Abbreviated Title: Model of Sequential Dengue

National Institutes of Health (NIH) Institutional Review Board (IRB) #: 001078

Version Date: January 29, 2026

Title: Phase 1 trial to model primary, secondary, and tertiary dengue using a monovalent vaccine

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Investigational Agents:

Drug Name:	rDEN3Δ30/31-7164
Investigational New Drug (IND) Number:	13886
Sponsor:	Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Manufacturer:	Charles River Laboratories (CRL) Biopharmaceutical Services

Data and Safety Monitoring Board (DSMB): NIAID DSMB

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR 46, 21 CFR 50, 21 CFR 60, 21 CFR 312, and/or 21 CFR 812)

NIH-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

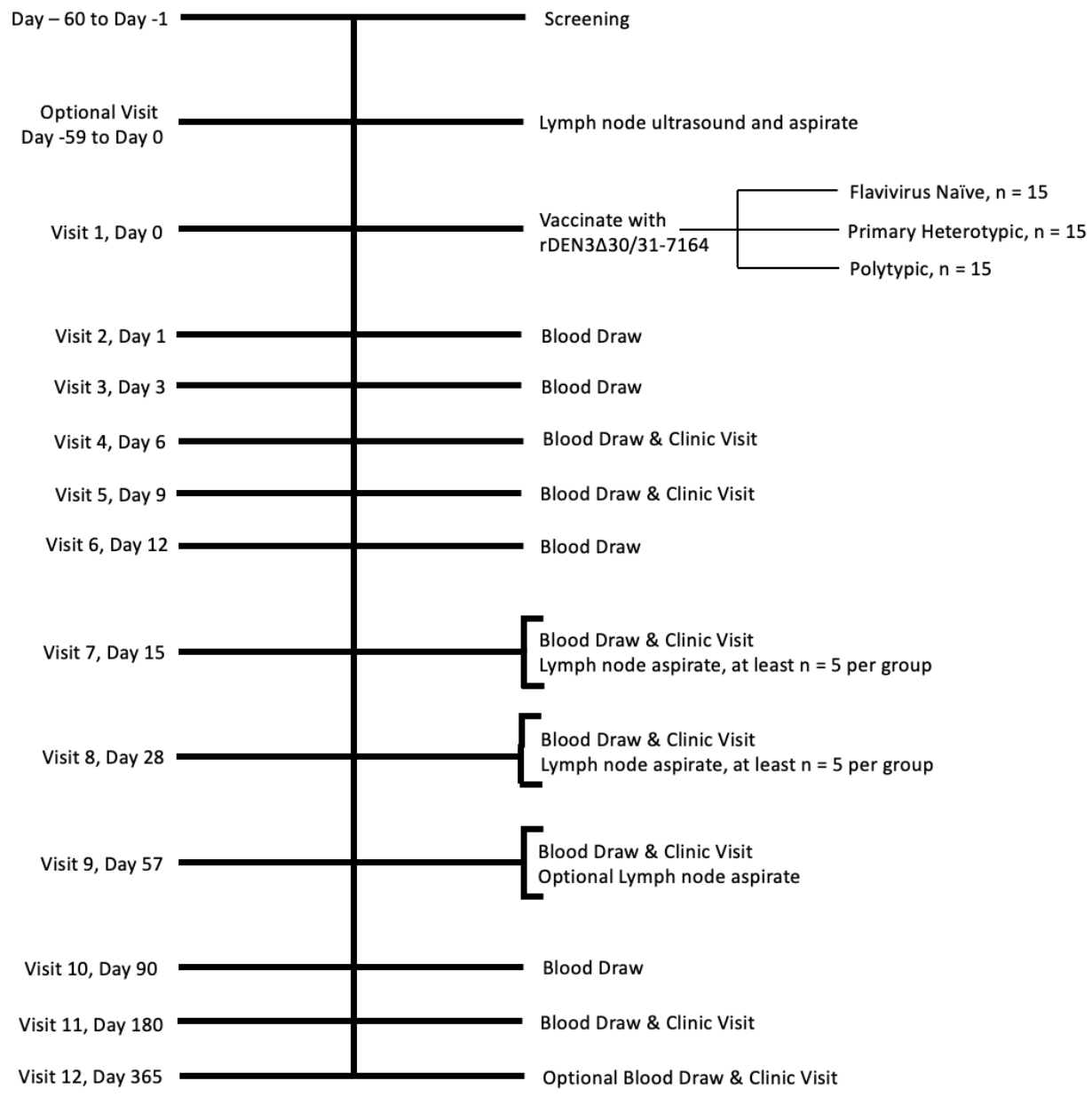
1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	Phase 1 trial to model primary, secondary, and tertiary dengue using a monovalent vaccine
Study Description:	Phase 1 trial wherein healthy adults with no (naïve), one (primary heterotypic), or more than one (polytypic) previous natural dengue virus (DENV) infection(s) will be immunized with the DENV3 monovalent vaccine rDEN3Δ30/31-7164. We aim to examine how pre-vaccine host immunity influences the safety and immunogenicity of monovalent DENV3 vaccination in a non-endemic population. We hypothesize that the vaccine will be safe and well tolerated, and all groups will have evidence of infection with the vaccine strain as indicated by a significant increase in the DENV neutralizing antibody geometric mean titer (GMT) between days 0 and 28. However, due to the immunity conferred by prior dengue exposure(s), the polytypic group will have the lowest and the heterotypic group will have the highest mean peak viremia, indicating protection and enhancement, respectively. Additionally, the polytypic group will have the strongest $CD8^+$ T-cell responses at day 15 and will only have a transient rise in GMT with no difference in GMT between days 0 and 57. In contrast, the change in GMT will persist at day 57 in the heterotypic and naïve groups. Finally, we expect that prior immunity will influence the vaccine response as evidenced by a significant association between the day 0 GMT and GMT at days 28 and 57.
Primary Objective:	Evaluate the safety of monovalent DENV3 vaccination in those with distinct natural DENV infection histories living in non-endemic areas, and how prior DENV immunity influences protection against vaccine strain infection evaluated by the change in GMT and mean peak viremia.
Secondary Objective:	Further evaluate how DENV infection history impacts the immunogenicity of the vaccine.
Primary Endpoints:	1. The frequency and severity of local and systemic reactogenicity signs and symptoms during the 28-day period after each vaccination, unexpected adverse events (AEs) up to 28 days after each vaccination, and serious adverse events (SAEs) through day 180. 2. Change in DENV neutralizing antibody GMT between days 0 and 28. 3. Mean peak viremia among groups as measured by viral quantitative reverse transcription polymerase chain reaction (qRT-PCR) between days 3 and 15.
Secondary Endpoints:	1. Change in DENV neutralizing antibody GMT between days 0 and 57. 2. DENV neutralizing antibody GMT at days 0, 28, and 57. 3. Magnitude of the $CD8^+$ T-cell response at day 15 among groups as measured by activation induced marker (AIM) assays.
Study Population:	Healthy adults aged 18 to 59 years who are flavivirus naïve (n = 15), primary heterotypic DENV antibody profile (n = 15), or polytypic DENV antibody profile (n = 15). The accrual ceiling is 200.
Phase:	Phase 1

Description of Sites/Facilities Enrolling Participants:	The study will be conducted at the NIH Clinical Center (CC) in Bethesda, MD.
Description of Study Intervention:	The rDEN3Δ30/31-7164 vaccine will be administered at a dose of 10^1 to $10^{3.5}$ plaque-forming units (PFU), with a goal range of $10^3 \pm 10^{0.5}$, into the subcutaneous tissue of the left deltoid area at day 0.
Study Duration:	Approximately 36 months
Participant Duration:	7 to 14 months

1.2 Schema


Day – 60 to Day -1
Screening
Optional Visit
Day -59 to Day 0
Lymph node ultrasound and aspirate
Visit 1, Day 0
Vaccinate with
rDEN3Δ30/31-7164
Flavivirus Naïve, n = 15
Primary Heterotypic, n = 15
Polytypic, n = 15
Visit 2, Day 1
Blood Draw
Visit 3, Day 3
Blood Draw
Visit 4, Day 6
Blood Draw & Clinic Visit
Visit 5, Day 9
Blood Draw & Clinic Visit
Visit 6, Day 12
Blood Draw
Visit 7, Day 15
Blood Draw & Clinic Visit
Lymph node aspirate, at least n = 5 per group
Visit 8, Day 28
Blood Draw & Clinic Visit
Lymph node aspirate, at least n = 5 per group
Visit 9, Day 57
Blood Draw & Clinic Visit
Optional Lymph node aspirate
Visit 10, Day 90
Blood Draw
Visit 11, Day 180
Blood Draw & Clinic Visit
Visit 12, Day 365
Optional Blood Draw & Clinic Visit

1.3 Schedule of Activities (SOA)

	Screen	Optional[a]	**Schedule of Activities**															Optional[b]
Visit Number	Screen		SC[c]	01	02	03	04	05	06	07	SC[c]	08	SC[c]	09	SC[c]	10	11	12
Day of Study[d]			−59 to 0	D0	D1	D3	D6	D9	D12	D15	D16	D28	D29	D57	D58	D90	D180	D365
Visit Window (days)	−60 to −1	−59 to 0	+2	0	0	±2	±2	±2	±2	±4	+1	±4	+1	+12	+1	±10	±15	±15
Clinical Evaluations/ Procedures				**Active Phase**										**Follow-up Phase**				
DENV3 vaccine screening consent	X																	
DENV3 vaccine full study informed consent[e]		X		X														
Demographics	X																	
Medical history/focused physical examination	X	X		X			X	X		X		X		X			X	X
Vital signs[f]	X	X		X			X	X		X		X		X			X	X
Height/weight	X																	
Review of health history	X			X						X		X		X			X	X
Exposure history assessment	X[g]			X[h]													X[i]	X[i]
Concomitant medication review	X	X		X			X	X		X		X		X			X	X
Pregnancy prevention counseling	X			X														
Communication for any aspirate-related AEs			X								X		X		X			

	Screen	Optional[a]	**Schedule of Activities**															Optional[b]
Visit Number	Screen		SC[c]	01	02	03	04	05	06	07	SC[c]	08	SC[c]	09	SC[c]	10	11	12
Day of Study[d]			−59 to 0	D0	D1	D3	D6	D9	D12	D15	D16	D28	D29	D57	D58	D90	D180	D365
Visit Window (days)	−60 to −1	−59 to 0	+2	0	0	±2	±2	±2	±2	±4	+1	±4	+1	+12	+1	±10	±15	±15
Clinical Evaluations/ Procedures				**Active Phase**										**Follow-up Phase**				
Administer rDEN3Δ30/31-7164 vaccine[j]				X														
AE assessment[k]				X			X	X		X		X		X			X	X
Optional lymph node fine needle aspiration[l]		X								X		X		X				
Optional photos of rash, if present							X	X		X		X		X				
CBC with differential	3	3		3	3	3	3	3	3	3		3		3		(3)[m]		
HbA1c	0[n]																	
Acute care and hepatic panel	3.5	3.5		3.5				3.5	3.5	3.5		3.5		3.5				
CRP[o]				0				0	0	0		0		0				
Ferritin[o]				0				0	0	0		0		0				
Immunoglobulins, **, Quantitative**[o]				0				0		0				0				
PT/PTT/INR	3	3		3				3	3	3		3		3				
Fibrinogen[p]				0				0	0	0		0		0				
IgE/tryptase				3.5					3.5					3.5				
HBsAg, anti-HCV antibody	8																	
Anti-HIV 1/2 antibody/Ag	3																	
HLA typing[q]				10														

	Screen	Optional[a]	**Schedule of Activities**															Optional[b]
Visit Number	Screen		SC[c]	01	02	03	04	05	06	07	SC[c]	08	SC[c]	09	SC[c]	10	11	12
Day of Study[d]			−59 to 0	D0	D1	D3	D6	D9	D12	D15	D16	D28	D29	D57	D58	D90	D180	D365
Visit Window (days)	−60 to −1	−59 to 0	+2	0	0	±2	±2	±2	±2	±4	+1	±4	+1	+12	+1	±10	±15	±15
Clinical Evaluations/ Procedures				**Active Phase**										**Follow-up Phase**				
Human chorionic gonadotropin, pregnancy[r]	X	X		X						X		X		X				
Drug abuse screen, urine	X																	
Research blood (flavivirus antibodies, neutralizing antibody assays, serum storage, viral qRT-PCR, and culture)[s]	7			7	7	7	7	7	7	7		7		7		10 (7)[t]	10 (7)[t]	10 (14)[t]
Research blood (T and B cell assays; plasma, PBMC storage)		8.5		68 (76.5)[u]	25.5	25.5	25.5	59.5		76.5		76.5		51		93.5 (51)[t]	93.5 (51)[t]	93.5 (51)[t]
Alternative resesearch blood volumes (including neutrophil assays)[v]		8.5		71.5 (80.5)[u]	37.5	25.5	25.5	63		68		68		51		93.5 (51)[t]	93.5 (51)[t]	93.5 (51)[t]
Daily volume (mL) of blood[w]	27.5	18		98 (106.5)[u]	35.5	35.5	35.5	76	20	93		93		71		103.5 (58)	103.5 (58)	103.5 (65)

	Screen	Optional[a]	**Schedule of Activities**															Optional[b]
Visit Number	Screen		SC[c]	01	02	03	04	05	06	07	SC[c]	08	SC[c]	09	SC[c]	10	11	12
Day of Study[d]			−59 to 0	D0	D1	D3	D6	D9	D12	D15	D16	D28	D29	D57	D58	D90	D180	D365
Visit Window (days)	−60 to −1	−59 to 0	+2	0	0	±2	±2	±2	±2	±4	+1	±4	+1	+12	+1	±10	±15	±15
Clinical Evaluations/ Procedures				**Active Phase**										**Follow-up Phase**				
Alternative daily blood volume (including neutrophil assays)[v]	27.5	18		101.5 (110)[u]	47.5	35.5	35.5	79.5	20	84.5		84.5		71		103.5 (58)	103.5 (58)	103.5 (65)

Abbreviations: AE, adverse event; CBC, complete blood count; CRP, C-reactive protein; DENV, dengue virus; HbA1c, hemoglobin A1c; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalized ratio; OTC, over the counter; PBMC, peripheral blood mononuclear cells; PT, prothrombin time; PTT, partial thromboplastin time; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SC, secure communication; X, to be performed.

a Optional visit for lymph node aspiration to obtain baseline cell populations.

b Optional blood draw on day 365 to assess antibody waning.

c All patients who undergo lymph node aspiration will receive a follow-up call or secure electronic communication (per participant preference) within 24 to 48 hours to assess for any procedure-related AEs.

d An early termination visit will be performed if the participant decides to discontinue participation or is withdrawn from the study prior to day 180. It will include a targeted history, focused physical exam, and assessment of any unresolved AEs, including abnormal laboratory results.

e Written informed consent for screening must be obtained prior to initiation of screening procedures and written informed consent for the DENV3 vaccination protocol must be obtained at the optional baseline lymph node aspiration day or day 0 prior to the initiation of any study procedures.

f Complete post-vaccination evaluation (temperature, blood pressure, pulse, respiratory rate, and injection site assessment) will be performed 30 to 60 minutes post-vaccination. For those undergoing lymph node aspiration, vital signs will also be assessed per Clinical Center and interventional radiology guidelines.

g Short travel history completed at pre-screening will only be reviewed by unblinded statistician to assist with screening target groups.

h Post-enrollment exposure history assessment can be completed any time between days 0 and 180. Study staff will not review the assessment until after study unblinding.

i To capture any potential exposures between the completion of the first assessment and final visits, a follow-up assessment will be completed within 3 weeks of the day 180 visit. If individuals opt to attend the day 365 visit, then they will complete the follow-up assessment again within 3 weeks of the day 365 visit.

j For participants who can get pregnant, confirm that pregnancy test is negative on day 0. Day 0 safety labs are for baseline data and will not determine eligibility. However, all safety laboratories drawn at day 0 will be reviewed by the principal investigator or designee to confirm there have been no clinically

	Screen	Optional[a]	**Schedule of Activities**															Optional[b]
Visit Number	Screen		SC[c]	01	02	03	04	05	06	07	SC[c]	08	SC[c]	09	SC[c]	10	11	12
Day of Study[d]			−59 to 0	D0	D1	D3	D6	D9	D12	D15	D16	D28	D29	D57	D58	D90	D180	D365
Visit Window (days)	−60 to −1	−59 to 0	+2	0	0	±2	±2	±2	±2	±4	+1	±4	+1	+12	+1	±10	±15	±15
Clinical Evaluations/ Procedures				**Active Phase**										**Follow-up Phase**				

significant changes prior to vaccination. Any changes that are concerning to the principal investigator or designee may result in cancellation of the vaccination.

k Day 0 evaluations, prior to the first vaccine dose, are the baseline for assessing subsequent AEs.

l Lymphatic cells will be collected, with minimal blood loss anticipated (< 5 mL). Participants will be monitored post-procedure until the responsible provider deems they are safe to leave the Clinical Center. Lymph node aspirations on days 15 and 28 are opt-out procedures, and pre-vaccine and day 57 aspirations are opt-in procedures. Participants can choose to undergo any number of aspirates: from zero to four.

m CBC will be collected at Day 90 for participants who experience Grade 1 or higher anemia or a Grade 2 or higher hemoglobin drop that does not resolve by Day 57.

n Hemoglobin A1c will be assessed as part of CBC on screening day only.

o CRP, quantitative immunoglobulins, and ferritin will be assessed as part of the acute care panel.

p Fibrinogen will be assessed as part of PT/PTT/INR

q HLA typing will only be performed once. If vaccination is rescheduled after day 0 blood draw, all day 0 safety labs will be repeated at the future vaccine date except HLA typing.

r Negative pregnancy test from day 0 will be confirmed prior to vaccination. Pregnancy testing will only be performed on days −59 to 0, 15, 28, and 57 if participants who can get pregnant undergo lymph node aspiration. Pregnancy test will be performed as part of the acute care panel, and additional volume is not required.

s Antibodies against DENV1-4 will be assessed in all individuals. If an individual requires rescreening, dengue antibodies may be repeated if any of the following have occurred since the initial screen: 1) 6 months have passed, 2) the individual reports travel to a flavivirus endemic area, 3) they report reciept of a travel vaccine, or 4) the investigator deems antibody retesting is appropriate. Flavivirus antibodies may include yellow fever virus, West Nile virus, Japanese encephalitis virus, and Zika virus, among others. These will be assessed at screening if necessary to confirm vaccination, travel, and/or medical history.

t For participants who experience Grade 1 or higher anemia or a Grade 2 or higher hemoglobin drop that does not resolve by Day 57 (as shown in the Day 57 CBC), a smaller volume of 58 mL will be drawn on Day 90 (indicated in parentheses). If anemia or hemoglobin drop remains unresolved by day 90, then a smaller volume (in parentheses) of 58 mL on Day 180 and 65 mL on Day 365 will be drawn.

u Volume in parentheses is for individuals who do not have the optional pre-vaccine visit.

v This alternative distribution of blood draw volumes will be used for individuals chosen for neutrophil evaluation. We are aiming for 5 individuals per group to have neutrophil evaluations.

w Blood draw volumes are approximate accounting for tube availability and variability in the collection of clinical specimens.

