for virus isolation (PCR/culture).
8. Obtain oral fluid for serology (CIR site only).

Study Day 9

1. Perform interim history and focused physical exam, concentrating on any acute complaints.
2. Record vital signs.
3. Obtain 13 mL of blood for virus isolation from serum and whole blood (PCR/culture).
4. Review pregnancy prevention and continued use of barrier contraceptives.
5. Provide volunteer with diary card to record temperature twice a day.
6. Provide education by study staff describing proper use of thermometer and memory card.
7. Provide education by study staff describing the signs and symptoms of potential AEs, and how and when to contact study staff.
8. Provide subjects with insect repellent and education on how to prevent mosquito bites (May – October).
9. **May discharge from inpatient unit if subject meets discharge criteria** (Section 6.4.2).

Study Day 10 (+ 1 day from 10 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.
2. Record vital signs from subject diary card and perform vital signs.
3. Obtain approximately 15 mL of blood for PAXGene and virus isolation from serum and whole blood (PCR/culture).
4. Obtain clean-catch urine for virus isolation (PCR/culture).
5. Obtain cervico-vaginal secretion sample for virus isolation (PCR/culture).
6. Obtain saliva sample for virus isolation (PCR/culture).
7. Obtain oral fluid for serology (CIR site only).
8. Perform serum β-HCG testing (females only).
9. Review pregnancy prevention and continued use of barrier contraceptives.

Study Day 12 (± 1 day from 12 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.
2. Record vital signs from subject diary card and perform vital signs.
3. Obtain approximately 22 mL of blood for CBC with differential, AST, ALT, serum creatinine, virus isolation from serum and whole blood (PCR/culture), and PAXGene.

4. Obtain clean-catch urine for virus isolation (PCR/culture).
5. Obtain cervico-vaginal secretion or semen sample for virus isolation (PCR/culture).
6. Obtain saliva sample for virus isolation (PCR/culture).
7. Obtain oral fluid for serology (CIR site only).
8. Perform serum β-HCG testing (females only).
9. Review pregnancy prevention and continued use of barrier contraceptives.

Study Day 14 (± 1 day from 14 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.
2. Record vital signs from subject diary card and perform vital signs.
3. Obtain approximately 45 mL of blood for immunology (serum, plasma, PBMCs), PAXGene, and virus isolation from serum and whole blood (PCR/culture).
4. Obtain clean-catch urine for virus isolation (PCR/culture).
5. Obtain cervico-vaginal secretion sample for virus isolation (PCR/culture).
6. Obtain saliva sample for virus isolation (PCR/culture).
7. Obtain oral fluid for serology (CIR site only).
8. Perform serum β-HCG testing (females only).
9. Review pregnancy prevention and continued use of barrier contraceptives.

Study Day 16 (± 1 day from 16 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.
2. Record vital signs from subject diary card and perform vital signs.
3. Obtain approximately 22 mL of blood for CBC with differential, AST, ALT, serum creatinine, virus isolation from serum and whole blood (PCR/culture), and PAXGene.
4. Perform serum β-HCG testing (females only).
5. Obtain clean-catch urine for virus isolation (PCR/culture).
6. Obtain cervico-vaginal secretion or semen sample for virus isolation (PCR/culture). (Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic).
7. Obtain saliva sample for virus isolation (PCR/culture).
8. Obtain oral fluid for serology (CIR site only).
9. Review pregnancy prevention and continued use of barrier contraceptives.

Study Day 21 (± 1 day from 21 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.

2. Record vital signs.
3. Perform serum β-HCG testing (females only).
4. Review pregnancy prevention and continued use of barrier contraceptives with all subjects.
5. Obtain approximately 40 mL of blood for immunology (serum, plasma, PBMCs), PAXGene and virology* from serum and whole blood (PCR/culture). CBC will be done if prior white blood cell count (WBC), ANC, platelet count, or hemoglobin results are a Grade I or above and unresolved, as defined by protocol, on Study Day 16.
6. Obtain clean-catch urine for virus isolation (PCR/culture).
7. Obtain cervico-vaginal secretion or semen sample for virus isolation (PCR/culture). (Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic).
8. Obtain saliva sample for virus isolation (PCR/culture).
9. Obtain oral fluid for serology (CIR site only).
10. Review pregnancy prevention and continued use of barrier contraceptives.

    *Whole blood/serum virology will be done only for those subjects who had detectable ZIKV in the blood at Study Day 16.

Study Day 28 (± 2 days from 28 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.
2. Record vital signs.
3. Obtain approximately 50 mL of blood for immunology (serum, plasma, PBMCs), PAXGene and virology* from serum and whole blood (PCR/culture).
4. Obtain clean-catch urine for virus isolation* (PCR/culture).
5. Obtain cervico-vaginal secretion or semen sample for virus isolation* (PCR/culture). (Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic).
6. Obtain saliva sample for virus isolation* (PCR/culture).
7. Obtain oral fluid for serology (CIR site only).
8. Perform serum β-HCG testing (females only).
9. Review pregnancy prevention and continued use of barrier contraceptives.

    *Whole blood/serum/CVS/semen/saliva/urine virology will be done only for those subjects who had detectable ZIKV in the blood at Study Day 21.

Study Day 35 (± 2 days from 35 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.

2. Record vital signs.
3. Collect 8 mL blood for whole blood/serum virus isolation* (PCR/culture). Assays will be performed only for those subjects who had detectable ZIKV in the blood at Study Day 28.
4. Obtain clean-catch urine for virus isolation* (PCR/culture).
5. Obtain cervico-vaginal secretion or semen sample for virus isolation* (PCR/culture). Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic).
6. Obtain saliva sample for virus isolation* (PCR/culture).
7. Perform serum β-HCG testing (females only).
8. Review pregnancy prevention and continued use of barrier contraceptives with all subjects.

*Whole blood/serum/CVS/semen/saliva/urine virology will be done only for those subjects who had detectable ZIKV in the blood at Study Day 28.

Study Day 56 (± 3 days from 56 days following test article administration)

1. Perform interim history, including sexual history, and focused physical exam, concentrating on any acute complaints.
2. Record vital signs.
3. Obtain approximately 40 mL of blood for immunology (serum, plasma, PBMCs)/serum virus isolation* (PCR/culture).
4. Collect 3 mL blood for whole blood virus isolation* (PCR/culture). Assays will be performed only for those subjects who had detectable ZIKV in the blood at Study Day 35 or 49.
5. Obtain clean-catch urine for virus isolation* (PCR/culture).
6. Obtain cervico-vaginal secretion or semen sample for virus isolation (PCR/culture)*. Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic).
7. Obtain saliva sample for virus isolation* (PCR/culture).
8. Obtain oral fluid for serology (CIR site only).
9. Perform serum β-HCG testing (females only).
10. Male volunteers: Review pregnancy prevention and continued use of barrier contraceptives.

*Whole blood/serum/CVS/semen/saliva/urine virology will be done only for those subjects who had detectable ZIKV in the blood at Study Day 49.

Study Day 70 (± 3 days from 70 days following test article administration)

1. Perform interim history for interim complaints. (Physical exam is optional).

2. Record vital signs.
3. Collect 8 mL blood for whole blood/serum virus isolation* (PCR/culture). Assays will be performed only for those subjects who had detectable ZIKV in the blood at Study Day 49 or 56.
4. Obtain clean-catch urine for virus isolation* (PCR/culture).
5. Obtain cervico-vaginal secretion or semen sample for virus isolation* (PCR/culture). (Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic).
6. Obtain saliva sample for virus isolation* (PCR/culture).
7. Obtain oral fluid for serology (CIR site only).
8. Perform serum β-HCG testing (females only).
9. Male volunteers: Review pregnancy prevention and continued use of barrier contraceptives with all subjects.

*Whole blood/serum/CVS/semen/saliva/urine virology will be done only for those subjects who had detectable ZIKV in the blood at Study Day 56.