2 INTRODUCTION

2.1 Study Rationale

In endemic areas, protective immunity against dengue is achieved following sequential infection with two of the four dengue virus serotypes (DENV1-4). In cohort studies, individuals with two or more previous DENV infections have a lower risk of future dengue disease compared to naïve individuals. In contrast, one previous heterotypic DENV infection increases disease risk [1, 2]. This poses a unique challenge to the design of dengue vaccines. To avoid inducing harmful immunity, dengue vaccines are tetravalent and aim to induce protective immunity against each serotype simultaneously. However, for some dengue vaccines, immunization only protects those with prior DENV infection, and the determinants of cross-serotypic protective dengue immunity are poorly understood [3, 4]. Moreover, although it is known that a longer interval between DENV infections is associated with a higher risk of severe disease [5], there is limited experience with dengue vaccines in naturally immune individuals living in non-endemic areas.

In this study, we will model primary and sequential DENV infections with a monovalent dengue vaccine. This will be the first protocol to simultaneously and extensively compare the immune responses after primary, secondary heterotypic, and tertiary or higher dengue exposures in naturally infected humans living in non-endemic areas [6]. This work aims to address two overarching questions. First, is a monovalent dengue vaccination safe and immunogenic in individuals with distinct DENV exposure histories living in non-endemic areas? Second, by what mechanism does sequential exposure induce broadly protective dengue immunity? By evaluating dengue vaccines in a new population and modeling the induction of cross-serotypic protective immunity, this work may inform vaccine evaluation and strategies and broaden potential target populations.

2.2 Background

Dengue is a viral disease transmitted by *Aedes* mosquitos, which inhabit tropical and subtropical regions [7]. Approximately 3.9 billion people are at risk of infection with any of the four globally co-circulating dengue virus serotypes (DENV1-4), and dengue is a leading cause of hospitalization and death in some regions of Asia and Latin America [8, 9]. In 2017, more than two million disability-adjusted life years were attributed to dengue globally, and in 2019, all World Health Organization regions reported some of the largest numbers of dengue cases ever recorded [9-12]. Dengue is one of the communicable diseases with an increase in global burden between 2005 and 2013, and in the coming years, dengue incidence is predicted to rise due to increasing population in endemic areas, urbanization, human migration, and warming climate [9, 12, 13].

Despite the high morbidity of dengue, a universally effective dengue vaccine has eluded scientists for decades [14]. The cocirculating and immunologically interactive DENV1-4 pose a unique challenge to vaccine design because a sub-protective vaccine can increase the risk of severe dengue disease. Primary dengue infection results in significant protection against the infecting serotype by inducing potently neutralizing serotype-specific antibodies [15] but also induces low titers of cross-serotypic reactive antibodies that are strongly associated with future severe dengue [16-18]. This phenomenon is hypothesized to be caused by antibody-dependent enhancement (ADE), where the weak, low-titer antibodies promote viral internalization rather than neutralization. ADE facilitates viral entry and replication in cells with Fc receptors such as

macrophages and monocytes, resulting in earlier and higher peak viremia and a dysregulated immunologic response [19].

To avoid inducing ADE, all three leading vaccine candidates—Dengvaxia, TAK-003, and TV003—were designed to be tetravalent, live attenuated, and to induce specific immunity against each serotype simultaneously. TV003 phase 1/2 trials indicate that it induces a tetravalent antibody response in about two-thirds of subjects [20-22]; phase 3 efficacy trials are ongoing. Neither Dengvaxia nor TAK-003 provide full protection against all four serotypes and both have significant waning of efficacy over time [4, 23-26]. Of greatest concern, Dengvaxia, which was licensed in 20 countries and introduced in mass vaccination programs in the Philippines and Brazil, significantly increased risk of hospitalized dengue in DENV-seronegative individuals in year 3 of the phase 3 clinical trial [3]. Surprisingly, while Dengvaxia and TAK-003 induced cross-reactive antibodies that neutralized all four DENV strains in vitro, vaccine efficacy varied by strain, and seroconversion alone did not predict protection. This most likely resulted from imbalanced infectivity among the vaccine strains, leading to a lack of homotypic antibody against each of the four serotypes [23, 27, 28]. There is an urgent need to identify immunologic measures that are strongly associated with protection against viral replication and disease for the evaluation and design of future interventions against dengue.

Observations of natural dengue infection and vaccination indicate that sequential exposure to two different DENV serotypes induces broad protection even against previously unexposed strains. Specifically, while second heterotypic infection has the highest risk of severe dengue, third and fourth infections are less likely to be symptomatic or serious [29]. One hypothesis is that the increased replication mediated by ADE during the second heterotypic infection leads to increased virulence and severe disease but also induces potent antibodies that target conserved epitopes and neutralize all four serotypes [30].

Induction of broad protection may be possible in the absence of severe disease. Both Dengvaxia and TAK-003 have higher efficacy in DENV-seropositive than seronegative individuals, suggesting that prior DENV infection improves the immunogenicity and protection conferred by the vaccination. A small vaccine trial conducted by Durbin et al has modeled heterotypic infection and demonstrated the induction of broad immunity. Sequential immunization with monovalent DENV1, 2, and 4 live attenuated vaccines resulted in earlier onset and higher mean peak viremia (Table 1) and higher rash frequency (Table 2) after secondary heterotypic versus primary vaccination for the DENV2 cohorts but no other increased adverse effects [6]. Primary immunization induced potent type-specific and weak cross-reactive antibodies, while secondary vaccination resulted in cross-reactive antibodies with high avidity to and strong neutralization of both exposed and nonexposed serotypes [31]. Studies of natural dengue infections have confirmed that type-specific antibodies correlate with protection against dengue disease [15], and cross-reactive antibodies targeting conserved epitopes can potently neutralize all four serotypes [30, 32]. Separately, sequential dengue exposures are associated with an evolution of the T-cell - response from targeting serotype-specific epitopes to a focus on conserved regions [33]. Together, these studies indicate that sequential dengue exposure induces a cross-serotypic protective response, but the mechanisms underlying this response are poorly understood.

Table 1. Magnitude, Onset, and Duration of Viremia in Persons Inoculated With rDEN1Δ30 or rDEN2Δ30 as a Primary or Secondary Vaccination

Vaccine	Previous inoculation	Vaccine dose, log_{10} PFU	No. of volunteers	Percentag eviremic (no.)	Mean peak titer, [b] log_{10} PFU/mL ± SE	Mean onset of viremia,day ± SE	Mean durationof viremia,days ± SE
DEN1[a]	None	3.0	71	61 (43)	1.0 ± .1	10.0 ± .3	3.2 ± .3
DEN1	DEN4[c]	3.0	8	63 (5)	1.0 ± .2	8.6 ± .5	3.6 ± .9
DEN1	DEN2	3.0	7	29 (2)	0.5 ± 0	10.5 ± 3.5	3.0 ± 0
DEN2[d]	None	3.0	40	60 (24)	0.5 ± .03	9.2 ± .6	3.3 ± .6
DEN2	DEN4[c]	3.0	8	75 (6)	1.1 ± 0.2[d]	5.0 ± 1.0[d]	5.0 ± 1.0
DEN2	DEN1	3.0	7	43 (3)	0.7 ± .2	11.0 ± 2.1	2.7 ± 1.7

NOTE. [a] Data are from two clinical trials in which volunteers received a single dose of 3 log_{10} PFU of live attenuated DEN1 vaccine candidate rDEN1Δ30.
[b] Calculated only for volunteers with detectable level of viremia (≥.5 log_{10} PFU/mL serum).
[c] Volunteers previously vaccinated with rDEN4Δ30 or rDEN4Δ30-200,201.
[d] Data are from two clinical trials in which volunteers received a single dose of 3 log_{10} PFU of live attenuated DEN2 vaccine candidate rDEN2/4Δ30.
[e] Statistically significant difference when compared to rDEN2/4Δ30 given as a single primary vaccination ($P < .01$, Tukey-Kramer post-hoc test).

Table 2. Clinical and Virologic Response of Volunteers Inoculated With rDEN1Δ30 or rDEN2Δ30 Given as Primary or Secondary Vaccination

					Percentage with indicated clinical sign (no.)						
Vaccine	Previous inoculation	Vaccine dose, log_{10} PFU	No. of participants	Percentag eviremic (no.)	Fever	Macular/ maculopapular rash	Arthralgia	Myalgia	Neutropenia[a]	Thrombo-cytopenia[b]	Increase in ALT level
DEN1[c]	None	3.0	71	60 (43)	1 (1)	32 (23)	3 (2)	13 (9)	44 (31)	1 (1)	1 (1)
DEN1	DEN4[e]	3.0	8	63 (5)	0	0	0	0	25 (2)	0	12[d] (1)
DEN1	DEN2	3.0	7	29 (2)	0	0	0	14 (1)	29 (2)	0	0
DEN2[f]	None	3.0	40	63 (25)	0	32 (13)[g]	2 (1)	5 (2)	28 (11)	0	12 (5)
DEN2	DEN4[e]	3.0	8	75 (6)	0	75 (6)[g]	0	0	13 (1)	0	0
DEN2	DEN1	3.0	7	43 (3)	0	43 (3)	0	0	0	0	0
Placebo	none	n/a	6	0	17 (1)	17 (1)	0	0	17 (1)	0	0

NOTE. Percentage of subjects with an increase in ALT level as defined as ≤ 1.25 times the upper limit of normal.[a] Neutropenia is defined as an absolute neutrophil count ≤ 1500 neutrophils/mm^3.
[b] Defined as a platelet count <120,000 platelets/mL.
[c] Data are from 2 clinical trials in which volunteers received a single dose of 3 log_{10} PFU of live attenuated DEN1 vaccine candidate rDEN1Δ30.
[d] ALT level elevation was determined to be unrelated to vaccination.
[e] Volunteers previously vaccinated with rDEN4Δ30 or rDEN4Δ30-200,201.
[f] Data are from 2 clinical trials in which volunteers received a single dose of 3 log_{10} PFU of live attenuated DEN2 vaccine candidate rDEN2/4Δ30.
[g] Frequency of rash statistically greater following inoculation of rDEN2/4Δ30 as a secondary vaccine compared to its use as a primary vaccine ($P < .05$, Fisher exact test).

In this study, we will leverage a controlled environment and comprehensive immunologic techniques to simultaneously and extensively model primary, secondary heterotypic, and tertiary DENV exposure. We aim to address fundamental questions in dengue immunology with respect to how distinct immunity profiles impact the innate, cellular, and humoral responses to a controlled dengue exposure. First, we will examine what baseline characteristics are associated with "protection" against the vaccine. Second, we will evaluate what baseline and early post-vaccination responses (eg, viremia, other measures) are associated with induction of potent, enduring cross-serotypic immunity. Additionally, our trial will evaluate dengue vaccines in naturally immune people living in non-endemic areas, many of whom had infections many years previously. Given that time from dengue exposure is associated with more severe disease [5], and travel and migration are common in our globalized society, evaluating dengue vaccine safety and immunogenicity in an immune, non-endemic population will be a first step towards dengue vaccination for travelers and expatriates.

2.2.1 Choice of rDEN3Δ30/31-7164 Over Other Candidates

The monovalent rDEN3Δ30/31-7164 is one of the four viral components of the tetravalent dengue vaccine candidate, TV003, developed by NIAID/NIH and CRL. It is derived from the wild-type DENV-3 Sleman/78 virus with two deletions in the 3' untranslated region (UTR) for attenuation: a Δ30 and a non-contiguous Δ31 mutation [34]. Additionally, the vaccine strain includes the 7164 Vero cell adaptation mutation (amino acid 115 in NS4B Val→Ala) [35].

We chose rDEN3Δ30/31-7164 over the other three strains for qualities specific to the strain, serotype, and the potential to expand on previous work. Specifically, of the four monovalent vaccine strains given as primary doses, rDEN3Δ30/31-7164 had one of the lowest mean peak viremia titers and standard errors (Table 3, which is from the clinical trial protocol published in the supplementary section of reference [36]). Additionally, in a trial of naturally dengue-immune individuals in Brazil dosed with Butantan-DV (a vaccine formulation that includes the rDEN3Δ30/31-7164 seed virus), DENV3 viremia was lower after secondary vs. primary exposure (Table 4) [22]. Thus, we expect that secondary rDEN3Δ30/31-7164 inoculation will maintain a relatively low mean peak viremia titer, which is important for vaccine safety in a new population at risk for ADE. Moreover, the low mean peak viremia titer standard error suggests that rDEN3Δ30/31-7164 is the most likely to have intrinsically consistent replication. As such, any differences in mean peak viremia titer we observe between groups can likely be attributed to host immunity rather than strain variability.

Table 3. Magnitude, Onset, and Duration of Viremia in Subjects Inoculated With Monovalent DEN Vaccine Candidates Included in TV003

Vaccine candidate	Dose (log_{10}PFU)	N	(%) with viremia	Mean peak titer ± SE (log_{10} PFU/mL)[1]	Mean day of onset of viremia ± SE	Mean # of days of viremia ± SE
rDEN1Δ30	3	71	60	1.0 ± 0.08	10.0 ± 0.3	3.3 ± 0.3
rDEN2/4Δ30(ME)	3	40	60	0.5 ± 0.03	9.2 ± 0.6	3.3 ± 0.6
rDEN3Δ30/31-7164	3	50	34	0.6 ± 0.01	7.6 ± 0.64	3.2 ± 0.5
rDEN4Δ30 (Lot 4-9)	5	20	70	1.6 ± 0.1	10.5 ± 0.7	3.6 ± 0.45
rDEN4Δ30 (Lot 4-9)	3	20	35	0.5 ± 0.1	9.1 ± 1.2	1.6 ± 0.8
rDEN4Δ30 (Lot 109A)	3	50	26	0.7 ± 0.1	10.8 ± 0.7	2.2 ± 0.4

1. Mean peak titer is calculated only for those subjects who were viremic. Lower limit of detection is 0.5 log_{10} PFU/mL.

Table 4. Viremia After Inoculation With Butantan-DV in Brazilian Recipients Stratified by Previous Exposure

	DENV-naive	DENV-exposed	p value
Participants assessed	18	25	
Viruses detected in a single participant			
0	6 (33%)	15 (60%)	0.124*
1	10 (56%)	8 (32%)	--
2	2 (11%)	2 (8%)	--
Identified serotypes			
DENV-1	5 (28%)	7 (28%)	> 0.999
DENV-2	2 (11%)	4 (16%)	> 0.999
DENV-3	7 (39%)	1 (4%)	0.006
DENV-4	0	0	--

DENV3 is a clinically relevant and immunologically unique serotype, and the use of rDEN3Δ30/31-7164 in this trial will expand on previous work. Specifically, the only sequential monovalent vaccination trial performed in humans utilized DENV1, 2, and 4, and monovalent rDEN3Δ30/31-7164 vaccination has only been assessed in flavivirus-naïve individuals [6, 37, 38]. The Walter Reed Army Institute of Research has performed two trials using a challenge strain of DENV3, and they have challenged seven flavivirus-naïve individuals and ten individuals who had been vaccinated with a live attenuated tetravalent dengue vaccine candidate [39, 40]. In sum, this will be one of the few trials to simultaneously and extensively assess secondary monovalent DENV3 exposure in subjects with natural primary heterotypic and polytypic immunity, thereby mimicking the sequential exposure associated with cross-serotypic protection.

Safely modeling secondary DENV3 infection in primary heterotypic individuals is particularly important because DENV3 has been associated with enhanced disease after secondary exposure [29, 41]. Additionally, we have previously shown that low levels of pre-existing anti-DENV antibodies can enhance natural DENV3 disease while high levels are protective, making it an ideal model for examining both enhancing and protective antibodies [42]. This contrasts with DENV1, where high cross-reactive immunity protects against DENV1 secondary disease, and DENV2, which is enhanced by a broad range of pre-existing antibody levels [37]. With regards to immunization, the TAK-003 vaccine failed to protect dengue-naïve subjects against DENV3 [43]. This may be related to the failed replication of the DENV3 component of TAK-003, but the vaccine did protect naïve subjects against DENV1 despite no detected replication of this component in the phase 1 trial [28]. Thus, the basis for the failure of TAK-003 to protect against DENV3 is not completely understood, and our study may identify relevant markers of serotype-specific protection. Additionally, the $CD8^+$ T-cell responses to DENV3 are unique as about 40% of all responses are directed against structural proteins, while only 10% to 30% of the responses to the other serotypes target structural proteins [43]. As such, using rDEN3Δ30/31-7164 will allow us to assess how different pre-existing immunity profiles impact

the development of $CD8^+$ T-cell immunity against both structural and nonstructural cross-reactive epitopes. In sum, scrutinizing the responses to a live attenuated full-length- DENV3 will help address clinically and immunologically relevant questions.

2.2.2 Summary of Nonclinical In Vitro or In Vivo Research

rDEN3Δ30/31-7164 has been evaluated in juvenile rhesus monkeys and in a rodent model consisting of severe combined immunodeficiency (SCID) mice bearing intraperitoneal tumors of the human liver cell line HuH-7 [34]. In the SCID-HuH-7 mouse model, the wild type DENV-3 Sleman/78 replicated to a mean peak virus titer of $10^{6.9}$ PFU/mL, and rDEN3Δ30/31-7164 replication was slightly less than 10-fold restricted. Juvenile rhesus monkeys were inoculated subcutaneously with monovalent doses of DENV-3 Sleman/78 or rDEN3Δ30/31-7164 at 10^5 PFU. All four monkeys inoculated with DENV-3 Sleman/78 became viremic for a mean number of 3.5 days, but 0/4 monkeys inoculated with rDEN3Δ30/31-7164 became viremic. Despite the lack of detectable viremia, the mean neutralizing antibody levels in monkeys infected with rDEN3Δ30/31-7164 were similar to those of the monkeys infected with DENV-3 Sleman/78. Monkeys were observed twice daily for mortality and morbidity, as well as for signs of neurologic, hemorrhagic, or dermal disease. Signs consistent with these diseases were not observed in any of the animals.

2.2.3 Replication in Mosquitos

Both rDEN3Δ30/31-7164 and its parent strain, DEN3-Sleman/78, are very poorly transmitted to mosquitoes. In one study, ingestion of $10^{4.1}$ PFU of DEN3-Sleman/78 by *Ae. aegypti* mosquitoes infected the midgut of only 4 of 28 (14%) mosquitoes tested and disseminated from the midgut in only 2 of 28 (7%) mosquitoes. Thus, transmission of DEN3-Sleman/78 virus to *Ae. aegypti* mosquitoes would require a dose of $>10^5$ PFU/mL in blood [35]. The mean peak viremia of rDEN3Δ30/31-7164 was found to be substantially lower than this, at 0.6 PFU/mL (standard error [SE] ± 0.01). Moreover, a 10-to-14-day incubation period within the mosquito is required for DENV to be transmitted from one person to another. Thus, for rDEN3Δ30/31-7164 to be transmitted to an in-house family member, the subject would have to be viremic with a peak virus titer greater than 10^5 PFU/mL, would have to be bitten by a viable vector mosquito at the peak of viremia, the mosquito would have to live for an additional 10 to 14 days, and the same mosquito would then have to bite another family member.

In a separate study, the highly sensitive *Toxorynchites amboinensis* mosquitoes were used to compare rDEN3Δ30/31-7164 replication to that of a recombinant form of DEN3-Sleman/78 (rDENV-3) [34]. Ten-fold serial dilutions of each virus were injected intrathoracically, and the resulting infections of head tissues were compared at day 14. Although the intrathoracic infectivity was similar for both strains, rDEN3Δ30/31-7164 had 5-to-30-fold lower replication in the head than DEN3-Sleman/78. Thus, in addition to the low replication intrinsic to DEN3-Sleman/78, the Δ30/31 mutation further restricts replication in mosquitos.

2.2.4 Summary of Relevant Clinical Research

rDEN3Δ30/31-7164 has been evaluated in one phase 1 clinical trial, and the most complete clinical data from this trial is available in the supplemental clinical protocol published with Kirkpatrick et al. [36]. TV003 has been evaluated in multiple phase 1 and 2 trials [20-22, 36, 44, 45], with phase 3 trial data pending (NCT02406729). In the phase 1 trial, 50 flavivirus-naïve

participants received 10^3 PFU of rDEN3Δ30/31-7164. Viremia was detected in 34% of participants, with a mean peak titer of 0.6 $\log_{10}$ PFU/mL. The most observed AEs were rash (52% of vaccinees vs. 3% of placebo recipients) and headache (36% vs. 36% of placebo recipients). Neutropenia was observed in 4% of vaccinees and 8% of placebo recipients, and no participants had fever versus 1% of placebo recipients. The vaccine was immunogenic with 81% seroconversion among participants [36].

TV003 has been consistently safe and well tolerated. Sixty flavivirus-naïve individuals received 1 dose of TV003 and 44 received a second dose 6 months later, and all tolerated the vaccine well with no SAEs [20]. The most common AEs were rash (62% after the first vaccine, 0% after the second vaccine), headache (37% and 25%), fatigue (17% and 11%), and neutropenia (8% and 0%). Only rash was significantly elevated in the TV003 group compared to placebo (likelihood ratio <0.001). None had fever after the first dose and 2% had fever after the second dose. After the first dose, viremia with rDEN3Δ30/31-7164 occurred in 38% of participants, with mean peak titer of 0.60 $\log_{10}$ PFU/mL (SE ±0.03). Following the second dose, rDEN3Δ30/31-7164 viremia was present in only one participant at 0.5 $\log_{10}$ PFU/mL, and no other serotypes were detected. Seroconversion to DENV3 occurred in 97% of participants after 1 dose of TV003, and the second dose did not boost antibody responses.

In a phase 1 trial of flavivirus-immune individuals, 41 participants received one dose of TV003 and 33 received a second dose 6 months later [21]. Eleven had previous dengue immunity from a monovalent DENV vaccine, 29 had yellow fever virus (YFV), and 8 had immunity to a different flavivirus. Again, the most common AEs were rash (66% after the first dose, 0% after the second dose), fatigue (15% and 12%), headache (44% and 30%), and neutropenia (10% and 0%). Only rash was significantly different from placebo ($p < 0.001$). Fever occurred in 2% of individuals after the first dose and in 0% after the second dose. The rDEN3Δ30/31-7164 peak viremia titer was significantly higher in flavivirus-experienced individuals versus flavivirus-naïve individuals (mean titer: 0.97 $\log_{10}$ PFU/mL vs. 0.60 $\log_{10}$ PFU/mL, $p = 0.04$; maximum titer: 2.4 $\log_{10}$ PFU/mL vs. 1.2 $\log_{10}$ PFU/mL). Additionally, the viremia onset day was slightly earlier in flavivirus-immune individuals, but with similar ranges (8.9 [7-14] vs. 9.4 [5-14]). Following one dose of TV003, the antibody response to DENV3 was significantly higher in flavivirus--immune versus naïve individuals ($p = 0.0003$), even after adjustment for multiple comparisons. However, after the second dose of TV003, there was no difference in the antibody response between the flavivirus-immune and naïve groups. Together, these TV003 trials suggest that rDEN3Δ30/31-7164 may undergo some enhanced replication in flavivirus-immune individuals as compared to naïve individuals with increased immunogenicity but no safety concerns.

The seed viruses used in TV003, including rDEN3Δ30/31-7164, have been licensed to outside groups, and two phase 2 trials have been published using these viruses [22, 45]. In Brazil, the Butanan Institute developed the seed viruses into a tetravalent formulation called Butantan-DV [22]. They compared the safety and immunogenicity of Butantan-DV in DENV-naïve (n = 85) and DENV-exposed (n = 101) participants and reported no safety concerns in either group. Of the 31 AEs assessed, only the frequency of rash differed between the DENV-naïve and DENV-exposed -individuals (65% in the DENV-naïve individuals vs. 45% in DENV-exposed, $p = 0.005$). The mean peak viremia titers and onset days were not compared between the DENV--naïve and DENV-exposed groups, but DENV3 viremia was more common in the DENV--naïve versus exposed group (37% vs. 3%, $p = 0.004$). Despite more detectable viremia

in the DENV-naïve group, the antibody response to DENV3 was lower in the DENV-naïve vs. DENV exposed group ($p < 0.0001$) and this is likely related to higher DENV-3 antibodies at baseline in the DENV-exposed group.

In India, the TV003 seed viruses were licensed to Panacea Biotec LTD, and they were formulated into a tetravalent vaccine called TDV [45]. A phase 1/2 trial was performed with 70 dengue-immune Indian adults receiving TDV and 24 receiving placebo. There were no significant differences in the frequencies of any AEs between the TDV and placebo groups, but the TDV group had significantly higher DENV3 antibody responses than the placebo group ($p = 0.003$), again perhaps due to higher DENV3 antibody levels at baseline. Viremia was not detected in any participant.

In sum, rDEN3Δ30/31-7164 monovalent vaccination was safe and well-tolerated in flavivirus--naïve individuals with mean peak viremia titers just above the limit of detection. Although rDEN3Δ30/31-7164 monovalent vaccination has never been tested in -flavivirus-exposed individuals, the virus has been tested in numerous flavivirus-exposed individuals as part of several tetravalent formulations. No significant AEs or safety concerns were noted in any of these trials. In a non-endemic area, where most individuals had YFV immunity rather than DENV immunity (60% YFV-immune vs. 23% DENV-immune), the mean peak vaccine viremia titer was higher in the flavivirus-immune group versus the naïve group with a maximum titer of $10^{2.4}$ PFU/mL. In Brazil, the viremia titer was higher in the DENV--naïve group versus the DENV-immune group, and in India, viremia was not observed in any participant, all of whom were DENV-immune. Thus, the type of underlying flavivirus immunity and the time from last exposure likely influence viremia frequency and peak titers, but the peak rDEN3Δ30/31-7164 viremia ($10^{2.4}$ PFU/mL) remained orders of magnitude lower than that required for mosquito transmission (10^5 infectious units/mL) or that observed in symptomatic (10^6 infectious units/mL) and severe dengue (10^8 infectious units/mL) [6, 35]. Significant AEs were not noted in the hundreds of participants vaccinated with rDEN3Δ30/31-7164 as a monovalent or part of a tetravalent formulation.

2.2.5 Importance of Clinical Trials and Relevant Treatment Issues or Controversies

Natural infection cohort studies and vaccine clinical trials have revealed notable patterns in the host responses to DENV, but the mechanisms underlying cross-serotypic immunity and consistently accurate correlates of protection have not been identified. This trial will leverage a controlled environment and comprehensive immunologic techniques to directly compare how differing histories of natural DENV infection impact the host response to an exposure. Additionally, evaluation of lymph node (LN) aspirates will allow us to assess how potent, cross-serotypic-immunity develops. We hypothesize that the broad protection observed following sequential heterotypic exposure arises from a two-part process: 1) primary infection produces low-affinity cross-reactive antibodies and memory B cells (MBCs) that can enhance disease while 2) secondary infection activates these MBCs to undergo affinity maturation and develop potent cross-reactive MBCs and antibodies that neutralize even previously unexposed serotypes [46, 47]. By monitoring the peripheral and germinal center responses in real time, we can compare the affinity maturation and antibody potency in each group pinpointing the cell traits associated with broad protective immunity.

This trial will also expand our understanding of DENV3, which has never been modeled in sequential monovalent vaccination. DENV3 is notable for its potential to undergo ADE, its unique impact on T-cell - responses with more of a focus on structural proteins compared to other serotypes, and its lack of a consistent correlate of protection [42, 43]. Specifically, in the TAK-003 vaccine trials, seronegative individuals were protected against DENV1 but not DENV3 despite similar antibody titers against both serotypes [4]. By comparing viral kinetics and the B- and T-cell - responses over time among the groups, we may identify more accurate correlates of protection for this serotype.

Dengue vaccination has been controversial since Dengvaxia was approved in 2016 and post--marketing analyses demonstrated an increased risk of severe dengue in vaccinated DENV-naïve -individuals [3]. The mechanism of this increased risk is unconfirmed, but experts have hypothesized that Dengvaxia may induce enhancing antibodies like a primary exposure and/or the lack of non-structural DENV proteins in Dengvaxia may result in incomplete DENV immunity [48]. Regardless, all DENV vaccine trials must consider the risks of ADE and the possibility of severe dengue with future exposures. Such a risk in this trial will be mitigated by several factors.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Risks from rDEN3Δ30/31-7164 Vaccination

Possible local vaccine reactions include pain, swelling, or erythema for 2 to 3 days, lymphadenopathy, or pruritus at the injection site [49]. Systemic reactions such as maculopapular rash and transient neutropenia have been observed in some individuals vaccinated with rDEN3Δ30/31-7164. Other potential systemic reactions include symptoms of dengue such as fever, headache, eye pain, photophobia, rash, generalized myalgias, arthralgias, elevated alanine transaminase (ALT), neutropenia, elevated partial thromboplastin time (PTT), or decreased platelet count. Immediate hypersensitivity reactions including urticaria, anaphylaxis, or other immunoglobulin E–mediated responses are possible, as with any vaccine.

Participants will be asked to defer all vaccinations (such as SARS-CoV-2 and influenza) from day −28 to day 28 (or day 57 for those opting for a LN fine needle aspiration [FNA]at day 57). This may increase the risk that the participant will be infected with SARS-CoV-2 or influenza virus during this period. As with any investigational vaccine, there is a theoretical possibility of risks about which we have no present knowledge. Participants will be informed of any such risks should further information become available.

Vaccination with rDEN3Δ30/31-7164 will also have group-specific risks. The primary heterotypic group is at the highest risk for ADE, which can result in an increased mean peak vaccine viremia titer. High viral titers are associated with mosquito transmission (10^5 infectious units/mL), symptomatic (10^6 infectious units/mL), and severe dengue (10^8 infectious units/mL) [6, 35]. However, in an analogous trial of flavivirus-immune individuals vaccinated with TV003 and living in a non-endemic area, the highest rDEN3Δ30/31-7164 viremia titer recorded was $10^{2.4}$ PFU/mL, and the vaccine was well-tolerated in this group [21]. Thus, we do not expect dangerously high viremia titers or significant AEs in this group. Monotypic vaccination, such as proposed with rDEN3Δ30/31-7164, will leave some participants, particularly those who are

dengue naïve, with an imbalanced dengue immunity. This immune profile could predispose participants to a more severe disease outcome following a subsequent natural DENV infection.

Given *Aedes spp* are endemic in Washington, DC, Maryland, and Virginia, and rDEN3Δ30/31-7164 is a live attenuated virus, there is a potential risk for mosquito transmission. However, this is unlikely since rDEN3Δ30/31-7164 and its parent virus do not replicate well in mosquitos, and the maximum rDEN3Δ30/31-7164 titer recorded in a vaccine trial was $10^{2.4}$ PFU/mL, which is much lower than the 10^5 infectious units/mL required for mosquito transmission [6, 35]. No rDEN3Δ30/31-7164 mosquito transmission has ever been recorded in any of the phase 1 or 2 trials performed with rDEN3Δ30/31-7164 as a monovalent or part of a tetravalent formulation. Of note, PFU is a well-established method of measuring infectious units, but other methods have also been used to assess the viral loads associated with mosquito transmission, and symptomatic and severe dengue [50, 51].

Participants who can become pregnant will be cautioned of the unknown risks of rDEN3Δ30/31-7164 to a fetus. They will be required to use effective birth control methods starting from the invitation to participate in the study through day 60 per FDA request, given this is a live-attenuated investigational vaccine [52].

Risk from Blood Drawing

Risks associated with blood drawing include excessive bleeding, pain, bruising at the site of the procedure, very rarely infection, and possibly fainting. We will not draw more than 550 mL of blood over an 8-week period, as per the NIH CC Medical Administrative Policy 95-9, Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf.

Risks from Lymph Node Aspiration

Risks associated with LN FNA include bleeding, pain, swelling, or bruising at the site of the procedure, very rarely infection, irritation or damage to nearby tissues such as nerves or blood vessels, and possibly fainting [53]. We anticipate minimal blood loss (< 5 mL) with this procedure since lymphatic cells only will be collected. Risks of infection, irritation or damage to nearby tissue, and accumulation of lymph fluid at the biopsy site will be minimized by performance of only easy-to-access superficial LN biopsies. The risks related to local anesthesia that may be used include some discomfort when injection is given, tingling, minor bruising, bleeding, or soreness at injection site. The risks of systemic anxiolytics that may be used include drowsiness, dizziness, lack of coordination, and slowed reaction time. The risks of conscious sedation include stinging or pain at the injection site, nausea and vomiting, headache, hiccupping, post-procedure drowsiness, and changes in heart or breathing rate and blood pressure.

Risks from HLA Typing

Return of HLA typing is described in section 8.4. Return of these results may pose some psychological implications for participants and family members regarding future health risks or incurable conditions.

2.3.2 Known Potential Benefits

There are no known benefits of monovalent rDEN3Δ30/31-7164 vaccination in any of the groups.

2.3.3 Assessment of Potential Risks and Benefits

The anticipated risks of rDEN3Δ30/31-7164 administration include those associated with the administration of any vaccine, the risk of future severe dengue in the flavivirus-naïve group, and the risk of higher viremia and increased reactogenicity in the heterotypic group. For those undergoing LN FNA, there is a risk of increased discomfort, and a minor risk of bleeding, swelling, and infection.

There are significant scientific merits to including a flavivirus-naïve group and LN FNAs. From a scientific and public health perspective, multiple vaccines have failed to successfully induce consistent, cross-serotypic -protection in flavivirus-naïve individuals [21]. By characterizing the immune response in flavivirus-naïve participants and comparing these to the DENV-exposed groups, we may identify the cell populations that are central to potent immunity and novel markers associated with protection. These can be used in future vaccine trials to assess the immunogenicity of vaccine candidates more accurately in flavivirus-naïve individuals. The risks to this group are expected to be the same as those in people who have been naturally infected with dengue. All groups will be advised to wear repellent and use precautionary measures such as screens when in DENV-endemic areas with high mosquito activity. Given the need to better protect flavivirus-naïve individuals and the relatively straightforward methods that can be utilized to avoid severe dengue, we feel that inclusion of flavivirus-naïve participants is justified. If a dengue vaccine is approved in the future, then we will attempt to contact all participants who underwent vaccination in this trial. We will advise participants that they may be able to receive the vaccine through their primary care or a travel medicine clinic.

This will be the first known trial to assess LN FNA post-DENV exposure. Studies in non-human primates (NHPs) have established that the samples obtained through LN FNAs are representative of the LN lymphocyte subsets and provide valuable and unique information regarding immune responses to vaccination [54]. This detailed immunologic characterization informed changes to vaccination schedules and strategies improving vaccine success [55, 56]. Given these benefits in NHPs, multiple groups have begun optimizing LN FNAs in humans and using them to examine immune responses to influenza and COVID vaccines [57-59].

Assessment of LN FNAs in humans have expanded knowledge regarding which cells are recruited to the germinal center and the extent of affinity maturation [58, 60]. In dengue, experts have proposed that sequential heterotypic DENV exposures induce broadly neutralizing antibodies through the recruitment of weakly cross-reactive B cells from the primary exposure to the germinal centers. Affinity maturation during secondary exposure then potentially results in B cells that effectively neutralize conserved epitopes [47]. By characterizing the cell populations in the germinal center after primary and sequential exposures, this protocol would be the first to directly assess this hypothesis.

LN FNA is a minor procedure requiring small needles (typically 25 gauge), and multiple studies have confirmed its safety and tolerability [61]. In one study of 70 women who underwent axillary LN FNA followed by core biopsy, FNA -associated bleeding was minimal in all but one

case, where it was moderate [62]. The mean pain score for LN FNA was 2/10, and LN FNA was significantly more likely to have a lower pain score than core biopsy ($p < 0.01$). A second study of 23 donors who had 2 to 4 LN FNAs each (n = 73 LN FNA attempts) reported that most subjects reported no to mild discomfort, with only 1 reporting moderate discomfort [57]. No major complications were reported in either study including no hematomas, infections, or activity limitations.

Additionally, because the immune response evolves over time post-vaccination, it is standard to sample the same LN 2 to 5 times post-vaccination, and no safety concerns have been reported after repeat sampling [57, 58, 61, 63, 64]. Our team has personally communicated with Dr. Ali Ellebedy (Washington University School of Medicine), who reported that some of his study participants have had the same LN sampled by FNA 15 to 20 times with consistent germinal center formation each time. This suggests no adverse impact on LN functionality despite many repeat samples. Given the favorable safety profile, low pain scores, sampling of peripherally accessible nodes, and high scientific value, we feel that LN FNA is justified in this trial.

Vaccinating the heterotypic group will allow us to safely mimic secondary heterotypic exposure and compare the immune responses and viral kinetics between this and the other groups. These assessments may reveal novel markers associated with risk and protection against severe dengue and identify cellular pathways that could be targeted to treat severe disease [65]. Overall, we feel that the scientific merit of vaccinating the heterotypic group outweighs the risks because a similar trial reported low mean peak rDEN3Δ30/31-7164 viremia with no increase in adverse effects [21], and there is significant scientific and public health value to understanding the determinants of severe disease.