Study Day 90 (± 7 days from 90 days following test article administration)

1. Perform interim history for interim complaints. (Physical exam is optional).
2. Record vital signs.
3. Obtain approximately 40 mL of blood for immunology (serum, plasma, PBMCs)/serum virus isolation* (PCR/culture).
4. Collect 3 mL blood for whole blood virus isolation* (PCR/culture). Assays will be performed only for those subjects who had detectable ZIKV in the blood at Study Day 56 or 70.
5. Obtain clean-catch urine for virus isolation* (PCR/culture). Assays will be performed only if Day 56 or 70 sample was positive.
6. Obtain cervico-vaginal secretion or semen sample for virus isolation*. Assays will be performed only if Day 56 or 70 sample was positive. (Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic).
7. Obtain saliva sample for virus isolation* (PCR/culture). Assays will be performed only if Day 56 or 70 was positive.
8. Obtain oral fluid for serology (CIR site only).
9. Perform serum β-HCG testing (females only).
10. Review pregnancy prevention.

*Whole blood/serum/CVS/semen/saliva/urine virology will be done only for those subjects who had detectable ZIKV in the blood at Study Day 70.

Study Day 120 (± 10 days from 120 days following test article administration)

1. Perform interim history for interim complaints. (Physical exam is optional)

2. Record vital signs.
3. Collect 8 mL blood for whole blood/serum virus isolation* (PCR/culture). Assays will be performed only for those subjects who had detectable ZIKV in the blood at Study Day 70 or 90.
4. Obtain clean-catch urine for virus isolation (PCR/culture)*. Assays will be performed only if Day 70 or 90 sample was positive*.
5. Obtain cervico-vaginal secretion or semen sample for virus isolation* (PCR/culture). Assays will be performed only if Day 70 or 90 sample was positive. (Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic)*.
6. Obtain saliva sample for virus isolation* (PCR/culture). Assays will be performed only if Day 70 or 90 sample was positive.*
7. Perform serum β-HCG testing (females only).
8. Review pregnancy prevention.

*Collection of blood, urine, cervico-vaginal secretion, semen, and saliva samples for virology will be done on Study Day 120 only if virus was recovered from the sample collected at the previous time-points (Day 70 or 90) or if virology results are not available.

<u>Study Day 150 ± 7 days</u>: The subjects will be contacted, and pregnancy prevention will be reviewed.

<u>Study Day 180 (± 14 days from 180 days following test article administration)</u>

1. Perform interim history for interim complaints.
2. Perform focused physical exam.
3. Record vital signs.
4. Obtain approximately 40 mL of blood for immunology (serum, plasma, PBMCs)/serum virus isolation (PCR/culture). Virus isolation will only be performed for those subjects who had detectable ZIKV in the serum at Study Day 90 or 120.
5. Collect 3 mL blood for whole blood virus isolation (PCR/culture), if necessary. Assays will be performed only for those subjects who had detectable ZIKV in the blood at Study Day 90 or 120.
6. Obtain clean-catch urine for virus isolation (PCR/culture). Assays will be performed only if the Day 90 or 120 sample was positive*.
7. Obtain cervico-vaginal secretion or semen sample for virus isolation (PCR/culture). Assays will be performed only if Day 90 or 120 sample was positive. (Cervico-vaginal cup may be inserted at home by the volunteer prior to presentation to the clinic)*.
8. Obtain saliva sample for virus isolation (PCR/culture). Assays will be performed only if Day 90 or 120 sample was positive*.
9. Obtain oral fluid for serology (CIR site only).
10. Perform serum β-HCG testing (females only).

11. Review pregnancy prevention.

*Collection of urine, cervico-vaginal secretion, semen, and saliva samples and virology (urine, vaginal secretion, saliva) will be done on Study Day 180 only if virus was recovered from the sample collected at the previous time-points (Day 90 or 120) or if virology results are not available.

Note: Blood, urine, saliva, semen, and cervico-vaginal secretion samples will be collected at the indicated visits. PCR and/or viral culture will be performed on the samples collected at the indicated time points from Study Day 21 through Day 180 if the sample was positive on samples collected on at least one of the previous time-points. If a sample has been negative for 2 consecutive time-points from Study Day 21 onward, then PCR/culture for ZIKV will not be performed on future samples unless there is an indication that the sample may be intermittently positive and additional samples need to be tested. Samples will be collected from all subjects through Study Day 90 because identifying which subjects may not require a sample would unblind the treatment assignment. After Study Day 90, if the samples at the 2 previous time points were negative then saliva, cervico-vaginal secretions, blood, semen, and urine will not be collected for virus isolation.

6.4.2 Discharge Criteria

Subjects will be discharged from the inpatient unit on Study Day 9 if they meet the following discharge criteria:

1. Did not develop fever during the inpatient stay.
2. If fever did develop, the subject has remained afebrile for 48 hours prior to discharge.
3. Any continuing solicited AEs are Grade 2 or lower.
4. The titer of ZIKV in serum, cervico-vaginal secretions, semen, urine, or saliva, is $\leq 1.4 \times 10^6$ genomic copies/mL by Study Day 8 or has been previously demonstrated to be non-infectious at the detected titer.

Because of the low inoculum dose to be given, the low titers recovered from NHP, our experience with the dengue CHIM, and reported ZIKV literature, subjects are expected to be free of infectious transmissible virus in the serum by Day 9.

6.4.3 Mitigation Plan Should a Subject Leave the Unit Early

Potential volunteers are carefully screened prior to enrollment to ensure they understand their responsibilities and are willing and able to carry them out, including remaining in the inpatient unit until discharge criteria are met. Subjects must be willing to stay in the inpatient unit until they meet discharge criteria as an inclusion criterion. Subjects are provided with several amenities during their inpatient stay including internet access, entertainment (movies, cable television, craft projects, games, and classes. In addition, catered meals are provided to subjects while on the unit. Should a subject express a desire to leave the unit early, the PI will counsel the subject about why he or she is being asked to remain on the unit and counsel him or her to stay. However, should a subject insist upon leaving the unit prior to discharge criteria being met, the study staff will:

- Reinforce the risk of mosquito-borne & sexual transmission of ZIKV to third parties
- Review ZIKV transmission preventive measures with the volunteer

- Perform pregnancy prevention counseling
- Provide mosquito repellent containing DEET and review with volunteer how to use it. This will be done during mosquito season (May through October).
- If possible, arrange daily outpatient follow-up visits for the subject for clinical examination and to obtain scheduled blood/urine/saliva/cervico-vaginal secretion/semen samples until Study Day 9, after which point they will resume usual outpatient schedule.
- If subject cannot physically return for follow-up visits, will contact the subject by phone to inquire about adverse events and perform pregnancy prevention counseling

6.4.4 Subject Temperature Memory Card

Subjects will be provided a thermometer and a "Temperature Memory Card", to use as a memory aid and will be asked to record temperatures 2 times a day on Study Day 9 - Study Day 16. Staff will review the temperature card on Study Days 10, 12, 14, 16, and 21 post-inoculation 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 (T_{max}) for each date 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 how to use the thermometers, to take their temperatures at approximately the same times 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.

6.4.5 Persistence of ZIKV in Bodily Fluids

If Zika virus is detected in any bodily fluid at Study Day 180, the subject will continue to be followed until Zika virus 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 virus 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 virus 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.

6.5 Clinical Laboratory Testing

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

1. CBC plus white blood cell 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, test is confirmed by testing for Hepatitis C RNA. If Hepatitis C antibody is positive but RNA is negative, the volunteer is assessed as having a past infection that has been cleared. In this case, the subject is eligible for the study.
7. Urinalysis (in the event of an abnormal urine dipstick test)
8. Serum β-HCG, if required
9. Urine toxicology screen for opiates.

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. SARS-CoV-2 testing will be performed at the site using a CLIA-waived test (GeneExpert Express) or will be sent to a CLIA-certified clinical laboratory for testing. HIV testing may be performed at the clinical trial site using an FDA-approved rapid test kit or sent to the clinical laboratory. In the event of a reactive rapid HIV test, a serum sample will be sent to the appropriate lab for confirmation. Determination of ZIKV virus titer by RT-PCR and virus culture, plaque reduction neutralization antibody assays, and cellular immune studies will be done at the clinical trial site laboratory.

6.6 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 following test article administration, the medical history will be updated for any new or changed significant or chronic conditions.

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 test article administration. 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 following test article administration will be recorded throughout the trial.

6.7 Mosquito Feeding

Feeding of mosquitoes on a subset of subjects enrolled in cohorts 3 and 4 will be conducted as described for rDEN4Δ30 except the days of feeding will be different [52]. Seven subjects from cohort 3 and seven subjects from cohort 4 (each randomized 5:2 ZIKV:placebo) will be selected for mosquito feeding. Inclusion of placebo recipients in the mosquito-feeding will be done to ensure the clinical team remains blinded to treatment assignment. Five to 7 day-old female *Aedes albopictus* mosquitoes will be brought to the inpatient unit from the insectory of the Laboratory of Viral Diseases, National Institutes of Health on study days 5, 6, and 7. Each subject will have a pint container holding ten to fifteen *Aedes albopictus* mosquitoes placed on the ventral surface of their forearm. The mosquitoes will be allowed to feed for 10 minutes. This will be performed on study days 5, 6, and 7. After feeding, the container will be placed at -20°C for a few minutes to “anesthetize” the mosquitoes. After removing the container from the -20° freezer, unfed and partially engorged mosquitoes will be removed from the containers and destroyed. Fully engorged mosquitoes will remain in the containers and will be immediately returned to the LVD

where they will be housed and maintained in the LVD insectory per LVD standard protocol. Twenty-one days later the head and midgut tissues of the mosquitoes will be examined for the presence of ZIKV antigens. Methods for detection of ZIKV may include polymerase chain reaction, immunofluorescence staining, and culture.

6.8 Immunology Testing

6.8.1 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 count of virus alone. Other neutralization assays such as microneutralization and flow-based neutralization assays may also be utilized as experimental endpoints.

6.8.2 Other Immunological Assays

Cytokine, and T-cell and B-cell stimulation assays, and PBMC phenotyping may be performed on peripheral blood mononuclear cells. RNA may be isolated from cells and run on microarrays to identify immune pathways of interest such as defining the innate immune response to ZIKV. In addition, human leukocyte antigen (HLA) typing of samples may be performed as part of assays to map the ZIKV epitopes that induce T or B cell responses.

6.9 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.

7 ADVERSE EVENT MONITORING

7.1 Definitions

7.1.1 Adverse Event

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 considered related to the research.

All AEs will be evaluated for severity, action taken, seriousness, outcome and relationship to the test article as described in Section 7.2 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 each inoculation and any test article-related AEs identified in the 28-day post-inoculation period will be followed until resolution.

AEs are categorized as **solicited AEs and other AEs**. Solicited AEs include local reactogenicity, systemic reactogenicity, and laboratory events. Solicited AEs are those events that the clinician is specifically evaluating during each 28-day post-inoculation period, listed in **Table 8.** All AEs are evaluated using the Adverse Event Grading Table in Appendix A**,** using severity definitions detailed in Section 7,

Table 9.

All abnormal laboratory findings will be reviewed on a routine basis by the PI to identify potential safety signals. An abnormal lab not included on the toxicity table should be assessed in a similar fashion to the criteria above.

7.1.2 Medically-attended Adverse Event

A medically-attended adverse event is defined as any visit (other than hospitalization or routine health maintenance visits) to an emergency room (ER), urgent care facility, doctor, nurse, or any other health care provider. Medically-attended adverse events from Day 29 through Day 180 will be collected.

7.1.3 Adverse Event of Special Interest

Guillain-Barré syndrome (GBS) is an adverse event of special interest for this protocol. Subjects will be examined for muscle weakness and hypo- or areflexia at each physical examination. Should subjects present with neurological complaints at visits that do not include a physical examination, an exam will be performed. The diagnostic certainty of a diagnosis of GBS will be determined using the Brighton Criteria (Appendix B) and the disability severity determined using the disability scale in Appendix C. Referral to neurology specialist will be issued if indicated.

7.1.4 Suspected Adverse Reaction (SAR)

An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. "Reasonable possibility" means that there is evidence to suggest a causal relationship between the test article and the adverse event. A suspected adverse reaction (SAR) implies a lesser degree of certainty about causality than adverse reaction that implied a high degree of certainty.

Table 8: Solicited Adverse Events

Systemic Reactogenicity	Laboratory Events	Local Reactogenicity
-Fever	-Leukopenia	-Injection site pain
-ZIKV-like rash	-Neutropenia	-Injection site erythema
-Headache	-Thrombocytopenia	-Injection site tenderness
-Retro-orbital pain (ROP)		-Injection site induration
-Photophobia		-Injection site pruritis
-Non-purulent conjunctivitis		
-Pruritus		
-Fatigue		
-Myalgia		
-Arthralgia		
-Muscle Weakness		
-Hypo- or areflexia		

7.1.5 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.

The pregnancy will be reported to the DSMB and/or IRB (if applicable).

Per Protocol Analysis in the Event of Pregnancy:

- If the pregnancy occurs prior to and including Study Day 28 after test article administration, the subject will not be included in the per-protocol analysis. The subject will remain in the study for safety follow-up. If the pregnancy occurs after Study Day 28 following administration of test article, the subject will be included in the per-protocol analysis.

Unblinding Due to Pregnancy:

- If a subject becomes pregnant within 56 days of test article administration, the subject will be unblinded to determine if she received ZIKV and aid in management. 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 occurred after Study Day 28, the subject will be included in the per-protocol analysis.
- Post Study Day 56, the subject will be offered the opportunity to have her treatment assignment unblinded in the case that this information would help her determine whether or not she wishes to continue her pregnancy. She will be included in the protocol per analysis.
- The research subject will be advised to notify her obstetrician of study participation and study agent exposure.

7.1.6 Serious Adverse Event (SAE)

An SAE is an AE that is determined to be "serious" whether considered related to the investigational agent or not. SAEs will be collected for the duration of the trial. An SAE results in 1 or more of the following outcomes:

- Death during the period of protocol-defined surveillance.
- 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 ward 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 event*

*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 result in 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." A SAE needs to meet only 1 or more of the above criteria to be considered serious.

7.1.7 Unexpected Adverse Events

An adverse event 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.

7.1.8 Serious and Unexpected Suspected Adverse Reaction (SUSAR)

A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

7.1.9 Unanticipated Problem

An Unanticipated Problem (UP) is any event, incident, experience, or outcome that is:

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research 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; and
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 harm) than was previously known or recognized. (An AE with a serious outcome will be considered increased risk).

7.1.10 Unanticipated Problem That Is Not an Adverse Event (UPnonAE)

An unanticipated problem that does not fit the definition of an adverse event, 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 a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

7.1.11 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 a major 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

7.1.12 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 has a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

Non-compliance: 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

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

Stable chronic conditions which 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 7.2 in this protocol.

7.2 Assessment of Adverse Events

7.2.1 Identification of Adverse Events

Assessment of safety will include clinical observations and monitoring of hematological, blood chemistry, and immunologic parameters. Safety will be evaluated by monitoring of the subjects for local and systemic adverse reactions during the course of the trial. Subjects will be closely monitored for 30 minutes following inoculation. Additionally, subjects will be evaluated while in the inpatient unit every day during Study Days 0 – 9 and again on Study Days 10, 12, 14, 16, 21, and 28. It is during this time period that we anticipate AEs related to infection with the ZIKV will manifest themselves, and hence, the subjects will be seen frequently. Study staff will record the subjects' temperature and assess for AEs. At each visit through Study Day 28 following inoculation, they will be queried about possible ZIKV-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. They will return to the clinic or inpatient unit on Study Day 35, 49, 56, 70, 90, 120, and 180 for sample collection and, at designated time points, physical examination.