Alternatives to participation: Individuals may opt not to join this study. Refusal to participate in this study will not affect participation in other studies at the NIH.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Evaluate the safety of monovalent DENV3 vaccination in those with distinct natural DENV infection histories living in non-endemic areas, and how prior DENV immunity influences protection against vaccine strain infection evaluated by the change in GMT and mean peak viremia.	The frequency and severity of local and systemic reactogenicity signs and symptoms during the 28-day period after each vaccination, unexpected AEs up to 28 days after each vaccination, and SAEs through day 180.	Assessment of these events effectively evaluates the safety of this vaccine in a new population: those with a history of dengue but living in non-endemic areas.
	Change in the DENV neutralizing antibody GMT between days 0 and 28.	The change in the DENV GMT is a measure of average humoral immunity before and after vaccination. GMT is an established measure of humoral immunity, and this metric will be comparable to other dengue trials. We anticipate that all groups will have an increase in GMT at day 28.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	Mean peak viremia among groups as measured by viral qRT-PCR between days 3 and 15.	Higher viremia has been associated with severe dengue and is thought to be due to the presence of low-level heterotypic antibodies and late T-cell - responses. Given the differences in dengue exposure history, we expect that the groups will have low magnitude but different mean peak viremia levels, which will safely mimic the enhancement of viral replication observed in natural infection.
Secondary		
Further evaluate how DENV infection history impacts the immunogenicity of the vaccine.	Change in DENV neutralizing antibody GMT between days 0 and 57.	Significant changes in GMT between days 0 and 57 represent the more sustained immune responses, which we expect to observe in the naïve and heterotypic groups. However, due to the protection incurred by previous infection, the polytypic group will have only a transient rise in GMT, and there will be no difference in GMT between days 0 and 57.
	DENV neutralizing antibody GMT at days 0, 28, and 57.	We expect that the day 0 GMT will be associated with vaccine response as measured by GMT at days 28 and 57. This association will help confirm the hypothesis that pre-vaccine host immunity influences the immunogenicity of monovalent DENV3 vaccination.
	Magnitude of the $CD8^+$ T-cell response at day 15 among groups as measured by AIM assays.	Early, potent $CD8^+$ T-cell responses have been associated with protection against severe dengue. We anticipate that the polytypic group will have earlier and stronger $CD8^+$ T-cell responses to vaccination than the other groups.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Tertiary/Exploratory		
Further evaluate how DENV infection history impacts the immune response to rDEN3Δ30/31-7164. Exploratory analyses may be conducted on any of the collected samples. The listed timepoints reflect those that may be of most interest, but the analyses may be performed at different timepoints pending the results of the initial analyses. Potential assays are also listed based on the available techniques. However, the assays may be altered if we develop novel techniques to measure the stated endpoints.	Change in DENV3 neutralizing antibody titer between day 0 and peak titer (measured at days 28, 57, or 90).	The DENV3 neutralizing antibody titer measures the serotype-specific immune response to vaccination. We anticipate that the naïve and heterotypic groups will have the strongest serotype-specific responses since the polytypic group may neutralize the vaccine.
	Neutralizing antibody titers against each serotype at all timepoints.	We will evaluate these additional timepoints depending on the results of the primary and secondary analyses. Comparisons of antibody levels at day 28 versus days 57, 90, 180, and 365 will allow assessment of antibody waning. Neutralizing antibody assays with different cell substrates and virus conditions will enable us to evaluate different populations of neutralizing and protective antibodies.
	Characterize the breadth of neutralizing antibodies against diverse DENV genotypes in each group at screening and at day 90 using antibody landscapes.	Antigenic cartography and antibody landscapes are techniques that enable measurement of the breadth of immunity across diverse variants. We expect that the heterotypic group will have the largest gain in breadth across their antibody landscape following vaccination.
	Characterize the broadly neutralizing antibodies observed in each group at screening and may be performed at days 15, 28, 57, 90, 180, and 365 after vaccination using E dimer competition enzyme-linked immunosorbent assay (ELISA) with previously isolated potent, cross-serotypic -antibodies.	The E dimer direct ELISA can detect antibodies that bind quaternary epitopes, which reflect conserved epitopes across serotypes. Competition ELISAs will allow us to detect whether serum contains antibodies that compete with monoclonals targeting quaternary epitopes on the E dimer.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	Assess whether broadly neutralizing antibodies and antibodies binding quaternary epitopes characterized by competition ELISAs at screening are associated with decreased rDEN3Δ30/31-7164 viremia.	If novel neutralizing antibody assays and competition ELISAs serve as correlates of protection against viremia, then they may be trialed in larger studies to assess whether they are better predictors than traditional plaque reduction neutralization assays.
	Assess whether ADE assays are associated with increased rDEN3Δ30/31-7164 viremia.	Conventional ADE assays have not been associated with increased viremia in humans. Using distinct assays, including with primary monocytes, may facilitate a mechanistic link between in vitro enhancement and viremia.
	Immunophenotyping at day 0 vs. day 1 post-vaccination in each group.	Early immune responses to dengue vaccination have not been well described. Differences in the innate immune response between groups may highlight pathways critical to early control of viremia.
	Characterize the phenotype, frequency, and magnitude of $CD4^+$ and $CD8^+$ T cells and T follicular helper (Tfh) cells in each group. These analyses may be performed at days 0, 3, 6, 9, 15, 28, 57, 90, and 180 using AIM assays.	Specific $CD4^+$ and $CD8^+$ T-cell populations have been associated with protection against dengue, and Tfh cells have been correlated with a higher frequency of plasmablasts. Comparisons of T-cell activities among groups may inform the potential mechanisms of protection against viremia and induction of antibody responses.
	Compare viremia onset day among groups.	Earlier onset day is likely a result of enhanced viral replication. We hypothesize that the heterotypic group may have an earlier onset day compared to other groups.
	Assess whether the magnitude of $CD8^+$ AIM assay–positive T cells at day 15 are associated with decreased rDEN3Δ30/31-7164 viremia.	Early, potent $CD8^+$ T-cell responses have been associated with protection against severe dengue. We anticipate that the stronger $CD8^+$ T-cell responses will be associated with less viral replication.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	Compare rash frequency among groups during the first 28 days post-vaccination.	Previous work with tetravalent vaccination indicated that post-vaccination rash is less common in DENV-exposed individuals. Thus, we anticipate that the polytypic group will have the lowest rash frequency.
	Characterize the transcriptome and cell surface proteins in each group using single--cell RNA sequencing (RNA-seq) and cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq). These analyses may be performed at days 0, 1, 3, 6, 9, 15, 28, and 57.	Comparisons of transcriptional responses over time in each group will highlight differentially expressed genes and may identify correlations between specific pathways and viremia/protective immunity.
	Determine the frequency and activity of atypical memory B cells across groups before vaccination and at may be performed at days 9, 15, 28, 57, and 90 using flow cytometric and CITE-seq analyses.	For other endemic infections, functional memory B cells expressing inhibitory markers called atypical memory B cells were enriched in immune individuals at baseline and after multiple infections. We anticipate an enrichment and maintenance of these subsets after vaccination with the highest frequency found in polytypic individuals, indicating a cellular specialization in response to recurrent antigenic exposure.
	Characterize cytokine and chemokine signaling.	Cytokine and chemokine levels provide a functional measure of immune activation and response. Differences among groups may highlight how prior dengue exposure influences the immune response. These may also be compared with signalling in future natural infection cohorts to assess similarities and differences in the host response to vaccine vs. natural exposure.
	Screen for broadly neutralizing antibodies targeting the E dimer in memory B collected at days 15 and 28 and plasmablasts collected at day 15 in the polytypic and heterotypic groups.	Identification of new classes of broadly neutralizing antibodies would significantly advance the field and our understanding of protective immunity.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	Evaluate neutrophil activity in vaccinated individuals. These assessments may be performed at days 0, 1, and 9, with the potential to limit or change the evaluation days pending results.	Small studies of natural dengue virus infection in humans have indicated that dengue severity is associated with increased neutrophil extracellular traps (NETs). Neutrophil activation has not been directly studied in previous dengue vaccine trials, and prior dengue exposure may influence their response. We expect that the heterotypic group may have a higher frequency of post-vaccine low density granulocytes, which produce NETs.
Evaluate how DENV infection history impacts the immune response to rDEN3Δ30/31-7164 in the LN.	Characterize the B- and T-cell frequencies and phenotypes in the peripheral blood versus germinal center in each group by surface staining, AIM assays, and single-cell- RNA-seq, and CITE-seq at days 15, 28, and 57.	Studies of influenza and SARS-CoV-2 vaccination have indicated that unique cell populations are recruited to the LN. Characterizing cells in the LN in each group may provide insight into the determinants of broad immunity.
	Assess the nucleotide mutation frequencies in the immunoglobulin heavy chain (IGHV) genes of germinal center B cells at days 15, 28, and 57 in each group using RNA-seq.	High mutation frequencies suggest increased affinity maturation. We hypothesize that the polytypic and heterotypic groups will have higher mutation frequencies than the naïve group at all timepoints.
	Assess whether the nucleotide mutation frequencies in the IGHV genes of germinal center B cells at days 15, 28, and 57 are associated with the presence of potent, cross-serotypic antibodies.	We hypothesize that broadly neutralizing antibodies arise from the affinity maturation of weakly cross-reactive B-cells. Thus, we expect that high mutation frequencies will be associated with potent cross-serotypic antibodies.
Evaluate the cells involved in viral replication.	Assess PBMCs for DENV non-structural protein 3 (NS3) and envelope (E) protein expression.	NS3 is only produced in cells with replicating DENV. Thus, staining for E and NS3 proteins can be used to assess which immune cells are targeted by DENV. NS3 positivity may be an additional measure of viral enhancement.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Characterize DENV and SARS-CoV-2 antigen-specific cellular immune profiles and antibody responses.	DENV- and SARS-CoV-2–specific responses may be evaluated by assessing: B-cell receptor mutational frequencies at days 0, 15, 28, and 57, binding antibodies (ELISA, neutralization assays) at days 0, 15, and 28, and plasmablast responses (ELISpot) at days 9 and 15 after primary, secondary, and tertiary DENV exposure.	This analysis will enable us to evaluate if repeat exposure to DENV induces similar cellular immunity and humoral immunity to that observed against SARS-CoV-2, providing further insights into the induction of broad anti-viral protective immunity against emerging viral diseases.
Assess whether clinical biomarkers associated with severe dengue are altered in vaccinated participants by immune group.	Assess CRP, ferritin, fibrinogen at days 0, 9, 12, 15, 28, 57; quantitative immunoglobulins at days 0, 9, 15, 57; tryptase, IgE at days 0, 12, 57.	Each of these biomarkers have been associated with severe dengue infections but have not been assessed in vaccine studies. Alterations in these biomarkers would suggest that vaccine studies mimic the dynamics of natural infection and could support these biomarkers as potential prognostics.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Study host response to dengue vaccine and characteristics of the vaccine in participant samples.	Various analyses of viral activity, immune function, and signaling molecules may be used.	This endpoint will allow us to leverage valuable patient samples to improve our understanding of host-pathogen interactions. This exploratory endpoint is aligned with the overall goal of the study, which was explained to the participants during the initial consent and written in the consent as follows: "The purpose of this research study is to see if a study vaccine for dengue virus type 3 is safe and can produce an immune response. We also want to see if side effects and immune responses are different between people who have never had a dengue infection and people who have had dengue infections in the past. Understanding how previous dengue infections affect vaccine responses is necessary for developing vaccines that can be effective for everyone.

4 STUDY DESIGN

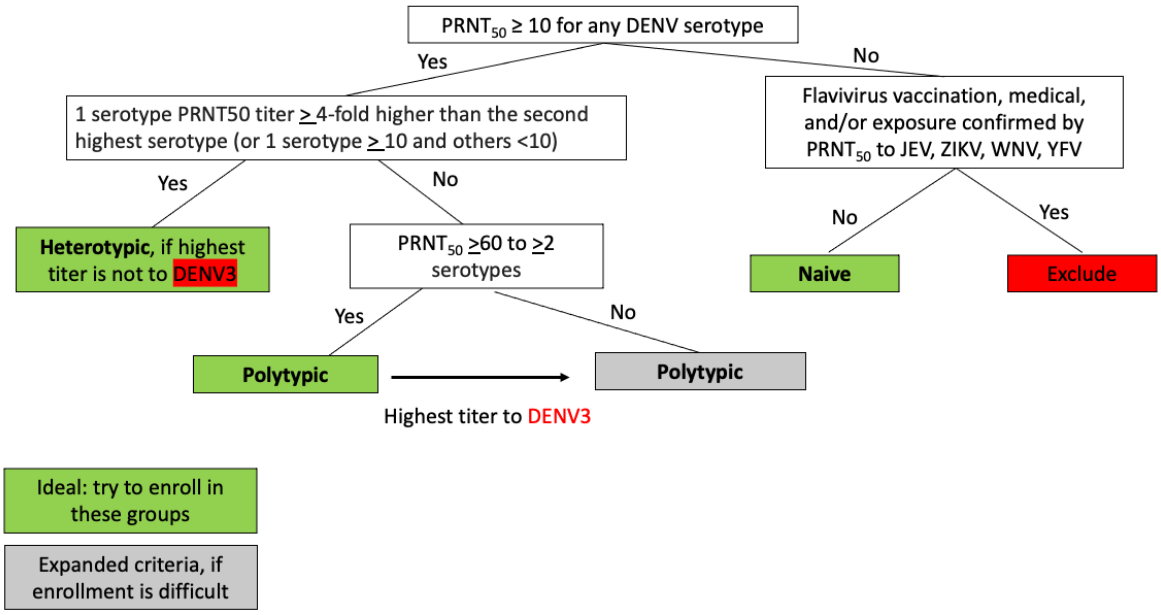
4.1 Overall Design

This is a phase 1, partially blinded, three-arm study that will be conducted at the NIH CC. We hypothesize that the vaccine will be safe and well tolerated, and all groups will have evidence of infection with the vaccine strain as indicated by a significant increase in the DENV neutralizing antibody GMT between days 0 and 28. However, due to the immunity conferred by prior dengue exposure(s), the polytypic group will have the lowest and the heterotypic group will have the highest mean peak viremia, indicating protection and enhancement, respectively. Additionally, the polytypic group will have the strongest $CD8^+$ T-cell responses at day 15 and will only have a transient rise in GMT with no difference in GMT between days 0 and 57. In contrast, the change in GMT will persist at day 57 in the heterotypic and naïve groups. Finally, we expect that prior immunity will influence the vaccine response as evidenced by a significant association between the day 0 GMT and GMT at days 28 and 57.

Potential participants will be 18 to 59 years of age and will undergo screening to determine if they meet eligibility criteria for this trial. Serotype-specific dengue immunity will be determined by a neutralizing antibody assay. All individuals with 50% plaque reduction neutralization test ($PRNT_{50}$) ≥10 for any DENV serotype will be considered immune. Those where the highest $PRNT_{50}$ titer is ≥4-fold higher than the second highest serotype (or 1 serotype ≥10 and others <10) will be considered monotypic. Among those where the highest $PRNT_{50}$ titer is <4-fold higher than the second highest serotype, those with a $PRNT_{50}$ ≥60 to at least two serotypes will be considered polytypic, while those with $PRNT_{50}$ titers between 10 and 60 will be categorized in the "expanded polytypic group" and may be included at the investigator's discretion. All polytypic individuals who have the highest titer against DENV3 will also be included in the expanded polytypic group and may be enrolled at investigator's discretion. DENV-seronegative individuals with exposure to other flaviviruses will be excluded (Figure 1).

If there is uncertainty about a previous flavivirus exposure in a candidate for the flavivirus-naïve group, then confirmatory testing will be performed via antibody testing against the virus of interest. The flaviviruses we are most likely to screen for based on travel and vaccination histories are YFV, West Nile virus, Japanese encephalitis virus, and Zika virus. The DENV-exposed -individuals will be eligible regardless of their exposure to other flaviviruses, but the flavivirus-naïve -group will not have detectable immunity against DENV and no history of exposure to other flaviviruses with confirmatory antibody testing as deemed appropriate by the investigator. Fifteen participants who meet enrollment criteria will be enrolled in each of the three groups: flavivirus--naïve, primary heterotypic (heterotypic), and polytypic. All investigators will be blinded to the group assignment of each participant except the medical advisory investigator (MAI) and study statistician (section 6.3).

Figure 1. Schema for Classifying Participants in Each Group



Because *Aedes spp.* are prevalent in the DC, Maryland, and Virginia area, there is a theoretical risk of transmission of the vaccine strain to local mosquitos. However, there has never been a case of vaccine strain transmission among the hundreds of people who have received

rDEN3Δ30/31-7164. Additionally, the peak viremia observed with this strain ($10^{2.4}$ PFU/mL) was significantly lower than that typically required for mosquito transmission (10^5 infectious units/mL) [6, 35]. Although we do not expect high viremia or SAEs in the heterotypic group, we will perform a safety run-in wherein viremia culture data will be collected on days 3, 6, 9, 12, and 15 from two heterotypic individuals and at least 4 individuals from the other groups. Once we confirm that mean peak viremia titers remain $<10^3$ PFU/mL and no halting rules are met (section 8.5.5), we will consent and vaccinate individuals ad-hoc and move to retrospective qRT-PCR assessments of viremia.

If any of the initial participants have a mean peak viremia titer $>10^3$ PFU/mL by culture, then this would increase our concern for potential mosquito transmission. In this case, we would review the data with the DSMB and the vaccine inventor, Dr. Steven Whitehead. If any of the initial participants has a mean peak viremia titer $\geq 10^6$ PFU/mL, we would halt the trial for safety concerns given this is the titer associated with symptomatic dengue. Regardless of viremia levels, the trial will be halted for SAEs and AEs as defined in section 8.5.5. If any of the 45 total participants develops a dengue-like syndrome (defined in section 8.5.5) or other AE or SAE that, in the opinion of the investigator, necessitates close clinical monitoring, then that participant will be admitted to the Clinical Center under the care of the principal investigator with the support of the appropriate inpatient team (NIAID Ward or Critical Care).

If a safety concern is noted by the principal investigator, then enrollment and dosing will be halted and the triggering event(s) will be reported in writing by the next business day to the sponsor medical monitor (SMM), theClinical Safety Office (CSO), the DSMB, the IRB, and other stakeholders, as appropriate. Additional reporting, review, and consideration of resumption of dosing will proceed per the study halting rules in section 8.5.5.