All AEs will be recorded during the period after the subject receives the study agent, through and including post-inoculation Study Day 28. Signs/symptoms of GBS will be assessed through Study Day 180.

All SAEs will be assessed appropriately as AEs and reported following SAE reporting guidelines outlined in Section 7.3.2 of this protocol.

7.2.2 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.

Headache: a pain located in the head, over the eyes, at the temples, or at the base of the skull.

Retro-orbital pain: Bilateral pain situated behind the orbits of the eyes

Photophobia: Abnormal sensitivity or intolerance to light as manifested as discomfort to light in a normal lighted room for longer than a few seconds (normal acclimation time).

Non-purulent Conjunctivitis: Redding of bilateral sclera of the eyes without evidence of exudate or crusting of the eyelids

Pruritis: Generalized itching

Fatigue: excessive tiredness following minimal exertion.

Myalgia: pain in the muscles, found in ≥ 2 muscle groups.

Arthralgia: pain in a joint, 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

Hypo- or areflexia: Tendon reflexes that are weaker than normal (hyporeflexia) or absent (areflexia). Reflexes that are graded as 1* or 0 will be classified as hypo- or areflexia. Grading of reflexes are as follows:

- **0:** absent reflex
- **1+:** trace, or seen only with reinforcement
- **2+:** normal
- **3+:** brisk
- **4+:** non-sustained clonus (i.e. repetitive vibratory movements)
- **5+:** sustained clonus

***Grade 1 tendon reflexes will only be considered hypo- or areflexia if this represents a change from the subject's baseline examination.**

Guillain-Barré Syndrome (GBS): acute or subacute onset of weakness in limbs or cranial nerve-innervated muscles, associated with hypo- or areflexia, and a characteristic profile in the cerebrospinal fluid (CSF) as defined by the Brighton criteria (Appendix B). A clinical case of GBS, evaluated by a health care professional with expertise in neurological examination, should meet the following criteria:

- Bilateral and symmetric weakness of the limbs (Grade 0 – 3 on the Oxford scale)
- Decreased or absent deep tendon reflexes in the weak limbs (Grade 0 or 1* on the above scale)
- Monophasic illness pattern; interval between the onset and nadir of weakness ranging from 12 hours to 28 days with a subsequent clinical plateau;
- Absence of an identified alternative cause for the weakness.

***Grade 1 deep tendon reflexes will only be considered acute or subacute muscle weakness if this represents a change from the subject's baseline examination.**

7.2.3 Determination of Severity

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

Table 9: Severity Definitions

Severity	Defined
Grade 1 (Mild)	Event that is easily tolerated, may require 1 dose of medication/treatment
Grade 2 (Moderate)	Event that interferes with daily activity or requires more than 1 dose of medication/treatment
Grade 3 (Severe)	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 a life-threatening event
Grade 5 (Death)	Any adverse event that results in the death of the subject

Solicited AE severity grading classifications are listed in

Table 10. All other AEs will be graded in severity using the Adverse Event Grading Table in Appendix A: Adverse Event Grading Table1.

Table 10: Assessment of Solicited Adverse Events

Local Reactogenicity	Grade	Severity
Injection Site Tenderness Injection Site Pruritis Injection Site Pain	1	Event that is easily tolerated, may require 1 dose of medication
	2	Event that interferes with daily activity or requires > 1 dose of medication
	3	Event that prevents daily activity
	4	Life-threatening
Injection Site Induration Injection Site 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	Rash is present but asymptomatic
	2	Rash is symptomatic (pruritus/pain) but does not interfere with function
	3	Rash is symptomatic and interferes with function
	4	Life-threatening
Headache Retro-orbital pain Photophobia Non-purulent conjunctivitis Pruritis Fatigue Myalgia Arthralgia Muscle weakness Hypo-, areflexia	1	Event that is easily tolerated, may require 1 dose of medication/treatment
	2	Event that interferes with daily activity or requires more than 1 dose of medication/treatment
	3	Event that prevents daily activity
	4	Life-threatening

Solicited Laboratory AEs	Grade	Severity
Leukopenia	1	2,500 – 3,500/mm^3
	2	1,500 – 2,499/mm^3
	3	1,000 – 1,499/mm^3
	4	<999/mm^3
Neutropenia (reduced ANC)	1	750 - 999/mm3
	2	500 – 749/mm3
	3	<500 mm3
	4	Life-threatening
Thrombocytopenia (decreased platelets)	1	100,000 – 120,000/mm3
	2	75,000 - 99,999/mm3
	3	<74,999/mm3
	4	Life-threatening

7.2.4 Relationship with Receipt of Test Article

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

Definitely related: Clear-cut temporal association, and 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 test article or the nature of the event is such that the test article 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 test article 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 administration of the study test article will be determined by how well the event can be understood in terms of 1 or more of the following:

- A reaction of similar nature having previously been observed with this type of test article and/or formulation or naturally occurring ZIKV-like illness
- All local injection-site reactions will be considered causally related to test article. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

7.2.5 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

7.2.6 Adverse Event Outcome

The investigator/designee will assess the outcome of the AE, either at resolution or at 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

7.2.7 Adverse Event Seriousness

The investigator/designee will categorize all AEs either as serious or non-serious, using the criteria defined in Section 7.1.6 of this protocol.

Any events defined as serious will also be reported following SAE reporting guidelines outlined in Section 7.3 of this protocol.

7.3 Adverse Event Reporting

7.3.1 Non-Serious Adverse Events

Non-serious AEs will be followed to resolution, or until the study ends, and 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. At the investigator/designee's discretion, subjects may be contacted up to 30 days after the study follow up period (Study Day 180) regarding non-serious AEs that are unresolved at Study Day 180.

AEs meeting the stopping criteria outlined in Section 7.5 of this protocol will be reported to the Sponsor following the SAE reporting guidelines.

7.3.2 Serious Adverse Events

Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
All SAEs (regardless of relationship and whether or not they are also UPs) 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 will 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 CRF 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.

DSMB

All SAEs will 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, E-mail: niaiddsmbia@mail.nih.gov

JHU Biosafety

All SAEs will 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

IRB Reporting

All SAEs will be reported to Johns Hopkins School of Public Health (JHSPH) IRB and University of Vermont (UVM) IRB as per guidelines, respectively for JHU and UVM as below:

- **JHSPH IRB Guidelines:**
  - JHSPH IRB Phone: 1-888-262-3242, Fax: 410-502-0584
  - https://www.jhsph.edu/offices-and-services/institutional-review-board/index.html for updated reporting guidelines

- **UVM IRB Guidelines:**
  - Investigators are required to report AEs that fit the following criteria:
    - Report timelines are based upon 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 and 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 ADVERSE EVENT is a negative side effect resulting from the study intervention that occurred to a subject enrolled at UVM, FAHC, or other 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 may have been 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 will be reported to the University of Vermont IRB (UVM IRB) according to UVM IRB guidelines.

- UVM IRB Phone: 802-656-5040, Fax: 802-656-5041

7.3.3 Unanticipated Problems

Unanticipated Problems that are also adverse events must be reported to the CSO and 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.

Report UPs that are also adverse events to the CSO according to the sponsor's reporting guidelines or a local IRB UP form.

7.4 Sponsor's Reporting Responsibilities

Serious, unexpected, suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA and all participating Investigators as IND Safety Reports.
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 UPs will be summarized by the IND Sponsor and distributed to Investigators.

7.5 Halting Criteria

If a dose of ZIKV is considered unacceptably reactogenic, as defined below, additional inoculations will be suspended for all subjects and suspension of enrollment until the DSMB and study Sponsor (OCRPRO) have reviewed the data and recommend that enrollment and study agent administration be continued.