Each participant will receive one dose of rDEN3Δ30/31-7164 on study day 0 and will have clinical evaluations and AE assessments on days 6, 9, 15, and 28 in the first month. These days were chosen to maximize the opportunity to observe rash, which is the AE that is most likely to differ between groups. Previous trials have indicated that rash onset after TV003 occurs from days 7 to 15, and typically lasts 5 to 10 days. No safety signals were observed prior to day 6 or between days 15 and 28 (clinical protocol from [36] supplementary material). Blood draws will occur on days 0, 1, 3, 6, 9, 12, 15, 28, 57, 90, and 180, and an optional draw on day 365 with safety labs and PBMCs collected as per the SOA. Viremia will be assessed on days 3, 6, 9, 12, and 15. Assessments for solicited injection site and systemic reactions related to vaccination will occur through day 28. Unexpected AEs and SAEs that are possibly, probably, or definitely related to rDEN3Δ30/31-7164 vaccination will be collected through day 180, the last required clinical visit. The primary endpoints for immunogenicity will be assessed at days 0 and 28 for the change in DENV neutralizing antibody GMT and from days 3 to 15 for mean peak viremia. The secondary endpoints will be collected at days 0 and 57 for the change in DENV neutralizing antibody GMT and at day 15 for the magnitude of the $CD8^+$ T-cell response.

LN FNA on days 15 and 28 will be opt-out procedures, and we are aiming for at least 5 participants per group. LN FNA pre-vaccination (to be performed sometime between days −59 and 0) and at day 57 will be opt-in procedures. Pre-vaccination LN FNA will be offered to all participants, but day 57 aspiration will only be offered to those who complete day 14 and/or day 28 aspirations. All participants who undergo LN FNA will receive a follow-up call or secure

electronic communication (per participant preference) within 24 to 48 hours to assess for any procedure-related AEs.

4.2 Scientific Rationale for Study Design

This study design will allow us to assess the safety and immunogenicity of rDEN3Δ30/31-7164 monovalent vaccination in individuals with differing histories of natural dengue infection living in a non-endemic area. If this vaccine is found to be safe and immunogenic, then future studies could consider tetravalent vaccination in traveler and expatriate populations. Controlled assessment of how natural infection history influences the immune response will allow us to address fundamental questions in dengue immunology, including what cell populations are associated with broadly protective immunity, how is the early host response associated with viremia, and what are more accurate markers associated with protection that may be applied to future vaccine studies? Given rDEN3Δ30/31-7164 has been well studied in naïve and DENV-exposed -populations in endemic areas (as part of TV003), we do not feel that a placebo group is necessary to compare vaccine-associated adverse effects.

4.3 Justification for Dose

The investigator's brochure for rDEN3Δ30 contains an updated summary of previous studies with rDEN3Δ30/31-7164 (see page 28). Notably, a dose of 10^1 PFU has been studied in 20 dengue-naïve individuals, and there was slightly improved immunogenicity with no safety concerns at this dose. Among the 20 individuals who received a dose of 10^1 PFU, 15% had a rash, 65% had viremia, and 90% had seroconversion. In contrast, of the 50 dengue-naïve individuals who received 10^3 PFU, 52% had a rash, 34% had viremia, and 81% had seroconversion. In the end, the NIAID/Johns Hopkins University team advanced the 10^3-PFU dose for use in the vaccine studies because it was easier to prepare from the highly concentrated virus. Thus, subsequent studies in flavivirus immune individuals and in dengue endemic areas were performed using the dose of 10^3 PFU.

Since there are data to support that a lower dose of this vaccine is safe and immunogenic, we will allow a range of 10^1 to $10^{3.5}$ PFU. However, our goal range will be $10^3 \pm 10^{0.5}$ PFU because this dose has the most available safety data and will allow for consistent comparison among our groups and between ours and other studies [21, 37]. Individuals who receive a low vaccine dose of 10^1 to $10^{2.4}$ PFU, will be managed as described in section 9.4.9.

5 STUDY POPULATION

5.1 Inclusion Criteria

To be eligible to participate in this study, an individual must meet all the following criteria:

1. Aged 18 to 59 years.
2. In good general health as evidenced by medical history, physical examination, and laboratory screening results (section 8.1).
3. Willing to allow storage of samples and data for future research.
4. Willing to forgo receipt of any vaccine in the 28 days preceding the vaccine or in the 28 days following the dose of vaccine. For participants opting for LN FNA on day 57, they must be willing to forgo any vaccine through final LN FNA.

5. For individuals who can become pregnant: use of at least one method of highly effective contraception from the invitation to participate in the trial through day 60 after vaccination.
6. Able to provide informed consent.
7. Willing to adhere to lifestyle considerations (section 5.4) for the duration of the study.
8. Willing to avoid travel to a dengue-endemic area as defined by the Centers for Disease Control and Prevention (CDC) from 1 month before vaccination through day 57 [66].
9. Baseline absolute neutrophil count (ANC) > 750 cells/μL.
10. Baseline creatinine < 1.5 mg/dL.
11. Baseline ALT < 1.25 × upper limit of normal.
12. Serologic evidence of previous dengue virus infection indicative of either one previous DENV1, 2, or 4 infection or infection with at least two different serotypes.
    a. For the flavivirus-naïve group, they must have no history of flavivirus vaccination, medical illness concerning for a flavivirus infection, or travel history that increases the likelihood of other flavivirus infections. If there is uncertainty about a previous flavivirus exposure, then confirmatory antibody testing against the virus of interest must be negative.
13. Agree to avoid participation in other clinical studies requiring investigational interventions for the duration of this study (180 days).
14. Agree to avoid blood and plasma donation outside this study through day 28.

Contraceptive requirements: Participants who can get pregnant must agree to use highly effective contraception as outlined below from the invitation to participate in the study (approximately 2 weeks after screening) through day 60. Day 0 will be scheduled at least 28 days after the initiation of effective contraception. Participants who can get pregnant must have a negative pregnancy test on day 0 before receiving rDEN3Δ30/31-7164. If a participant becomes pregnant or suspects they are pregnant by day 60, then they should inform the study staff and their primary care physician immediately (section 8.5.2.3.4). Acceptable forms of contraception are:

- Intrauterine device or equivalent.
- Hormonal contraceptive (eg, consistent, timely, and continuous use of contraceptive pill, patch, ring, implant, or injection that has reached full efficacy prior to dosing).
- Condom, diaphragm, or cervical cap plus spermicide.
- A stable, long-term monogamous relationship with a partner who does not pose any potential pregnancy risk, eg, has undergone a vasectomy at least 6 months prior to vaccination or is of the same sex as the participant.
- A hysterectomy and/or a bilateral tubal ligation, bilateral oophorectomy, or post-menopausal status defined as age ≥45 years and at least 1 year since last menstrual period.

Pregnancy after day 60 will not be exclusionary as this will not impact our primary or secondary endpoints. Although we may observe pregnancy-associated differences in the transcriptome [67], these endpoints are exploratory and we have chosen to prioritize safety and inclusivity for people who can become pregnant. Study blood draw volumes after day 28 are less than the recommended volumes for research blood in critically ill patients [68]. Pregnant participants will be excluded from LN FNA due to the potential risks of anesthetics that may be used. Pregnancy

testing will be performed on each LN FNA day, with a negative result required to proceed to aspiration.

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Pregnant at screening.
2. History of or positive test result for HIV, hepatitis B, or hepatitis C.
3. History of previous dengue vaccine.
4. Has any of the following:
    a. More than 10 days of systemic immunosuppressive medications (≥10 mg prednisone dose or its equivalent) or cytotoxic medication within the 30 days prior to vaccination or immunomodulating therapy (see section 6.5) within 180 days prior to vaccination.
    b. Received blood products, including immunoglobulin products, within 120 days prior to vaccination.
    c. History of serious reactions to vaccines.
    d. Hereditary, acquired, or idiopathic forms of angioedema.
    e. Idiopathic urticaria within the past year.
    f. Asthma that is not well controlled or required emergency care, urgent care, hospitalization, or intubation during the past two years or that requires the use of oral or intravenous steroids.
    g. Type 1 or type 2 diabetes mellitus that is not well controlled (hemoglobin A1c > 8).
    h. Clinically significant autoimmune disease or immunodeficiency.
    i. Blood pressure ≥ 180/110 (stage 3 hypertension) on at least 2 measures.
    j. Documented diagnosis of a bleeding disorder (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions).
    k. Significant bruising or bleeding difficulties with subcutaneous injections or blood draws.
    l. Malignancy that is active or treated malignancy for which there is no reasonable assurance of sustained cure, or malignancy that is likely to recur during the study period.
    m. Asplenia or functional asplenia.
    n. Current alcohol or drug abuse or addiction and/or positive drug screen with substances other than marijuana.
5. Any medical, psychiatric, or social condition that, in the judgement of the investigator, is a contraindication to protocol participation.

5.3 Inclusion of Vulnerable Participants

5.3.1 Children

Individuals aged <18 years are excluded from this protocol because the safety of the study vaccine has not yet been evaluated in children, and a dedicated pediatric study would be better suited to assess the safety of this product. Additionally, children are likely unable to adhere to the study requirements.

5.3.2 Adults Who Cannot Provide Informed Consent

Adults who lack capacity to consent will be excluded from the study due to likely difficulty adhering to the number of clinic visits.

5.3.3 Pregnant People, Fetuses, or Neonates

Pregnant people are excluded from this study because the effects of the rDEN3Δ30/31-7164 vaccine on the developing human fetus are unknown.

If a study participant becomes pregnant or suspects they are pregnant, the participant should notify the study staff immediately. Pregnancies occurring during study participation will be managed as outlined in section 8.5.2.3.4.

Because there is an unknown but potential risk for AEs in nursing infants secondary to administration of the study vaccine to the nursing parents, no breastfeeding should occur for 28 days after the dose is administered.

5.3.4 NIH Staff or Family of Study Team Members

NIH staff and family of study team members may be enrolled in this study as these populations meet the study entry criteria. Neither participation nor refusal to participate in the research will have an effect, either beneficial or adverse, on the participant's or their family member's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The NIH investigator will provide and request that the NIH staff member review the *NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* and the *Leave Policy for NIH Employees Participating in NIH Medical Research Studies* (NIH Policy Manual 2300-630-3). Please see section 10.1.1 for consent of NIH staff and family of study team members.

5.4 Lifestyle Considerations

During this study, participants are asked to:

- Abstain from excessive alcohol intake for 24 hours before vaccination and blood collection.
- Abstain from strenuous exercise for 12 hours before each blood collection.
- Participants who can get pregnant must maintain pregnancy prevention measures (section 5.1) from the time they are invited to participate in the study (approximately 2 weeks after screening) through day 60 and notify the study staff prior to any change. In addition, notify the study staff immediately for any concerns, including but not limited to the possibility of a pregnancy between day 0 and day 60.
- Willing to avoid travel to a dengue-endemic area as defined by the CDC from 1 month before vaccination through day 57 [66].
- Although there is no evidence of transmission to wild mosquitos in large phase 1/2/3 studies with this product in endemic areas [69-71], this is the first study to vaccinate naturally infected individuals living in non-endemic areas. Thus, we will ask participants

who are vaccinated between May 15 and October 30 (*Ae. aegypti* season) to use mosquito repellant with 20% to 30% DEET when they will be outdoors for more than 15 minutes (eg, outdoor meals, walks, concerts). We will ask that they do this only for the first 15 days after vaccination, as this is when viremia typically occurs.

5.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who have a screening laboratory test that is out of the acceptable range may have a repeat blood draw to verify the results at a follow-up screening visit within the 60-day screening period. If individuals do not meet other screening criteria for participation in this trial (screen failure) or all screening procedures cannot be completed within the 60-day time period, then they can be rescreened at a later time. Rescreened participants should be assigned the same screening identification number as for the initial screening.

5.6 Strategies for Recruitment and Retention

The NIH CC will be the only site for this study. We will recruit persons of any gender, race, and ethnicity from ages 18 to 59 to participate in this study. We chose this age range because this is the range used in previous safety and immunogenicity studies for rDEN3Δ30/31-7164. Recruitment of flavivirus-naïve individuals will be the most straightforward in a non-endemic area, especially if individuals have had limited travel histories and no known flavivirus vaccination. Separately, compared to the United States' averages, Montgomery County, MD has a higher median income and number of non-native-born residents, suggesting that there are likely more travelers and expatriates with previous DENV exposure in this area. Per the United States Census Bureau and a compilation of these data by Deloitte, the median household income in the United States is $65,712 versus $110,389 in Montgomery County. Additionally, 13.7% of United States residents are born outside of the country versus 32.1% of Montgomery County residents [72, 73]. Of the foreign-born Montgomery County residents, the top three most common countries of origin are all dengue endemic: 35.5% from El Salvador, 19.9% from India, and 12.9% from Guatemala.

The Viral Epidemiology and Immunity Unit (VEIU) of the LID, NIAID has a small observational cohort of local residents who have lived in or traveled to dengue endemic areas (NIH IRB# 11-I-0109, "Viral infections in Healthy and Immunocompromised Hosts"). Despite very limited recruitment effort, we have screened 33 individuals, enrolled 24, and 17 have had assessment of their dengue status to date. Of these, 6 were DENV-naïve, 3 were homotypic, 6 were heterotypic, and 2 were polytypic. With more aggressive recruitment, we anticipate screening up to 200 individuals and plan to enroll all 45 participants approximately within the first year of this proposed study protocol. Participants enrolled on 11-I-0109 have given permission to be contacted in the future, and we will recruit from this cohort. Only one associate investigator knows the names and immune history of the 11-I-0109 participants, and she will be blinded to participant names for this protocol.

Additionally, we will work with BuildClinical to develop a recruitment strategy and deploy advertisements across web platforms. Individuals who respond to a BuildClinical advertisement and complete the BuildClinical screening form will be referred to the study team. The study team will then reach out to referred individuals to gauge their interest and appropriateness for a screening visit. Participant information (including personally identifiable information) to be collected through this screening method includes the participant's first and last names, phone number, email address, date of birth, current city and state of residence, race/ethnicity, history of dengue infection, and travel history. To help maintain group assignment blinding, study staff will not review history of dengue infection or travel history. This information may be used by the BuildClinical team to help narrow screening. We will work with BuildClinical, as needed, to help support recruitment.

We will also work with the NIH Office of Patient Recruitment to establish a recruitment strategy and develop flyers and advertisements for the NIAID website, NIH/NIAID social media sites, and certain NIH email listservs. The study will also be registered with ResearchMatch, ClinicalTrials.Gov, and the NIH Office of Patient Recruitment, from where we will be able to obtain a list of healthy volunteers interested in our study. Since this study requires long-term participation, enrolled participants will receive visit reminders by phone, including possible text messages, and/or reminders by email. Participants will be compensated for their time and inconvenience for attending study visits.

If accrual to any of the groups is not complete by 18 months after the start of the study, then the feasibility of meeting the overall accrual goal of 15 participants per group will be re-evaluated. If it is determined that 15 participants per group will not be accrued by 20 months, then the accrual goal may be decreased to 12 per group. The requirement of 5 individuals with LN FNAs per group may also be dropped. These changes will be implemented via protocol modification.

5.6.1 Costs

Participants will not be responsible for costs related to participation in the research.

5.6.2 Compensation

Participants will be compensated for time and inconvenience in accordance with standards for compensation at the NIH CC. Payment will be provided by debit card, direct deposit, or automated clearing house in five installments, including one payment during the 60-day screening window and the others at approximately days 28, 57, 180, and 365. The compensation per visit will be as follows:

- $100 for a screening visit involving a history, physical examination, and blood draw.
- $60 for a blood draw–only visit on days 1, 3, and 12 or for a follow--up screening visit.
- $250 for day 0 visit with injection of the vaccine. If a participant arrives for the day 0 visit but cannot be vaccinated for a previously unforeseen reason, they will receive $60 for that visit.
- $200 for each blood draw and clinic visit on days 6, 9, 15, and 28.
- $75 for a blood draw–only visit on day 90.
- $225 for each blood draw and clinic visit on days 57, 180, and 365.
- $300 for each optional LN FNA on days −59 to 0, 15, 28, and 57. This will replace compensation for days 15, 28, and/or 57.

- $100 for unplanned study visits, such as visits for the assessment of AEs or for an early termination visit.

The maximum payment for a participant who completes the study as scheduled, including all optional LN FNAs and day 365, is $2655. The maximum payment with no LN FNAs and no optional visits is $1855.

Travel expenses will be reimbursed for those living more than 50 miles away from the Clinical Center, per NIAID Central Travel Policy for Research Protocol Participants. If a participant is found to have a urine drug abuse screen that is positive for an illegal substance (other than marijuana) and prohibits their ability to participate in the trial, they will not be compensated for the study visit.

6 STUDY INTERVENTION

6.1 Study Interventions Administration

6.1.1 Study Intervention Description

The rDEN3Δ30/31-7164 vaccine is a live attenuated virus constructed by creating two deletions in the 3' UTR of the DENV3 Sleman/78 strain, which are each 31 nucleotides long [34, 49]. The vaccine is supplied in 0.6-mL aliquots of $10^{7.7}$ PFU/mL of live attenuated rDEN3Δ30/31-7164 in Leibovitz L-15 medium containing 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; monosodium glutamate, 0.0054 M). Both the vaccine and the diluent were prepared under current Good Manufacturing Practices conditions by CRL Biopharmaceutical Services (Malvern, PA).

6.1.2 Dosing and Administration

The rDEN3Δ30/31-7164 vaccine will be delivered subcutaneously into the left deltoid area on day 0. If participants have a strong preference, vaccination can be performed in the right deltoid area instead. However, those who have vaccination in the right arm will no longer be eligible for LN FNA due to the need for consistency for this procedure. The rDEN3Δ30/31-7164 will be administered at a dose of 0.5 mL of 10^1 to $10^{3.5}$ PFU, with a goal range of $10^3 \pm 10^{0.5}$ PFU, plus Plasma-Lyte A pH 7.4 diluent.

If a participant has recently experienced febrile illness, then the day of vaccination will be scheduled at least 14 days after resolution of the fever.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

The study drug will be distributed and accounted for by the NIH pharmacy according to standard pharmacy procedures.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The study agent will be individually labeled with the name of the material, volume, lot number, concentration, storage instructions, Investigational Use Statement ("Caution: New Drug - Limited by Federal law to investigational use"), and manufacturer information. The Final Drug Product is dispensed as 0.6-mL aliquots of approximately $10^{7.7}$ PFU/mL of Live Recombinant Dengue Virus Type 3 rDEN3Δ30/31-7164 Vero Grown Virus Vaccine into 2.0-mL sterile

cryovials and stored at −70 °C ± 15 °C. The Final Drug Product composition is a concentration of live attenuated rDEN3Δ30/31-7164 in Leibovitz L-15 medium containing 1X SPG (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; monosodium glutamate, 0.0054 M). The potency of rDEN3Δ30/31-7164 (Lot DEN3#113B) is $10^{7.7}$ PFU/mL.