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 their site.

Following administration of ZIKV, the following criteria will be used to define unacceptable reactogenicity of the ZIKV strain or dose:

1. One or more subjects experience an SAE (as defined in Section 7.1.6. in this protocol) that is determined to be possibly, probably, or definitely related to ZIKV (as defined in Section 7.2.4. in this protocol), **OR**
2. One or more subjects experience anaphylaxis that is possibly, probably or definitely related to ZIKV, **OR**
3. One or more subjects experience GBS that is possibly, probably or definitely related to ZIKV, **OR**
4. Two or more subjects experience the same objective physical finding of severity Grade 3 that is definitely, probably, or possibly related to ZIKV, with the exception of Grade 3 erythema at the injection site, as defined in Section 7.2.3**.** in this protocol, **OR**
5. Two or more subjects experience the same Grade 3 laboratory abnormality that is possibly, probably, or definitely related to ZIKV **OR**
6. Two or more subjects experience an ANC <500/mm^3 for any duration[1] **OR**

7. Two or more subjects experience a ZIKV-like syndrome following administration of ZIKV, defined as infection[2] associated with fever **and 2 or more** of the following symptoms:
   a. Grade 2 or greater headache lasting ≥48 hours
   b. Grade 2 or greater photophobia lasting ≥48hours
   c. Grade 2 or greater generalized myalgia lasting ≥48 hours

[1] These halting rules resulted from previous discussions between NIAID and the FDA on Phase 1 clinical trials of investigational live attenuated DENV vaccines (reference INDs 8463, 13730, and 13886).
[2] Infection is defined as recovery of ZIKVs from the blood, urine, cervico-vaginal secretions, or saliva of a subject and/or seropositivity or seroconversion to ZIKV.

7.5.1 Reporting a Study Halt

If a halting rule is identified by the Study PI/Protocol Chair and/or the CSO, a description of the adverse event(s) or safety issue will be reported by the Study PI within 1 business day of sponsor awareness to the other Site Investigator. The Study PI will notify the DSMB. The Site Investigators will notify their local IRBs. Safety data reports and changes in study status will be submitted to the applicable IRB promptly, in accordance with institutional policy. This constitutes a minimum criterion, and the decision to halt the trial may be made on the basis of any other criteria that, in the judgment of the investigators, FDA, IRB, DSMB, or Sponsor, indicate a potentially serious safety concern.

7.5.2 Resumption of a Halted Study

The IND Sponsor, in collaboration with the PI and DSMB will determine if it is safe to resume the study. The CSO or designee will notify the Site Investigators of the decision on resumption of the study. The Site Investigators will notify their local IRB(s) of the decision.

7.5.3 Discontinuation of Study Agent/Intervention

Subjects who do not resume study agent/study intervention will continue to be followed for safety per protocol or as clinically indicated, whichever is more conservative.

7.6 Safety Oversight

7.6.1 Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plans (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the IND Sponsor CSO, which 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.

The IND Sponsor and the PI will sign a formal Transfer of Regulatory Obligations (TORO)/SRCP which documents that the PI has accepted the responsibility for periodic safety surveillance assessments as outlined in 21 CFR 312.32(b).

7.6.2 Sponsor Medical Monitor

A Medical Monitor representing the IND Sponsor (OCRPRO), will be appointed for oversight of safety in this clinical study. The Sponsor Medical Monitor will be responsible for performing safety assessments as outlined in a Safety Review and Communications Plan (SRCP) as defined in Section 7.6.1.

7.6.3 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 Intramural NIAID clinical studies that require DSMB oversight, and consists of experts in infectious diseases, biostatistics, and clinical trials. The PI/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 and deaths 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 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 DSMB will receive summary data in an unblinded manner to determine whether or not dose expansion or dose escalation will occur. The DSMB will be responsible for:

1. Determining if the criteria for a suitable CHIM strain has been met. These criteria are:
    a) ≥ 80% of inoculated subjects develop detectable viremia by virus culture ***and***
    b) The mean peak titer of ZIKV recovered is 2 – 3 $\log_{10}$ PFU/mL.
    c) The committee will review the clinical signs and symptoms induced by the ZIKV strain, but signs and symptoms will not be required for dose escalation.
2. Recommending whether or not dose-escalation should occur. Dose escalation may occur even if the above criteria for a suitable CHIM strain have been met if it is believed that more consistent outcomes would be achieved with a higher dose, or if evaluating the shedding of virus given at a higher dose would improve the scientific understanding of ZIKV infectivity, shedding, and possibly transmission.

8 DATA COLLECTION AND MONITORING

8.1 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 inoculation 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 the 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 shall 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 the CRIMSON Data System. 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.

8.2 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 the Code of Federal Regulations (CFR), Title 21, Part 312.33 (21 CFR 312.33), and will include any revisions of the protocol if not previously submitted.

The PI will maintain adequate records to account for the disposition of the investigational product, including dates of receipt and quantity, current inventory, and dispensation to subjects. If the study is terminated, suspended, or completed, all unused study product will be disposed of per sponsor’s instructions.

8.3 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 ID numbers of the subjects that, 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. 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.

8.4 Retention of Records

The PI is responsible for retaining all essential documents listed in the International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) Guideline. Trial-related documents will be maintained by the investigator in a secure storage facility for a period of at least 2 years since the formal discontinuation of clinical development of the product. 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 law.

It is the PI's responsibility to retain copies of source documents until receipt of written notification to the contrary from the OCRPRO of the NIAID. Study documents should not be destroyed without prior written agreement between OCRPRO/NIAID 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 OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their 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.

8.5 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 of, 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 data and safety monitoring plan of the study.

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 shall 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 to the regulatory authorities.

8.6 Clinical Investigator's Brochure

Investigators will receive the current versions of the Clinical Investigator's Brochures for the ZIKV strains used in this protocol, which comprehensively describes all the available preclinical experience with the ZIKVs. If relevant new information becomes available during the trial, the investigators will receive a revised brochure, or an amendment to the current version.

8.7 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 notifications 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 also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP, 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 the 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, FDA, the site monitors, and the 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.

9 STATISTICAL CONSIDERATIONS

9.1 General Design

The major objectives of this Phase 1 ZIKV trial are to identify a suitable ZIKV CHIM strain to aid in the selection of vaccine candidates and to characterize the clinical and virologic characteristics of ZIKV infection.

9.2 Statistical Methods

This study, like other Phase 1 studies, is exploratory rather than confirmatory. As such, the primary analyses will assess frequencies of adverse events and describe patterns of challenge virus replication and immune response within individuals at multiple timepoints (e.g. pre vs post challenge), and within and between groups (challenge virus vs. placebo).

Continuous outcomes (e.g. peak titer of virus shed, antibody titers over time) will be presented with the use of summary statistics such as means, medians, quartiles, ranges, etc. as appropriate, with data displayed in both tables and figures. Student's T-tests and linear regression will be used to assess differences in continuous outcomes between subjects in each study arm. Correlations between 2 continuous outcomes will be evaluated with the use of Pearson or Spearman correlation coefficients and graphed as scatterplots.

Proportions of subjects with dichotomous outcomes of interest (e.g. presence or absence of: specific AEs, viral shedding, seropositivity, four-fold rise in antibody response, etc.) observed in each group will be summarized in tables and figures. Differences in proportions between groups will be compared with X^2 tests, Fisher's exact tests, and logistic regression as appropriate. Outcomes will be summarized by severity and/or category where applicable (e.g. immediate, systemic, local AE).

The study is powered to detect a difference in the occurrence of virus in blood, urine, cervico-vaginal secretions, or saliva following administration of one of 2 ZIKV at different doses, compared with placebo recipients. A sample size of 10 ZIKV recipients and 4 placebo recipients will be able to detect a statistically significant difference ($\alpha = 0.05$) in recovery of infectious ZIKV from the blood between the ZIKV and placebo groups at a power of 0.99 with ≥80% of ZIKV-inoculated subjects having detectable ZIKV viremia and 0 placebo recipients having detectable ZIKV in blood and body secretions. The detection of ZIKV by RT-PCR and by virus culture from blood, urine, cervico-vaginal fluids, semen and saliva in recipients of each ZIKV strain will be compared to placebo recipients. In addition, the magnitude and duration of ZIKV presence in blood, urine, cervico-vaginal secretions, semen and saliva will be compared to one another at each dosing level. The effect of dose escalation on the presence of virus in blood, urine, saliva, cervico-vaginal secretions and semen will also be evaluated. Multiple comparisons analysis will be used to compare the magnitude and duration of virus presence at each dose level of each candidate ZIKV and placebo recipients.

Measuring the transmissibility of ZIKV-SJRP and ZIKV-Nicaragua from infected volunteers to mosquitoes is an exploratory endpoint. Following feeding on volunteers, mosquitoes will be returned to the NIH for analysis (Section 6.7). The heads and thoraces of the mosquitoes will be examined for ZIKV antigen. If antigen is detected in the head of the mosquitoes, it will be assumed that the virus escaped the mid-gut barrier and is present in the salivary glands.

9.3 Safety Endpoint

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

9.4 Immunogenicity

ZIKV neutralizing antibody titers will be measured on Study Days 0, 28, 56, 90, and 180. Seroconversion to ZIKV will be defined as a $PRNT_{50}$ of ≥ 1:10 based on a pre-inoculation $PRNT_{50}$ of <1:5 (seronegative to ZIKV).

9.5 Per Protocol Analysis

9.5.1 Safety Data

The adverse event data will be included in the per-protocol analysis of all subjects who received an inoculation, even if one or more study visits during safety follow-up period were missed. This will assure that the largest body of safety data are included in the analysis.

9.5.2 Virology
Subjects who were enrolled through Study Day 180, even if they missed one or more visits during this time period will be included in the per-protocol analysis of viral shedding. Because there are frequent visits and an inpatient stay, missing these time-points is not expected to affect the analysis of the duration of virus shedding.

9.5.3 Immunogenicity Data
The frequency of seroconversion and peak neutralizing antibody titers will be included in the per-protocol analysis for all subjects who were enrolled through Study Day 90 and have samples from at least 2 of the following 3 time-points (Day 28, 56, 90).

9.6 Intent-to-Treat Analysis

9.6.1 Virology
Subjects who were not enrolled through Study Day 180 but who had samples collected for ZIKV isolation will be included in the intent-to-treat analysis of viral shedding.

9.6.2 Immunogenicity Data
Subjects who did not complete Study Day 90 but did complete Study Day 28 or 56 will be included in the intent-to-treat analysis of seroconversion and peak neutralizing antibody titer.

10 Protection of Human Subjects

10.1 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.

10.2 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. A copy of the informed consent document will be given to the subject for his or her records. The subject may withdraw consent at any time during the course of the trial. 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 or if they withdraw from the study this study.

10.3 Risks

The risks to the subjects are minimal and are generally associated with venipuncture and ZIKV infection. These risks are outlined below. There is a potential risk of ZIKV transmission to the community by mosquito as well as by sexual transmission. In addition, questions asked regarding a subject's sexual history may make the subject uncomfortable. These questions will be asked in a private area and the subject will be made aware that they do not have to answer any

question that they do not feel comfortable answering. Subjects will be informed of the known risk of ZIKV and birth defects in the fetus. Subjects will be advised to use effective birth control methods while at risk for ZIKV infection (we will follow CDC guidelines of 8 weeks for people of childbearing potential and 12 weeks for men post-infection time of infection is defined as inoculation with challenge virus). Those of child bearing potential will also be required to use barrier contraception through study day 56 to prevent Zika transmission, in accordance with CDC guidance. Males will be advised to use barrier contraception through study day 90 to prevent Zika transmission in accordance with CDC guidance.

10.3.1 Venipuncture

The total amount of blood to be drawn throughout the 6-month duration of the primary study is approximately 700 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.

10.3.2 Local Risks Associated with Test Article Administration

Local risks associated with subcutaneous administration of test article (either PlasmaLyte or ZIKV) include injection site pain, injection site pruritus injection site erythema or injection site induration.

10.3.3 Risk of Use of Cervico-vaginal Cup

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

10.3.4 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.

10.3.5 Risk from Mosquito Feeding

The mosquito bites that will result from mosquito feeding may cause itching, erythema, and welt-formation (normal responses to mosquito bites). Some volunteers may be more sensitive to mosquito bites and may have more intense symptoms. Severe allergy to mosquito bites is extremely rare.

10.3.6 Risk Due to Infection with ZIKV

10.3.6.1 Zika Illness

ZIKV may cause a mild, transient illness in those who are infected, although the majority of infections are asymptomatic. Possible local inoculation reactions include pain, swelling, or erythema for 2 to 3 days, lymphadenopathy, or pruritus at the injection site. Systemic reactions such as fever, maculopapular, itchy rash, red eyes, headache, and muscle and joint pains have been described in patients diagnosed with ZIKV infection.

10.3.6.2 Zika Congenital Syndrome

Zika virus has been causally linked to congenital microcephaly [12]. Microcephaly and other severe birth defects in the fetus have been linked to ZIKV infection during pregnancy. The risk of birth defect in the fetus is highest when infection occurs during the first month of pregnancy but can occur with infection in any trimester. There is growing evidence that developmental delay, hearing abnormalities, and vision abnormalities may be associated with intrauterine ZIKV infection and may become apparent after birth, even when the baby appears to be normal at birth.

10.3.6.3 Guillain-Barré Syndrome (GBS)

GBS is an autoimmune disorder characterized by varying degrees of weakness, autonomic dysfunction, and sensory abnormalities. Typically, GBS is manifested by bilateral lower limb numbness, tingling, and weakness that can then spread to include the upper extremities. In severe cases, the muscles of the diaphragm can be affected which man affect the ability of the patient to breath and may require mechanical ventilation. Gradually these symptoms resolve over weeks, but it can sometimes take months for resolution to occur. Some patients never fully recover. These are due to peripheral nerve or nerve root damage thought to be caused by an autoimmune reaction. The annual incidence of GBS worldwide is estimated to be ~ 0.6 – 4 cases/100,000 population and varies by geography and age group [53-55]. GBS has been associated with various infectious agents including influenza, cytomegalovirus, *Campylobacter jejuni*, chikungunya and ZIKV (see Section 1.1 for more detail). Several studies have observed that the risk of GBS appears to increase with older age, particularly older than 50 [56-58]. The risk of GBS following ZIKV infection is not fully known but is estimated to be around 1 – 2 cases per 10,000 ZIKV infections in the general population and is irrespective of health status at time of infection [15, 59]. This is slightly lower than the estimated risk of GBS following Campylobacter infection [15, 60].

10.3.6.4 Risk of Transmission

ZIKV can be sexually-transmitted from men-to-women, men-to-men, and women-to-men (see Section 1.1 for more detail). The majority of cases of sexual transmission reported to date have occurred just before or during the period of time when the person was symptomatic with ZIKV; however, one reported case of sexual transmission has occurred from an individual who did not recall any previous symptoms associated with ZIKV infection [28]. The risk of sexual transmission appears to be much higher from men-to-women than for women-to-men as ZIKV is rarely shed from the female genital tract and when it is shed, it is of short duration [37]. The reported cases of sexual transmission have occurred within 3 weeks of return from a ZIKV-endemic area with most of those occurring within 2 weeks of return [42] (see Section 1.1 for more detail). Study subjects will be housed in an inpatient unit for Study Days 0 – 9 (discharged on Study Day 9). All subjects will be counseled on the risks of congenital Zika syndrome and will be required to use barrier contraception through Study Day 56 per CDC guidelines. Condoms will be provided at no cost to subjects in the study.