Investigational Product Label for rDEN3Δ30/31-7164 Lot DEN3#113B

Live Recombinant Dengue Virus Type 3 rDEN3Δ30/31-7164 VERO Grown Virus CAUTION: NEW DRUG LIMITED BY FEDERAL (USA) LAW TO INVESTIGATIONAL USE Store at -70±10°C Charles River Laboratories, Malvern, PA	**Date: 18JAN2012** **Vial#: 0000** **Lot: DEN3#113B**

Evaluation of live attenuated dengue virus vaccines (developed at the NIAID/NIH) in both laboratory and clinical situations indicate that these viruses are stable and potent when stored at a temperature of −70 °C ± 15 °C. Therefore, −70 °C ± 15 °C is the recommended long-term storage condition for the Final Drug Product.

6.2.3 Product Storage and Stability

The rDEN3Δ30/31-7164 vaccine is stored at −70 °C ± 15 °C [49]. The diluent is stored at 2 °C to 8 °C. The diluted vaccine will be transported on ice to the clinic and must be administered within 4 hours of being removed from the freezer. Vaccine should never be refrozen for reuse in vaccine preparation.

6.2.4 Preparation

The vaccine will be prepared as described in the pharmacy manual.

6.3 Measures to Minimize Bias: Blinding

Participants will not be randomized because the primary endpoints are safety and immunogenicity, and the groups are defined by pre-existing immunity. To avoid biased assessment of AEs and immunogenicity markers, all study staff except the statistician, the MAI, and one laboratory scientist will be blinded to the final group assignment of each participant. The laboratory member will have primarily a computational role and will not perform laboratory assays or work directly with the clinical team.

To narrow the screening pool to potentially flavivirus-naïve and dengue-immune individuals, all participants will receive a short, pre-screening Research Electronic Data Capture (REDCap) survey with questions about travel, residential, and flavivirus vaccine history. These will be reviewed by the unblinded study staff, who will recommend individuals for screening to help meet the goal of 15 per group. Unblinded study staff will also provide the lab with sample IDs that require screening for other flaviviruses. Once individuals consent to screening, more detailed information regarding history of flavivirus infection and vaccine exposure will be collected via

REDCap. This information will not be reviewed until the study is unblinded. For those participants who are unable to complete the REDCap surveys electronically, an unblinded study team member will contact the participant by phone to obtain the information and enter the data into REDCap. A language interpreter will be provided for participants who are non-English speaking.

To maintain blinding to final group assignments, the following procedure will be implemented. All screening samples will be de-identified and labelled with a barcode/sample ID by the clinical team, clinical staff, or Fredrick National Laboratory. The clinical team will maintain a list of study names with corresponding sample IDs, and this list will be password-protected and only available to the clinical team and unblinded study staff. The laboratory team will receive anonymized samples, generate, and interpret the screening antibody data, and create a document that contains the sample ID and group assignment. This document will also be password protected -and only available to laboratory team and unblinded study staff. Throughout screening, these two documents (1. name and sample ID and 2. group assignment and sample ID) will be shared with the statistician. The statistician will then link participant names to group assignments and provide a list of individuals who are eligible for enrollment based on serology results only. All other eligibility criteria will be evaluated by the clinical team. The document containing participant names, group assignment, and sample IDs will be kept in a password protected -file that can only be accessed by the unblinded staff. All samples will be deidentified and labeled with sample ID/barcode by the clinical team and/or Fredrick National Laboratory such that the laboratory team will not know participant names until unblinding.

Routine unblinding of group assignment will occur after all 45 participants have completed study day 57. Study day 57 was chosen for unblinding because all primary and secondary endpoints will be assessed by that point. AE assessments between days 58 and 180 are less likely to be influenced by baseline immune status. Additionally, immunogenicity assessments at days 90 and 180 focus on antibody waning, which is an exploratory endpoint. The principal investigator will request group assignments in writing from the unblinded study staff once all participants have completed study day 57.

Intentional unscheduled (clinically driven) unblinding: Emergency unblinding (eg, needed for participant welfare/treatment) should be limited to those who need the information to accomplish the participant management. There will be no delay in intentional unblinding performed in the interest of participant safety. This may be accomplished through the principal investigator or designee by direct written request to the statistician or MAI.

In no case is unblinding to be significantly delayed for approval, and the default will be to unblind any reasonable request made in the interest of participant safety.

Unintentional unblinding: If unintentional unblinding occurs, then the principal investigator will create a plan for ongoing management of the participant(s) involved and for preventing the recurrence of a similar incident, as appropriate. If the protocol team determines that the unintentional unblinding may have a significant impact on the study plan (eg, if the codes for multiple participants were accidentally broken), then the need for a protocol amendment will be addressed as soon as possible.

Management of unscheduled unblinding: All cases of unscheduled unblinding (ie, intentional and unintentional) will be documented in the appropriate source and/or research record and will

include the reason for the unscheduled unblinding, the date it occurred, who approved the unblinding, who was unblinded, who was notified of the unblinding, and the plan for the participant. The principal investigator will report all cases of intentional and unintentional unscheduled unblinding to the DSMB in writing within 1 business day after site awareness via email to the DSMB mailbox (niaiddsmbia@niaid.nih.gov) outlining the reason for the unblinding and the date it occurred. The report will also be submitted to the SMM. If the unblinding meets the definition of a reportable event, then it will be reported to the IRB according to NIH Human Research Protection Program (HRPP) Policy 801.

If an SAE or other event merits unblinded review for safety (ie, per the principal investigator, regulatory authority, or the SMM), then the unblinding occurrence and a summary of how unblinded review and assessment was conducted for safety (by the DSMB) will be summarized in the SAE or regulatory safety report. However, the actual underlying immunity will not be disclosed unless the particular participant is being globally unblinded per study leadership and sponsor.

6.4 Study Intervention Compliance

Compliance with administration of the vaccine will be assured with a standard operating procedure checklist that will be reviewed and completed by the research nurse administering the vaccine. The research nurse will fill out a vaccination record form that will include the pre--administration vitals, documentation that the information on the label of the vaccine dose syringe was verified by two staff members, the expiration date and time and volume in the dose syringe, site and time of administration of the vaccine dose, vital signs approximately 30 minutes to 1 hour after administration, and documentation of any complaints reported by the participant after dosing. In addition, the principal investigator or designee will review the form and assess the participant prior to discharge. The study team will document the date and time of the administered rDEN3Δ30/31-7164 vaccine in the Clinical Research Information System of the NIAID (CRIMSON) database.

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in CRIMSON are concomitant prescription medications, over-the-counter medications, and supplements. At each study visit or contact, a study team member will ask the participant about any medication that is taken.

Immunosuppressive or immune-modifying agents (cumulatively known as immunomodulating) that are prohibited during the trial include but are not limited to:

- ≥10 mg prednisone equivalent per day planned for ≥14 days.
- Cytotoxic drugs (eg, methotrexate, cyclophosphamide, doxorubicin, vincristine).
- Alemtuzumab.
- Azathioprine.
- Cyclosporine and tacrolimus.
- Mycophenolate mofetil.
- Rapamycin.
- Anti-lymphocyte serum and anti-thymocyte globulins (eg, ATG, OKT3).

- Rituximab.
- Anti-TNF-α inhibitors (eg, infliximab, adalimumab, etanercept).
- Anti-IL-2 receptor monoclonal antibodies (eg, basiliximab).
- Purine analog therapy (eg, cladribine, pentostatin).
- Intravenous immunoglobulin.

These medications can be restarted after completion of the trial.

Participants may not use investigational drugs starting 30 days before receiving rDEN3Δ30/31-7164 through the end of study participation. Participants may not receive any vaccine other than rDEN3Δ30/31-7164 from day −28 through day 28 (or day 57 for those opting for LN FNA on day 57).

Participants will be instructed to avoid aspirin NSAIDs between days 0 and 28. For participants undergoing LN FNA, aspirin and NSAIDs should be discontinued at least 7 days pre-procedure. They can be restarted 48 hours post-procedure as long as the participant has no significant swelling or bruising in the area. Participants on more potent blood thinners, such as direct--acting oral anticoagulants, will not be eligible for LN FNAs.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Study intervention may be discontinued for all participants and enrollment suspended (ie, halting). Halting rules and procedures are described in section 8.5.5.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

Criteria and procedures for withdrawal and replacement of a participant by the investigator are provided in section 8.5.3.

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if they fail to return for a scheduled or rescheduled visit and are unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 30 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, a study team member will make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address). These contact attempts should be documented in the participant's study file.

Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures

Screening visits will occur no more than 60 days prior to vaccination for participants to be considered for eligibility. Screening will be performed during 1 to 3 screening visits. Due to the need to maintain balance between vaccine groups, some qualified participants may not be selected for a vaccine group prior to their 60-day cut-off window. If the 60-day window has elapsed but a participant remains a good candidate for enrollment, they will be invited to rescreen and all medical and laboratory assessments will be repeated with the exception of dengue antibodies. However, at re-screening, dengue antibody testing may be repeated if any of the following have occurred since the initial screen: 1) 6 months have passed, 2) the individual reports travel to a flavivirus endemic area, 3) they report reciept of a travel vaccine, or 4) the investigator deems antibody retesting is appropriate.

Participants must meet all eligibility criteria for this protocol to be considered for enrollment. Participants will be informed of their screening results performed in the clinical laboratory. Clinical information obtained through review of existing data may include but is not limited to any documentation of previous flavivirus vaccination, previous dengue laboratory testing (this is never serotype-specific), and/or clinic visits or hospitalizations due to dengue infections.

8.1.1 Screening Activities Performed Prior to Obtaining Informed Consent

Minimal risk activities that may be performed before the participant has signed a consent include the following:

- Email, written, in person or telephone communications with prospective participants.
- Completion of medical history and separate short (or brief) travel questionnaire (REDCap) including questions that address eligibility for the trial. The medical information sheet will include the study team's contact information, so potential participants can contact them with any questions or concerns.
    - The short (or brief) travel questionnaire will be reviewed by unblinded study statistician to assist with screening target groups.
- Review of existing medical records to include history and physical, laboratory studies, etc. to help confirm eligibility

8.1.2 Screening Activities Performed After a Consent for Screening Has Been Signed

The following activities will be performed only after the participant has signed the screening consent for this study:

- Medical history and physical examination.
- Concomitant medication review.
- Vital signs.
- Measurement of height and weight.
- Demographics.
- Review pre-screening medical history.
- Urine drug screen.
- Blood draw for the following clinical and research laboratory evaluations:
    - Complete blood count (CBC) with differential.

- Hemoglobin A1c.
- Acute care and hepatic panels.
- Prothrombin time (PT), PTT, and INR.
- Hepatitis B surface antigen and anti–hepatitis C virus antibody.
- Anti–HIV 1/2 antibody/antigen.
- Neutralizing DENV1-4 antibody titers
- Flavivirus antibody titers may be performed if there is uncertainty about medical, vaccination or travel history. This may include YFV, West Nile virus, Japanese encephalitis virus, and Zika virus, among others.
- Participants who can get pregnant will have a serum pregnancy test.

- Pregnancy prevention counseling and signing of the reproductive counseling form.

8.2 Study Evaluations & Procedures

The following evaluations and study procedures will be performed to evaluate the safety and immunogenicity of the rDEN3Δ30/31-7164 vaccine:

Medical history and physical examination: A targeted medical history and history-directed physical examination will be performed at screening and days 0, 6, 9, 15, 28, 57, 180, and 365.

Vital signs*:* Temperature, pulse, respiratory rate, and blood pressure will be obtained prior to vaccine dose, 30 minutes to 1 hour after vaccine dose, and at every clinical visit.

Exposure history assessment: After enrollment, participants will complete a more extensive travel history assessment that may include questions about exposures to other pathogens. This will be reviewed by study staff after study unblinding. This can be completed via REDCap any time between days 0 and 180.

Follow-up exposure assessment: To capture any potential exposures between the completion of the first assessment and final visits, a follow-up assessment will be completed within 3 weeks of the day 180 visit. For logistical ease, both the initial exposure and follow-up assessment will be sent to all individuals. However, the follow-up assessment does allow individuals to note if any of the exposures have changed since the initial exposure assessment. Thus, if an individual completes the initial exposure assessment within three weeks of the day 180 visit, they will be able to indicate on the follow-up survey that each exposure is unchanged since the initial assessment. This will limit the number of questions they need to answer at follow-up. If individuals opt to attend the day 365 visit, then they will complete the follow-up assessment again within 3 weeks of the day 365 visit.

Biological specimen collection and laboratory evaluations. Blood will be collected for clinical and research evaluations. We will typically collect approximately 40 to 100 mL of blood at each visit. In total, CBC, acute care and hepatic panels, and PT/PTT/INR will be performed on days 0, 9, 12, 15, 28, and 57. CBC will also be drawn at Day 90 for participants who experience Grade 1 or higher anemia or a Grade 2 or higher hemoglobin drop that does not resolve by Day 57. In previous studies with sequential monovalent DENV vaccination and one with a DENV2 challenge strain, the only consistent laboratory abnormality observed was mild neutropenia with the nadir at day 12 [6, 74].

The following clinical biomarkers have been identified as altered in severe dengue and suggested as potential prognostic indicators, but they have not been evaluated in previous vaccine studies:

C-reactive protein (CRP), ferritin, immunoglobulins (IgG, IgA, IgM), fibrinogen, IgE, and tryptase [75-80]. Given the heterotypic group is designed to safely mimic the conditions of enhancement, it would be valuable to assess whether these biomarkers are altered in this or the other two groups. Alterations in these biomarkers would suggest that vaccine studies mimic the dynamics of natural infection and could support these biomarkers as potential prognostics.

All biomarkers will be evaluated at day 0 for a baseline assessment. Because CRP, ferritin, and fibrinogen may change rapidly and require no extra blood volume, they will be assessed at days 9, 12, and 15, which are the timepoints associated with viremia and most likely to reflect symptomatic natural infection. Evaluation at day 28 will provide a convalescent state, and day 57 will provide information about the resting state. Quantitative immunoglobulins will be assessed at days 9 and 15 to align with our research plasmablast assessment, and at day 57 to assess a return to resting state. Because IgE and tryptase require extra blood, they will be assessed only at day 12, which is most likely to capture active viral replication and immune responses in all groups, and at day 57 to test for a return to resting state.

The majority of this laboratory testing will be performed in the Department of Laboratory Medicine of the NIH CC, which is a laboratory compliant with the Clinical Laboratory Improvement Amendments (CLIA) of 1988. Testing for infection with hepatitis B, hepatitis C, and HIV will be conducted in the Department of Transfusion Medicine.

LN FNA: Aspiration of the left axillary LNs on days 15 and 28 will be opt-out procedures, and we are aiming for at least 5 participants per group. LN FNA pre-vaccination (to be performed sometime between days −59 and 0 and at day 57) will be opt-in procedures. Pre-vaccination LN FNA will be offered to all participants, but day 57 aspiration will only be offered to those who complete day 15 and/or day 28 aspirations. Local anesthesia will be used, and participants may also receive systemic anxiolytics or conscious sedation per participant and clinician preference, and within NIH CC practice guidelines. Drugs used for sedation could include fentanyl and/or midazolam. The left axillary LNs will be sampled.

For each aspirate sample, typically up to six passes will be made under continuous real-time ultrasound guidance using the appropriate needle and washings, each of which will typically be flushed with 3 mL of RPMI 1640 supplemented with 10% FBS and 100 U/mL penicillin -streptomycin, followed by three 1-mL rinses. Doppler ultrasound will be used to localize nearby vasculature to minimize risk.

After each aspiration, participants will be assessed by a clinical provider from the study team, consistent with CC practice guidelines. Discharge will typically be on the same day (with family member or friend if sedation or anxiolytic administered). Vital sign monitoring will be per typical CC and Radiology & Imaging Sciences Department practice guidelines and standard Clinical Research Information System order sets in place. All participants who undergo LN FNA will receive a follow-up call or secure electronic communication (per patient preference) within 24 to 48 hours to assess for procedure experience, comfort level, and any potential procedure-related AEs, or the need for in-person interim assessment or exam.

Assessment of AEs. Assessment of AEs will occur throughout study participation. Participants are allowed to send photographs of the injection site and any rashes to study team members' work cellphone or email photos via secure communication. If the participant complains of signs or symptoms that are significantly uncomfortable, they will be assessed by a clinician and the

participant may be invited for an interim visit at the Clinical Center for evaluation. Ongoing AEs or SAEs will be followed until resolution or stabilization, depending on the nature of the AE. Assessment and recording of SAEs will occur through day 180 or (day 365 for those who opt for long-term follow-up). If participants would like the results of their screening laboratory testing and any other clinical lab testing obtained during study visits, then these results will be provided to them. If the participant signs up for the patient portal, they will be able to see the clinical lab test results.

Photographs of the skin may be taken if a rash is present. Form NIH-661: Authorization for Recording, Filming, and/or Photographing of Patients in the Clinical Center for Educational or Research Purposes will be signed prior to any photos being obtained.

8.2.1 Biospecimen Evaluations

We will evaluate viremia and the immunogenicity of this vaccine as described in the objectives (section 3). Serum obtained from the participants will be used to determine neutralizing antibody titers at screening and days 15, 28, 57, 90, 180, and 365 with a neutralization assay. Screening titers will be performed within 6 months of vaccination, but the titers from the post-vaccination timepoints will be batched and evaluated retrospectively. Viremia will be assessed by qRT-PCR at days 3, 6, 9, 12, and 15. The first six participants, including two with heterotypic immunity, will have their viremia measured by culture after all have completed day 15. If the viremia levels in these six participants remain below 10^3 PFU, then all subsequent samples will be batched and performed retrospectively by qRT-PCR.

PBMC samples collected at days 0, 1, 3, 6, 9, 15, 28, 57, 90, and 180 may undergo immunophenotyping classification using multiparameter flow cytometry. Memory T-cell subsets and antigen-specific activity may be further characterized through flow cytometric analyses using AIM assays, and supernatants from stimulated PBMCs will be assessed for cytokine signaling. Intracellular staining techniques may be used to assess for NS3 and E protein at days 1, 9, and 15. Antibody activity profiles may be identified in each group pre-vaccination and at days 15, 28, 57, 90, 180, and 365 using E dimer, competition ELISA assays, neutralization assays, and enhancement assays. All cellular characterizations and ELISA assays will be batched and retrospective and, along with viremia and neutralizing antibody assays, will be performed by qualified staff and scientists in the VEIU of the LID.

Human leukocyte antigen (HLA) typing will be performed on all individuals at day 0 using the CC clinical laboratory. Sequencing techniques may be used to characterize the transcriptome (RNA-seq), B- and T-cell receptors (BCR and TCR sequencing), and protein expression profiles (CITE-seq) of individuals' PBMCs at a single-cell level from days 0, 1, 3, 6, 9, 15, 28, and 57. Bulk RNA sequencing may also be performed to further explore the differences between groups at various time points. Hashtag antibodies will be used to resolve the time points, and single nucleotide polymorphisms (SNPs) will be used to group cells by donor [81]. Additionally, LN aspirates will be evaluated by RNA-seq, CITE-seq, and BCR and TCR sequencing at all timepoints. Sequencing analyses will be retrospective. Cell preparation, including cell hashing and CITE-seq antibody labelling, will be performed by the VEIU, and single-cell partitioning, library preparation, and sequencing will be performed by the VEIU with the assistance of the Research Technologies Branch. Bioinformatic analyses, including transcript alignment, donor

and timepoints demultiplexing, differential gene and protein expression analyses, and BCR and TCR usage analyses, will be conducted by members of the VEIU.