The major route of ZIKV transmission is by mosquito. It is believed that *Aedes aegypti* is the primary vector for ZIKV but that *Aedes albopictus* may also transmit the virus. *Aedes aegypti* mosquitoes are not known to inhabit the study areas, although *Aedes albopictus* species are present. *Aedes albopictus* mosquito-pools have not been identified as positive for ZIKV in the current outbreak. The virus must achieve a sufficient level of viremia in infected volunteers to support transmission by mosquitoes (generally >3 $\log_{10}$ PFU/mL). It is not anticipated that subjects will achieve this level of viremia for any extended length of time during this study. As an added protection, ***subjects will be housed in an environmentally-controlled inpatient unit until Study Day 9 to prevent any possible transmission by mosquito until mosquito-transmissibility studies have been performed and the data from these studies support outpatient studies.***
In accordance with American Red Cross blood donation guidelines, we will inform volunteers that they cannot donate blood for at least 6 months following ZIKV infection and that they

should inform blood or plasma donation centers that they were enrolled in the trial and received ZIKV challenge (for those who were not placebo recipients).

10.3.6.5 Risk of More Severe Dengue Disease

Previous infection with one DENV serotype can predispose to more severe dengue following subsequent infection with a second, different DENV serotype ≥ 18 months after the first infection [61, 62]. Modeling studies from a cohort study from Nicaragua suggested that a previous Zika infection may increase the probability of symptomatic and more severe DENV-2 infection occurring 3 years after Zika infection [63]. It is unknown how long the enhanced risk of severe dengue following Zika infection persists. However, it is thought that the risk of severe dengue following subsequent heterotypic DENV infection can last years to decades.

10.3.6.6 Other Risks

Subjects may be asked to defer routine immunization (such as influenza) until after 21 days following inoculation. Subjects will be offered influenza vaccination at no charge before they are enrolled in the study (≥ 14 days prior to enrollment [killed vaccine] or ≥ 21 days prior to enrollment [live vaccine]), or after Study Day 21 of the study. This may increase the risk that the subject will be infected with an influenza virus during this period. As with any investigational product, 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.

10.3.7 Risk Mitigation

10.3.7.1 Prevention of Congenital Zika Syndrome

Female volunteers who are pregnant or breast-feeding are not eligible for participation in the study. Female volunteers will be screened for pregnancy on 2 occasions prior to enrollment, on the day of ZIKV administration, and at regular intervals thereafter. All volunteers will be counseled on pregnancy prevention at regular intervals throughout the study and they will be provided with barrier contraception through study day 56 (women) or study Day 90 (men). All volunteers must agree to the use of barrier contraception for the specified duration. Female volunteers of child-bearing potential must also agree to the use of effective birth control (defined in Section 4.1) while at risk for ZIKV infection (we will follow CDC guidelines of 8 weeks post-infection (Study Day 56); time of infection is defined as inoculation with challenge virus). Female volunteers who exclusively have sex with females and females who are postmenopausal will be granted an exception to this requirement as they are not at risk for pregnancy from sexual intercourse.

10.3.7.2 Mitigation of Risk of GBS

It is estimated that the overall risk of GBS associated with ZIKV is 1 – 2 cases per 10,000 ZIKV infections. GBS is known to occur more commonly in subjects older than 50 and in subjects with history of GBS or autoimmune disease [54]. To reduce the risk of GBS, enrollment will be limited to subjects 18 – 40 years of age without history of autoimmune disease or history of GBS. Subjects will be examined daily while in the inpatient unit, approximately weekly from Day 14 – Day 70, then again at Study Day 90, 120, and 180. This examination will include a neurological assessment. Should a volunteer exhibit neurologic signs or symptoms suggestive of GBS or other neurologic disorder, the subject will be evaluated by a neurologist and treatment

recommended. If hospitalization is needed, the volunteer will be hospitalized at the sponsor's expense.

10.3.7.3 Mitigation of Risk of Transmission of ZIKV

To reduce the risk of ZIKV transmission, subjects will be housed in the inpatient unit from Study Day 0 through Study Day 9 (discharged on Study Day 9 if discharge criteria are met). ZIKV is generally able to be recovered from the blood for only a few days before and after ZIKV symptoms occur. A recent longitudinal study detected ZIKV by PCR in 88% of ZIKV-infected subjects [29]. ZIKV was detected in the serum at a level thought to be infectious ($C_T \leq 27$) only until approximately day 8 post-symptom onset. It is unlikely that infectious ZIKV will be detected in blood at a level that could be transmitted beyond Day 8 post-infection. Volunteers will not be discharged from unit unless the titer of ZIKV in serum, urine, saliva, cervico-vaginal secretions and semen is $< 1.4 \times 10^6$ genomic copies/mL by Study Day 8. At discharge from the inpatient unit, subjects will be given commercially available mosquito repellent containing DEET during mosquito season (May – October) and will be instructed to use it when outdoors through Study Day 28. In addition, this trial will be conducted as a dose-escalation study to ensure that only the lowest dose necessary to induce the desired virologic and clinical outcomes will be used. A very low dose of ZIKV (100 PFU) will first be evaluated. Lower doses of ZIKV are likely to induce lower titers in the blood and therefore reduce the risk of transmission. It is anticipated that the level of viremia induced in this study is below the level required for mosquito transmission (~ 3.0 $\log_{10}$ PFU/mL). To reduce the risk of sexual transmission of ZIKV, subjects will be counseled on the use of barrier contraception through Study Day 56 (women) or Study Day 90 (men) and must agree to the use of barrier contraception when they engage in intercourse during this time period. Condoms will be provided to all subjects at no cost for the duration of the study. In addition, virus inoculum will be delivered by needle and syringe and is considered less infectious than natural mosquito-borne virus which is augmented by saliva factors.

10.3.7.4 Other Risks

Subjects will be counseled regarding the timing of routine immunizations such as the flu vaccine. When available, subjects will be offered the flu vaccine at no cost to them during screening or within the acceptable time periods during the study. Topical anti-itch medication (diphenhydramine and hydrocortisone cream/ointment) will be available to the volunteers upon request if the mosquito bite symptoms are bothersome to the volunteer. Systemic antihistamines will be available to treat the itch from the Zika-like rash or, if needed, the itch from mosquito bites.

10.3.7.5 Escalation of Care Plan

Should a volunteer develop new onset muscle weakness, change in deep tendon reflexes, or evidence of dysautonomia, or any other change from baseline exam that the clinician feels warrants further evaluation, the volunteer will be assessed by a formal consultation with a neurologist, or will be transferred by ambulance to the Emergency Room associated with the University of Vermont Medical Center or the Bayview Medical Center. Following evaluation, should a volunteer require hospitalization, she will be admitted to the University of Vermont Medical Center or the Bayview Medical Center for further management.

10.4 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 to ZIKV. It is hoped that information gained in this study will contribute to the development of safe and effective ZIKV vaccines and to a better understanding of ZIKV infection, ZIKV disease, and the risk of ZIKV sexual transmission. Should a potential volunteer wish to begin using hormonal contraception or an IUD, she will be referred to a practitioner to discuss her contraception options. Should she decide to use either hormonal contraception or an IUD and be otherwise eligible for enrollment in the study, the Sponsor will pay for her contraception for the duration of the study (oral contraception, NuvaRing®, hormonal patch, Depo-Provera injection, etc.), or for placement of longer-acting contraception (hormonal implant or an IUD). The Sponsor will also pay for removal of the long-term contraception should the subject desire.