To perform these analyses, we will collect approximately 35 mL of blood at screening and 485 mL of blood between days 0 and 28, which is a total of approximately 520 mL. Approximately 71 mL will be drawn at day 57, approximately 103.5 mL will be drawn at days 90, 180, and at the optional day 365 visit.

8.2.2 Samples for Genetic/Genomic Analysis

8.2.2.1 Description of the Scope of Genetic/Genomic Analysis

HLA typing will be performed in the CLIA-certified Department of Transfusion Medicine in the NIH CC. Results from the HLA typing will become part of each participant's medical record at the NIH and will be available to participants through the online patient portal. Medical records containing this information are maintained in a secure place. Given the limited scope of genetic testing on this protocol, genetic counseling will not be offered, and no incidental findings are expected.

Transcriptomic analysis will be performed as part of the exploratory objectives, including bulk RNA sequencing focused on identifying gene pathways with altered transcriptional activity after vaccination. Single-cell transcriptomics will be performed on blood samples and LN samples. Bulk RNA sequencing of PBMC samples will be used to generate SNP calls for each donor, and these will enable grouping cells by donor [82]. Limited DNA sequencing may be performed for characterization of the T- and B--cell receptors. Limited DNA sequencing, transcriptomics, and SNP analyses will be performed on a research-only basis and will not be reported to the participant. We will not evaluate SNPs for known disease associations.

8.2.2.2 Description of How Privacy and Confidentiality of Medical Information/Biological Specimens Will Be Maximized

Genetic data will be coded and stored in secure electronic research records (section 10.3) and may be shared as described in section 10.12.2.

8.3 Safety and Other Assessments

Information collected from clinical and biospecimen evaluations (section 8.2) will be reviewed for ongoing safety assessments.

8.4 Management of Results

Results of clinical procedures or evaluations (including incidental findings) will be shared with participants throughout the study if they are assessed by the principal investigator to be medically actionable. Such results will be discussed with the participant along with guidance for appropriate follow-up with their healthcare provider. Results from the HLA typing will become part of each participant's medical record at the NIH and will be available to participants through the online patient portal. The results from all assays done for research purposes including neutralizing antibody titers, transcriptomics, viremia levels, and immunophenotyping will not be released to the participants as these will not performed in CLIA-certified laboratories.

8.5 Safety Definitions, Management, and Sponsor Reporting

8.5.1 Definitions

The NIAID Clinical Safety Office (CSO) is responsible for sponsor safety oversight of this study, and the definitions below comply with CSO requirements.

Adverse Event (AE): An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (eg, 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.

Adverse Reaction (AR): An AR means any AE caused (see "Causality" below) by a study agent. ARs are a subset of all suspected adverse reactions (SARs; defined below) where there is reason to conclude that the study agent caused the event.

Suspected Adverse Reaction (SAR): SAR means any AE for which there is a reasonable possibility that the study agent caused the AE.

Per US Food and Drug Administration (FDA) guidance:

For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal (see "Causality" below) relationship between the study agent and the AE. A SAR implies a lesser degree of certainty about causality than an AR, which means any AE caused by a study agent.

SARs are the subset of all AEs for which there is a reasonable possibility that the study agent caused (see "Causality" below) the event. Inherent in this definition, and in the requirement to report SARs, is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the study agent actually caused the AE.

The sponsor is responsible for making the causality judgment.

Serious Adverse Event (SAE):

- is an AE that results in death.
- is an AE that is a life-threatening event (places the subject at immediate risk of death from the event as it occurred).
- is an AE that requires inpatient hospitalization or prolongs an existing hospitalization.
  NOTE:
  - Hospitalization is considered required if outpatient treatment would generally be considered inappropriate.
  - Same-day surgical procedures that are required to address an AE are considered hospitalizations, even if they do not involve an overnight admission.
  - Hospitalization due to a condition that has not worsened and that pre-dates study participation (eg, elective correction of an unchanged baseline skin lesion), or due to social circumstance (eg, prolonged stay to arrange aftercare), or that is planned/required "per protocol" AND that proceeds without prolongation or complication, is NOT considered an SAE by this criterion.
- is, or results in, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- is a congenital anomaly/birth defect/miscarriage/stillbirth.
  NOTE: This definition is more inclusive than some commonly published definitions. It includes an affected conceptus/neonate whose:
  - biological mother was exposed to a study agent at any point from conception through the end of the pregnancy, AND/OR, if breastfeeding, the 30-day neonatal period; or
  - biological father was exposed to a study agent at any point during the 90 days prior to conception.

  This is separate from, and in addition to, general reporting of pregnancy in a study participant or female partner of a male participant (see section 8.5.2.3.4 below).
- is a medically important event.
  NOTE: Medical and scientific judgment should be exercised. Events that significantly jeopardize the subject and/or require intervention to prevent one of the SAE outcomes listed above are generally considered medically important, and are thus SAEs.

Unexpected Adverse Event: An AE is unexpected if it is not listed in the investigator's brochure or package insert (for marketed products) at the frequency, AND specificity, AND severity that has been observed.

NOTE:

- Such events should also be evaluated for possible reporting as unanticipated problems (UPs) (see section 8.5.2.3.3 below).
- Unexpected, as used in this definition, also refers to AEs or SARs that are mentioned in the investigator's brochure as occurring with a class of drugs/biologics, or as anticipated from the pharmacological properties of the study agent but are not specifically mentioned as occurring with the particular study agent under investigation.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is an SAR (defined above) that is both serious and unexpected.

Unanticipated Problem (UP): A 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 (including but not limited to risks) as 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 is**
2. possibly, probably, or definitely related (see "Causality" below) to participation in the research; **and**
3. suggests the research places subjects or others at a **greater risk** of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, per the documents currently approved by the IRB.
   NOTE:

- Per the sponsor, an SAE always meets this "greater risk" criterion.
- An incident, experience, or outcome that meets the definition of a UP generally will warrant consideration of changes to the protocol or informed consent form, or to study procedures (eg, the manual of procedures for the study), in order to protect the safety,

welfare, or rights of participants or others. Some UPs may warrant a corrective and preventive action plan at the discretion of the sponsor or other oversight entities.

Unanticipated Problem that is not an Adverse Event (UPnonAE): A UPnonAE belongs to a subset of UPs that:

- meets the definition of a UP, AND
- does NOT fit the definition of an AE or an SAE.
  NOTE: Examples of UPnonAEs include, but are not limited to:
  - a breach of confidentiality
  - prolonged shedding of a vaccine virus beyond the anticipated timeline
  - unexpectedly large number of pregnancies on a study
  - subject departure from an isolation unit prior to meeting all discharge criteria
  - accidental destruction of study records
  - unaccounted-for study agent
  - overdosage, underdosage, or other significant error in administration or use of study agent or intervention, even if there is no AE/SAE
  - development of an actual or possible concern for study agent purity, sterility, potency, dosage, etc.
  - NOTE: A decision to temporarily quarantine, or to permanently not use all or part of study agent supply due to an unexpected finding or event (eg, particulate, cloudiness, temperature excursion), even if there is no known or proven issue (ie, out of an "abundance of caution"), is considered a UPnonAE.

Protocol Deviation: Any change, divergence, or departure from the IRB-approved research protocol.

1. **Major Deviations:** Deviations from the IRB-approved protocol that have, or may have the potential to, negatively impact the rights, welfare, or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
2. **Minor Deviations:** Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Non-compliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

1. **Serious non-compliance:** Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially affects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
2. **Continuing non-compliance:** A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare, and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (eg, due to lack of understanding, knowledge, or commitment), or

intentional (eg, due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

8.5.2 Documenting, Assessing, Recording, and Reporting Events

All AEs, including those that may appear to have a non-study cause (see "Causality" below), will be documented (eg, on the clinical chart/progress notes/clinical laboratory record), recorded (eg, in the study-specified case report form [CRF]/research database), and reported (eg, cumulatively from the research database, or according to protocol-specified expedited reporting mechanism) to the sponsor from the time informed consent is obtained through the timeframe specified below. At each contact with the subject, information regarding AEs will be elicited by open-ended questioning and examinations.

AEs and SAEs will generally be recorded, assessed, and reported according to the timeframes outlined in Table 5.

Table 5. Standard Event Recording, Assessment, and Reporting Timeframes

Event type	Record, assess, and report through
Related SAEs	End of individual participation in study, or if study personnel become aware thereafter
Unrelated SAEs	End of individual participation in study
Related non-serious AEs of grade 1 to 3	End of individual participation in study
All other related non-serious AEs	End of individual participation in study
Unrelated non-serious AEs	End of individual participation in study

8.5.2.1 Investigator Assessment of Adverse Events

The investigator will initially assess all AEs with respect to **seriousness** (according to SAE definition above), **severity** (intensity or grade, see below), and **causality** (relationship to study agent and relationship to participation in the research, see below).

Note: The CSO/SMM is responsible for final/regulatory determinations of expectedness and causality.

8.5.2.1.1 Severity Grading

The investigators will use a modification of the FDA document "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (September 2007) for grading of AEs/SAEs throughout the study (https://www.fda.gov/media/73679/download and Appendix 1).

NOTE: A participant death should always be reported as grade 5.

Laboratory Value Assessment

All abnormal (ie, not within normal range, or else grade 1 or greater, per local lab standard) lab values are reportable.

8.5.2.1.2 Causality

Causality (likelihood that the event is caused by the study agent) will be assessed by the principal investigator considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship
  AND/OR
- there is good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship
  AND/OR
- definitely due to an alternative etiology

Note: Other factors (eg, dechallenge, rechallenge, if applicable) should also be considered for each causality category when appropriate. 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.

Causality assessment will be reviewed by the sponsor. The sponsor may make a separate and final determination on the "reasonable possibility" that the event was "related" (comprising definitely, probably, and possibly related) or "unrelated" (comprising unlikely and not related) to the study agent, in keeping with applicable (US FDA) guidance on sponsor IND safety reporting.

8.5.2.1.3 Expectedness

The principal investigator may make an initial investigator determination as to whether an AR is expected or unexpected.

Expected ARs are AEs that are known to occur, at the specified severity and frequency, with the intervention being studied, based on information in the most current IB [49].

The sponsor will review these determinations and may make an alternative and regulatorily overriding determination, particularly if the event appears to qualify as a suspected unexpected adverse reaction, ie, one not listed in the IB or that is not listed at the specificity or severity that has been observed. A SUSAR meets the definition in section 8.5.1. The ARs in Table 6, Table 7, and Table 8 are expected to occur in participants who receive rDEN3Δ30/31-7164 and will be recorded in the database. The AEs expected to occur in participants who undergo LN FNA are listed in Table 9 and will be recorded in database.

Table 6. Expected and Unexpected Injection Site ARs

	Expected		Unexpected	
Adverse Reactions	Grade	Severity	Grade	Severity
Injection site pain	1 2	No interference with activity, may require one dose of medication/treatment Some interference with activity or requires >1 dose of medication/treatment	3 4	Prevents daily activity and requires medical intervention Hospitalization
Injection site tenderness	1 2 3	Mild discomfort to touch Discomfort with movement Significant discomfort at rest	4	Hospitalization
Injection site redness	1 2 3	2.5-5 cm 5.1-10 cm >10 cm	4	Necrosis or exfoliative dermatitis requiring medical attention
Injection site bruising	1 2 3	2.5-5 cm 5.1-10 cm >10 cm	4	Hematoma requiring medical attention
Injection site swelling/induration	1 2 3	2.5-5 cm and does not interfere with activity 5.1-10 cm or interferes with activity >10 cm or prevents daily activity	4	Necrosis requiring medical attention
Injection site pruritis	1 2	No interference with activity, may require one dose of medication/treatment Some interference with activity or requires >1 dose of medication/treatment	3 4	Prevents daily activity and requires medical intervention Pruritis requiring medical attention

Table 7. Expected and Unexpected Systemic ARs

	Expected		Unexpected	
Adverse Reactions	Grade	Severity	Grade	Severity
Fever	1 2 3	100.4-101.1 101.2-102.0 102.1-104	4	>104
Headache Retro-orbital pain Photophobia Nausea Fatigue Myalgia Arthralgia	1 2 3	No interference with activity, may require one dose of medication/treatment Some interference with activity or requires >1 dose of medication/treatment Prevents daily activity and requires medical intervention	4	Hospitalization

Dengue-like rash	1 2 3	Rash is present but asymptomatic Rash is symptomatic (pruritis/pain) but does not interfere with function Rash is symptomatic and interferes with function	4	Hospitalization

Table 8. Expected and Unexpected ARs of Laboratory Parameters

	Expected		**Unexpected**	
Adverse Reactions	Grade	Severity	Grade	Severity
Hemoglobin (g/dL) – female **Report as anemia*	1 2	11-2 9.5-10.9	3 4	8.0-9.4 <8.0
Hemoglobin (g/dL) – male **Report as anemia*	1 2	12.5-13.5 10.5-12.4	3 4	8.5-10.4 <8.5
ANC (cc/mm3) **Report as neutropenia*	1 2 3	1,000-1,499 750-999 500-749	4	<500
ALT, increase by factor	1 2	1.1-2.5x ULN >2.5-5.0x ULN	3 4	>5-10x ULN >10x ULN
Platelets (cells/mm3) **Report as thrombocytopenia*	1 2	100,000-120,000 75,000-99,999	3 4	25,000-74,999 <25,000
Creatinine (mg/dL)	1 2	1.5-1.7 1.8-2.0	3 4	2.1-2.5 >2.5 or requires dialysis
PT, increase by factor	1 2	1.1x ULN >1.1-1.25x ULN	3 4	>1.25-1.5x ULN >1.5x ULN
PTT, increase by factor	1 2	1.1-1.4x ULN >1.4-1.6x ULN	3 4	>1.6-2.33x ULN >2.33x ULN

Table 9. Expected and Unexpected AEs of Lymph Node Aspiration Site

	Expected		Unexpected	
Adverse Reactions	Grade	Severity	Grade	Severity
Aspiration site pain	1 2	No interference with activity, may require one dose of medication/treatment Some interference with activity or requires > 1 dose of medication/treatment	3 4	Prevents daily activity and requires medical intervention Hospitalization
Aspiration site redness	1 2 3	2.5 – 5 cm 5.1 – 10 cm > 10 cm	4	Necrosis or exfoliative dermatitis requiring medical attention
Aspiration site warmth	1 2 3	2.5 – 5 cm 5.1 – 10 cm > 10 cm	4	Warmth requiring medical attention
Aspiration site bruising	1 2 3	2.5 – 5 cm 5.1 – 10 cm >10 cm	4	Hematoma requiring medical attention
Aspiration site swelling/induration	1 2 3	2.5 – 5 cm and does not interfere with activity 5.1 – 10 cm or interferes with activity >10 cm or prevents daily activity	4	Necrosis requiring medical attention
Aspiration site bleeding	1 2	Mild symptoms and no intervention required Moderate symptoms and/or intervention required	3 4	Transfusion and/or invasive intervention and/or hospitalization required Life-threatening bleed
Aspiration site drainage	1 2	Mild symptoms and no intervention required Moderate symptoms and/or intervention required	3 4	Invasive intervention required Hospitalization
Fever within 48 hours of aspiration	1 2	100.4-101.1 101.2-102.0	3 4	102.1-104 >104

8.5.2.2 Recording of Events

AEs will be promptly recorded in the research database, regardless of possible relationship to study interventions. If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or laboratory abnormalities will be recorded as the AE. The investigator will review events regularly to ensure they have been captured correctly and to perform assessment of events individually and cumulatively to assess possible safety trends.

8.5.2.3 Investigator Reporting Responsibilities

The principal investigator and/or equally qualified designee will check daily for events that may require expedited reporting.

The principal investigator and/or equally qualified designee will also monitor all accumulating data no less than weekly, or according to superseding NIH or NIAID policy, whichever is more frequent.

Data will be reviewed by the principal investigator/designee on a regular basis for accuracy and completeness.

Data will be submitted to the sponsor in keeping with all applicable agreements and when requested, such as for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

The principal investigator will ensure prompt reporting to safety oversight bodies (eg, CSO, DSMB), regulatory entities, and stakeholders as specified below, and according to any additional requirements or agreements.

8.5.2.3.1 Adverse Events

Unless otherwise specified above, AE data will be entered into the research database no less than every other week and will include all data through one week prior to database entry.

8.5.2.3.2 Serious Adverse Events

Unless otherwise specified above, all SAEs (regardless of relationship and whether or not they are also UPs) must be reported to the CSO as specified by the CSO (eg, REDCap system; use the safety expedited report form [SERF]/email if REDCap is not available). If the preferred/indicated mechanism for reporting is not available, the CSO/SMM should be contacted by telephone, fax, or other reasonable mechanism to avoid delays in reporting.

CSO CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
Email: rchspsafety@mail.nih.gov
https://crimsonredcap.cc.nih.gov/redcap/index.php

Unless otherwise specified above, deaths and immediately life-threatening SAEs must be reported to the CSO promptly, and no later than the **first business day** following the day of study personnel awareness.

All other SAEs must be reported to the CSO no later than the **third business day** following the day of study personnel awareness.

If an individual subject experiences multiple SAEs in a closely timed/overlapping "cause -and -effect" (cascade) sequence, the principal investigator, after careful evaluation, will report ONLY primary/precipitating event(s) individually. SAEs that are determined to be definitely secondary to other SAEs will be detailed in the narrative portion of the report of the relevant primary/precipitating SAE. A clinical rationale and findings to support such reporting should be part of the narrative.

For each SAE report, the research database entry MUST match the corresponding entries on the SAE report (eg, start and stop dates, event type, relationship, and grade), and **must be updated if necessary** (eg, if the SAE report was generated after the corresponding AE was entered in the research database).

Unless otherwise specified above, SAEs that have not resolved by the end of the per-protocol follow-up period for the subject are to be followed until final outcome is known (to the degree

permitted by the IRB-approved informed consent form). If it is not possible to obtain a final outcome for an SAE (eg, the subject is lost to follow-up), and to update the CSO, the last known status and the reason a final outcome could not be obtained will be recorded by the investigator on an SAE report update and the CRF.

8.5.2.3.3 Unanticipated Problems

Unless otherwise specified above, UPs (as defined in this protocol, or as defined by the IRB of record, whichever definition is more conservative) that are also AEs or SAEs, must be reported to the CSO (by REDCap, or by email and SERF if REDCap is not available) no later than when they are due to be reported to the IRB.