10.5 Compensation

Subjects will be compensated up to $200 for screening, up to $300 for each inpatient day & night (Study Days 0 through 8, discharged on Day 9), up to $125 for each completed scheduled follow-up visit (Study Days 9, 10, 12, 14, 16, 21, 28, 35, 56, 70, 90, 120 and 180), and up to $50 for the scheduled phone call on Study Day 150. They will receive $100.00 for each mosquito-feeding session. They will also receive a $300 bonus if all study visits and procedures are completed on time and if they adhere to all inpatient policies and the code of conduct. Subjects may only receive a portion of the study visit payment and/or bonus if not all study visits or procedures are completed on time or for failure to follow the code of conduct agreement. 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. We may invite more potential volunteers to come to the unit on Study Day 0 than we will enroll at that time. This is to ensure that we are able to enroll the planned number of volunteers in the case that someone is not eligible on Study Day 0 or does not come to the unit. Alternates who come to Day 0 but who are not inoculated due to fulfillment of enrollment for that day will be compensated for screening and for one outpatient day ($325.00). Subjects enrolled in the study will receive a maximum total compensation of $5,175.00. Payment will be distributed by check or other mechanism such as Clincard®.

10.5.1 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.

10.6 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.

10.7 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.

11 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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Appendix A: Adverse Event Grading Table[1,2]

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe
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**
Local Reactogenicity	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Injection Site Tenderness	Tenderness that is easily tolerated	Tenderness that interferes with daily activity	Tenderness that prevents daily activity
Injection Site Pain	Pain that is easily tolerated	Pain that interferes with daily activity	Pain that prevents daily activity
Injection Site Pruritus	Pruritus that is easily tolerated	Pruritus that interferes with daily activity	Pruritus that prevents daily activity
Injection Site Induration	>0 - 20 mm	>20 - 50 mm	>50 mm
Injection Site Erythema	>0 - 20 mm	>20 - 50 mm	>50 mm
Systemic Reactogenicity	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Fever (Oral)	100.4° F – 101.4°F	101.5°F – 102.4°F	≥102.5°F
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
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
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
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

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe
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
Pruritus	Pruritus that is easily tolerated, may require 1 dose of medication/treatment	Pruritus that interferes with daily activity or requires >1 dose of medication	Pruritus that prevents daily activity
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
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
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
Muscle Weakness	Muscle weakness that is easily tolerated, may require 1 dose of medication/treatment	Muscle weakness that interferes with daily activity of requires >1 dose of medication	Muscle weakness that prevents daily activity
Hypo/areflexia	Hypo/areflexia that is easily tolerated, may require 1 dose of medication/treatment	Hypo/areflexia that interferes with daily activity of requires >1 dose of medication	Hypo/areflexia that prevents daily activity
Solicited Lab AEs	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Leukopenia	2,500-3,500/mm^3	1,500-2,499/mm^3	1,000-1,400/mm^3
Neutropenia (Reduced ANC)[2]	≥750-999/mm3	≥500-749/mm3	≤500/mm3
Thrombocytopenia (Reduced Platelets)	100,000 – 120,000/mm3	75,000 - 99,999/ mm3	≤74,999/mm3
Other Laboratory Values	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Leukocytosis (Increased WBCs)	11,500 - 13,000/mm3	13,001 - 15,000/mm3	≥15,000 or <1,000/mm3

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Hemoglobin (female)	9.5 - 10.7 gm/dL	8.0 - 9.4 gm/dL	≤7.9 gm/dL
Hemoglobin (male)	11.0 - 12.5 gm/dL	9.0 – 10.9 gm/dL	≤8.9 gm/dL
PT	>1.0 - 1.25 x upper limit of normal (ULN)	>1.25 - 1.5 x ULN	>1.5 x ULN
PTT	>1.0 - 1.66 x ULN	>1.66 - 2.33 x ULN	>2.33 x ULN
ALT	>1.25 - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 x ULN
Creatinine	1.5 - 1.7 mg/dL	>1.7 – 2.0 mg/dL	>2.0 mg/dL
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
Fibrinogen, Increased	>ULN to 600 mg/dL	>600 mg/dL	N/A
Creatine phosphokinase (CPK)	≥4 x ULN- 6 x ULN	>6 x ULN- 10 x ULN	>10 x ULN
Sodium: Hyponatremia	130 – 134 mEq/L	123 – 129 mEq/L	<122 mEq/L
Sodium: Hypernatremia	145 – 150 mEq/L	151 – 157 mEq/L	>158 mEq/L
Potassium: Hypokalemia	3.1 – 3.2 mEq/L	2.9 – 3.0 mEq/L	<2.8 mEq/L
Potassium: Hyperkalemia	5.2 – 5.5 mEq/L	5.6 – 6.0 mEq/L	>6.1 mEq/L
Phosphate: Hypophosphatemia	2.0 – 2.2 mg/dL	1.5 – 1.9 mg/dL	<1.4 mg/dL
Calcium (Corrected for Albumin): Hypocalcemia	1.95 – 2.04 mmol/L	1.75 – 1.94 mmol/L	<1.74 mmol/L
Calcium (Corrected for Albumin): Hypercalcemia	2.51 – 2.88 mmol/L	2.89 – 3.13 mmol/L	>3.14 mmol/L
Magnesium: Hypomagnesemia	0.60 – 0.74 mmol/L	0.45 – 0.59 mmol/L	<0.44 mmol/L
Bilirubin (hyperbilirubinemia)	>1.0 – 1.5 x ULN	>1.5 – 2.5 x ULN	>2.5 ULN
Glucose: Hypoglycemia (Nonfasting, No Prior Diabetes)	55 – 69 mg/dL	40 – 54 mg/dL	<39 mg/dL

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Glucose: Hyperglycemia (Nonfasting, No Prior Diabetes)	116 – 160 mg/dL	161 – 250 mg/dL	>251mg/dL
Triglycerides	—	400 – 750 mg/dL	>751 mg/dL
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
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
Other Cardiovascular	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Cardiac Arrhythmia	Asymptomatic; transient dysrhythmia, no therapy required	Recurrent/persistent dysrhythmia; symptomatic therapy required	unstable dysrhythmia, hospitalization and therapy required
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
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
Pericarditis	Mild/moderate asymptomatic effusion, no therapy	Symptomatic effusion, pain, EKG changes	Tamponade or pericardiocentesis or surgery required
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

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe
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
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
Oral Discomfort/Dysphagia	Difficulty swallowing but able to eat and drink	Unable to swallow solids	Unable to drink fluids; IV fluids required
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
Other Respiratory	Grade I – Mild	Grade II – Moderate	Grade III - Severe
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
Dyspnea	Dyspnea on exertion	Dyspnea with normal activity	Dyspnea at rest
Other Neurologic	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Nuchal Rigidity	--	--	Presence of Nuchal rigidity
Neuropsychological	Mild confusion or cognitive impairment	Moderate confusion or cognitive impairment	Severe confusion or cognitive impairment
Neurocerebellar	Slight incoordination or dysdiadochokinesia	Intention tremor, dysmetria, slurred speech, or nystagmus	Ataxia requiring assistance to walk or arm incoordination interfering with ADLs

Event	Grade I – Mild	Grade II – Moderate	Grade III - Severe
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
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
Paresthesia (Burning, Tingling, etc.)	Mild discomfort; no therapy required	Moderate discomfort; non-narcotic analgesia required	Severe discomfort; or narcotic analgesia required with symptomatic improvement
Other Dermatologic	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Dermatitis	Rash is present but asymptomatic	Rash is symptomatic (pruritus/pain) but does not interfere with function	Rash is symptomatic and interferes with function
Other Urinalysis	Grade I – Mild	Grade II – Moderate	Grade III – Severe
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 Cervico-vaginal Bleeding)	Microscopic only, 6-10 rbc/hpf	>10 rbc/hpf	Gross, with or without clots; or RBC casts
Other Miscellaneous	Grade I – Mild	Grade II – Moderate	Grade III - Severe
Malaise	Malaise that is easily tolerated	Malaise that interferes with daily activity	Malaise that prevents daily activity

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

2. These values for neutropenia are values that have been used in dengue controlled human infection model studies and dengue live attenuated vaccine studies

Appendix B: 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	

Appendix C: 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