UPnonAEs are NOT reported to the CSO but must be reported to the Clinical Trials Management (CTM) group and the IRB according to their requirements and preferred methods. If the UPnonAE raises a significant potential participant safety concern, then the SMM should be consulted by email or phone no later than when reports are made to the IRB and/or CTM.

8.5.2.3.4 Pregnancy

Unless otherwise specified above, all pregnancies that occur by day 60 will be reported (by REDCap, or by email and SERF if REDCap is not available) to the CSO no later than the first business day following the day of study personnel awareness.

Pregnancy outcome data will be reported to the CSO no later than the third business day following the day of study personnel awareness (by REDCap, or by email and SERF if REDCap is not available).

Pregnancy itself is not an AE. Events that meet AE or SAE criteria in relation to pregnancy, delivery, or the conceptus/neonate (see section 8.5.1) are reportable (by REDCap, or by email and SERF if REDCap is not available).

In the event of pregnancy in a study participant by day 60, the following actions will be taken, with the goal of ensuring maternal and fetal wellbeing, in consultation with the SMM and DSMB:

- Discontinue the study procedures but continue to follow-up for safety.
- Request to unblind the participant, if applicable, AND if doing so would offer a benefit to the participant.
- Report, no later than the first business day after study personnel awareness, to the DSMB and the IRB.
- Advise the participant to notify their obstetrician of study participation and study agent exposure, providing contact information for the obstetrician to contact the study principal investigator, should this be required, and with the participant's consent.

8.5.2.4 Sponsor's Reporting Responsibilities

Events reported to the sponsor will be promptly evaluated and will be reported as required according to FDA IND safety reporting guidance and regulations. IND safety reports will be sent to other investigators conducting research under the same IND and will be shared with other stakeholders according to applicable agreements.

The sponsor will also submit an IND annual report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

All UPs will be evaluated by the sponsor, and a summary of the event, and any necessary (corrective/preventative) actions, will be distributed to investigators conducting research under the same IND as may be relevant and appropriate.

8.5.3 Withdrawal Criteria for an Individual Participant

An individual participant will be withdrawn from the study for any of the following:

- An individual participant's decision. (The investigator should attempt to determine the reason for the participant's decision.)
- The participant initiates immunomodulating therapy (see section 6.5).
- The participant permanently loses capacity to provide ongoing informed consent.
- Non-compliance with study procedures to the extent that it is potentially harmful to the participant or to the integrity of the study data.
- The investigator determines that continued participation in the study would not be in the best interest of the participant.

8.5.3.1 Re-enrollment and Unplanned Procedure Repetition

Unless otherwise specified within this protocol, each person who is a participant in this study may be enrolled and may pass through each step and process outlined in the protocol, only **ONCE** (ie, participants may not "go back" and repeat a protocol step already completed). On a case-by-case basis, a request for re-enrollment, or for repetition of a protocol step or procedure already completed, may be submitted to, reviewed by, and approved by the SMM in writing. The SMM may also recommend or require consultation of the IRB and/or DSMB.

8.5.3.2 Replacement of Withdrawn Participants or Participants Who Discontinue Study Agent

Participants who withdraw or are withdrawn from the study or discontinue study agent prior to day 28 will be replaced. If a participant is replaced, then all the data collected from that participant will still be included for the safety assessment.

All participants exposed to study agent MUST be included in the safety dataset.

8.5.4 Additional Safety Oversight

8.5.4.1 Medical Advisory Investigator

The medical advisory investigator (MAI) is the person appointed to assist the principal investigator in the development of clinical aspects of the protocol and is responsible for ensuring that the provision of any clinical interventions mandated by the protocol are conducted appropriately and safely [83]. MAIs are typically appointed when the principal investigator is a clinical fellow or junior faculty, and this study has an MAI.

8.5.4.2 Safety Review and Communications Plan

A safety review and communications plan (SRCP) is required for this protocol. The SRCP is an internal communications document between the principal investigator and the CSO, as sponsor representative, which delineates key safety oversight responsibilities of the principal investigator,

the CSO, and other stakeholders. The SRCP includes a plan for conducting periodic safety surveillance assessments by the CSO.

8.5.4.3 Sponsor Medical Monitor

An SMM, representing the sponsor, has been appointed for oversight of safety in this clinical study. The SMM will be responsible for performing safety assessments as outlined in the SRCP.

8.5.4.4 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 interest as defined by NIAID policy. The DSMB will review the study protocol, consent documents, and investigator brochure prior to initiation, and will review study progress and the accumulating data approximately twice a year thereafter, or as may be determined by the DSMB.

The DSMB may convene additional reviews as necessary. The DSMB will review the study data as needed to evaluate the safety, efficacy, study progress, and conduct of the study.

All deaths, SAEs, UPs, pregnancies, and IND safety reports will be reported to the DSMB at the same time they are submitted to the IRB and CSO unless otherwise specified herein.

All cases of intentional or unintentional unblinding will be reported to the DSMB not later than one business day from the time of study personnel awareness.

The principal investigator will notify the DSMB at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The principal investigator will submit the written DSMB summary reports with recommendations to the IRB.

8.5.5 Halting Rules for the Protocol

"Halting" is discontinuation of study intervention/treatment/dosing (agent/placebo/procedure, etc.) for all participants in a study and suspension of enrollment until a decision is made to either resume or permanently discontinue such activity. Participants continue to be followed for safety during a halt.

The halting rules are:

- One or more participants experience an SAE that is possibly, probably, or definitely related to the study agent or research procedure (except for neutropenia – see specific guidelines below; probabilities of observing neutropenia are detailed in section 9.2).
- Two or more participants experience the same or similar grade 3 or greater AEs that are unexpected and possibly, probably, or definitely related to a study agent. There are two exceptions to this rule:
    - Neutropenia: halting rules are specific and listed below (probabilities of observing neutropenia are detailed in section 9.2).
    - Local reactions to vaccine: The study will not be halted for any grade 3 or lower AEs classified as local reactions to the vaccine.
- One or more of the first 6 participants experience a mean peak viremia titer of $\geq 10^6$ PFU/mL by viral culture.

- Two or more participants experience an ANC ≥500/µL but <750/µL for >5 days duration.[a]
- Two or more participants experience an ANC <500/µL for any duration.[a]
- Two or more participants experience a vaccine-associated dengue-like syndrome, defined as infection[b] associated with fever **and 2 or more** of the following symptoms:
    a. Grade 2 or greater headache lasting ≥12 hours.
    b. Grade 2 or greater photophobia lasting ≥12 hours.
    c. Grade 2 or greater generalized myalgia lasting ≥12 hours.
- Any safety issue that the principal investigator or the CSO determines should halt the study. The DSMB may recommend a halt to the CSO.

[a] These halting rules resulted from previous discussions between NIAID and the FDA on phase 1 clinical trials of rDEN3Δ30/31-7164 (reference IND 13886).

[b] Infection is defined as recovery of vaccine virus from the blood or serum of a participant and/or seropositivity or seroconversion to any dengue virus.

In addition, the IRB, FDA, or any regulatory body having oversight authority may halt the study at any time.

8.5.5.1 Reporting a Study Halt

If a halting criterion is met, then a description of the AE(s) or safety issue must be reported by the principal investigator within 1 business day to the CSO and the IRB according to their requirements. The principal investigator will also notify the DSMB.

8.5.5.2 Resumption of a Halted Study

The CSO, in collaboration with the principal investigator and DSMB, will determine if study activities, including enrollment, study agent administration, and/or other study interventions, may be resumed and any additional modifications or requirements that may apply.

The CSO or sponsor designee will notify the principal investigator of the decision. The principal investigator will notify the IRB of the decision according to the IRB's process.

8.5.5.3 Discontinuation of Study Agent

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

8.6 Unanticipated Problems

8.6.1 Definition of UP

The definition of a UP is provided in section 8.5.1.

8.6.2 Unanticipated Problem Reporting

The investigator will report UPs to the NIH IRB as per HRPP Policy 801.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

Primary hypotheses are:

Safety hypotheses

1) The SAE rate (rate as specified in halting criterion 1, section 8.5.5) is less than 0.10.

2) The unexpected AE rate (rate as specified in halting criterion 2, section 8.5.5) is less than 0.15.

Immunologic hypotheses:

1) Each group will have a significant rise in DENV neutralizing antibody GMT between days 0 and 28.

2) There will be a significant difference in mean peak viremia among groups.

Primary endpoints consist of:

- The frequency and severity of local and systemic reactogenicity signs and symptoms during the 28-day period after each vaccination, unexpected AEs up to 28 days after each vaccination, and SAEs through day 180.
- Change in DENV neutralizing antibody GMT between days 0 and 28.
- Mean peak viremia among groups as measured by viral qRT-PCR between days 3 and 15.

Secondary endpoints consist of:

- Change in DENV neutralizing antibody GMT between days 0 and 57.
- DENV neutralizing antibody GMT at days 0, 28, and 57.
- Magnitude of the $CD8^+$ T-cell response at day 15 among groups as measured by AIM assays.

9.2 Sample Size Determination

The primary immunologic comparisons in this study are to determine whether there is an increase in DENV neutralizing antibody GMT between days 0 and 28 for each group, and to compare mean peak viremia titer among groups measured from days 3 to 15. The sample size of 15 per group is based on these comparisons. With 15 participants in each group, there will be 90% power to detect a fold-increase in GMT of 5.36 (or $\log_{10} = 0.73$) assuming a standard deviation (std) of 0.81 on the $\log_{10}$ scale using a 2-sided paired t-test with a significance level of 0.05. This std is based on the previous work of Russell, et al, where they assessed the change in DENV3 GMT between days 0 and 28 after TV003 vaccination in DENV-naïve and exposed groups [71]. In the exposed group, they reported that the change in GMT was 9 ($\log_{10} = 0.95$) with a std of 0.81. In the naïve group, the change in GMT was 15.6 ($\log_{10} = 1.19$) with a std of 0.56. Therefore, this study is well powered to detect differences that are smaller than those was observed by Russell, et al.

With regards to viremia, previous work reported a difference in the mean peak titer of 0.6 between a naïve and exposed group after monovalent DENV2 vaccination with std of 0.19 in the naïve group and 0.57 in the exposed group [6]. With 15 per group, there will be 80% power to detect a difference of 0.55 if std are 0.19 and 0.57 or a difference of 0.47 if std are the average of these two values at 0.38 using a 2-sided t-test with a significance level of 0.05/3 to compare all three groups. We anticipate screening 200 participants to enroll all 45 participants. A formal

statistical analysis plan will be developed. (The power calculations were performed using the function power.welch.t.test in R.)

Safety is another primary aim of the study. It is important to halt the study as soon as possible if a safety signal emerges. Thus, we calculate the number of people that would be entered into the study before the halting rules are met (as defined in section 8.5.5) with high probability 0.8 or 0.9 (Table 10). For example, if the true SAE probability is 0.10, then there is a probability of 0.90 that at least one SAE will be observed by the time 16 participants have been enrolled. This calculation is based on the exact binomial distribution.

Table 10. Number of Participants Enrolled to Observe One SAE with 0.80 or 0.90 Probability

True SAE Probability	0.80 probability of observing first event by n participants enrolled	0.90 probability of observing first event by n participants enrolled	Probability of observing at least 1 event out of 45 participants
0.15	10	15	0.99
0.1	16	22	0.99
0.05	32	46	0.90
0.005	-	-	0.20
0.002	-	-	0.09

Similarly, we calculated the number of people that would be entered into the study before the halting rule 2 is met (as defined in section 8.5.5) with high probability, 0.8 or 0.9 (Table 11). For example, if the true AE probability is 0.15, then there is a probability of 0.80 of observing at least 2 AEs of similar types in the first 19 participants. Of note, as per discussions between NIAID and the FDA (see IND 13886), all halting due to neutropenia will require two or more participants to experience neutropenia as defined in section 8.5.5 and thus will follow the probabilities listed in Table 11. These calculations are based on the exact binomial distribution.

Table 11. Number of Participants Enrolled to Observe Two AEs of Same Type with 0.80 or 0.90 Probability

True AE Probability	0.80 probability of observing 2 events by n participants enrolled	0.90 probability of observing two events by n participants enrolled	Probability of observing 2 events out of 45 participants
0.15	19	25	0.99
0.1	29	38	0.95
0.05	-	-	0.66
0.005	-	-	0.028
0.002	-	-	0.005

9.3 Populations for Analyses

9.3.1 Evaluable for Toxicity

Each participant who receives the single dose of vaccine will be evaluated for the primary safety endpoint. We anticipate a similar frequency of injection site reactions to vaccination in all four

groups. However, we expect the heterotypic group will have the highest mean peak viremia and rash frequency.

9.3.2 Evaluable for Immunologic Outcome

All participants who receive the single dose of vaccine will be evaluated for primary and secondary immunogenicity endpoints. Missing immunologic data will be assumed to be missing at random and we will evaluate all available data.

9.4 Statistical Analyses

9.4.1 General Approach

All participants who receive the single dose of vaccine will be analyzed for safety and immunogenicity.

Dengue groups will be assigned by the dengue antibodies measured at screening. However, some individuals may have changes in their dengue antibody levels between screening and day 0. This could be related to the natural dynamics of the immune response or assay variability. To address this issue, primary and secondary endpoints will be analyzed using two subsets for group categorization. The 'intention to treat' subset will keep individuals in the group they were assigned to by the screening antibodies. The 'per protocol subset' will evaluate individuals according to their group assignment determined by day 0 antibodies.

SAEs and AEs will be tabulated by grade and proportions of people with each AE. Comparisons of the SAEs and AEs among groups will be performed using Fisher's exact for the overall test and Fisher's exact unconditional tests and tests of proportions for 2 group comparisons. (Using R with fisher.test or uncondExact2x2 in exact2x2 library.)

All immunologic data will be analyzed using a $\log_{10}$ transformation. Observations below the lower limit of detection (LLOD) will have a value of ½ the LLOD imputed. Paired t-tests will be used to assess changes in continuous endpoints within each group, and t-tests will be used to assess changes in continuous endpoints among groups. Continuous data will be presented as means with standard deviations and confidence intervals. Regression models will also be used for continuous data to control for other variables.

We plan to perform t-tests to compare continuous endpoints. However, we will examine the distribution of the data in a blinded fashion and determine whether, due to a skewed distribution, a Wilcoxon test will be more appropriate.

Categorical data for immunologic endpoints will be presented as percentages with confidence intervals or relative risk or logistic regression models may be used to estimate odds ratios. AE comparisons between groups will be performed at the 2-sided 0.05 level of significance. Comparisons of the primary and secondary immunogenicity endpoints will be performed at a 2-sided 0.05 level of significance. All tertiary comparisons will be considered exploratory and will be performed at a 2-sided 0.05 level of significance. As secondary analyses, regression models will also be performed to adjust for age, sex, gender, time since dengue exposure, and day 0 GMT.

9.4.2 Analysis of the Primary Endpoints

Fisher's exact tests and Fisher's exact unconditional test and tests of proportions will be used to compare AE rates between groups. Fisher's exact tests will be used to determine if there is a difference between groups. Significant Fisher's exact tests may be followed up with individual group comparisons using tests of proportions with Fishers exact unconditional test. Safety tests will be performed at a 0.05 level of significance, except rash, which will performed at a 0.05/3 level of significance.

The change in the DENV neutralizing antibody GMT from day 0 to 28 in each group will be assessed using a paired t-test with a 2-sided 0.05 level test. The mean peak viremia from days 3 and 15 among groups will be assessed using t-tests with Bonferroni correction.

9.4.3 Analysis of the Secondary Endpoint(s)

The change in the DENV neutralizing antibody GMT from day 0 to 57 in each group will be tested using paired t-tests with a 2-sided 0.05 level test. The magnitude of activated CD8+ T cells at day 15 will be compared between groups with each test performed at the 2-sided 0.05 level. As secondary analyses, regression models will also be performed to adjust for age, sex, gender, time since dengue exposure, and DENV neutralizing GMT at day 0.

9.4.4 Safety Analyses

9.4.4.1 Solicited Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom (injection site pain, redness, swelling, bruising and headache, muscle pain, fatigue, chills, oral temperature, joint pain, nausea, vomiting, diarrhea) will be tabulated by local or systemic reactogenicity events. For each AE, the start and stop dates, severity, relationship, and outcome will be reported.

9.4.4.2 Adverse Events

AEs will be recorded using the Medical Dictionary for Regulatory Activities (MedDRA) as the coding dictionary for this study and graded as described in section 8.5.2.1.1 and Appendix 1. MedDRA preferred terms will be used to record solicited events that we anticipated following vaccination (eg, myalgia instead of muscle aches). The number and percentages of participants experiencing each specific AE and the severity, frequency, and relationship of these AEs to vaccination will be presented by system organ class and preferred term groupings. For calculations of events in these tables, each participant's AEs will be counted once under the maximum severity or strongest recorded causal relationship to the vaccine injection. Because rDEN3D30/31-7164 is known to be well tolerated in DENV-naïve individuals [37], but safety in naturally infected heterotypic and polytypic individuals in non-endemic areas has not been well characterized, we will compare AE rates in the combined heterotypic plus polytypic groups to the rates in the naïve group as a secondary analysis.

9.4.5 Baseline Descriptive Statistics

The groups will be characterized and compared for the following using descriptive statistics: age, sex, race, ethnicity, date of dengue disease (if known), and time from last exposure to an endemic area.

9.4.6 Sub-Group Analyses

The primary and secondary endpoints will be analyzed based on time from last dengue exposure if there are sufficient differences in time from exposure to create sub-groups and make valid comparisons.

9.4.7 Tabulation of Individual Participant Data

Individual participant data will be tabulated for AEs and immunogenicity assay results (neutralizing antibody, viremia, CD8+ T-cell assays) for each study timepoint.

9.4.8 Exploratory Analyses

Exploratory analyses that will be considered based on results from the primary and secondary endpoints are listed in the table in section 3.

9.4.9 Statistical Considerations for Individuals Who Receive Low Vaccine Dose

Individuals who receive a vaccine dose between 10^1 and $10^{2.4}$ PFU will be considered to have received a low vaccine dose. All individuals, even those who receive a low vaccine dose, will be included in the primary and secondary analyses. However, to assess for possible effects of a low vaccine dose, sensitivity analyses will be performed wherein these individuals will be removed from primary and secondary endpoint analyses. Given our primary endpoints are powered for n=12 per group, we will allow up to three individuals per group to receive a low dose vaccine. If >3 individuals in any group receive a low dose vaccine, we will discuss the need for individual replacement with the DSMB.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 Informed Consent Process

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort, and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members, and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place. A copy of the informed consent document will be given to the participants for their records. The consenting investigator will document the informed consent process in the participant's medical record.

The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. The participants may withdraw consent at any time throughout the course of the study.

The initial consent process as well as reconsent, when required, may take place in person or remotely (eg, via telephone or other NIH-approved remote platforms used in compliance with policy, including NIH HRPP Policy 303) per the discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the participant will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (eg, clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to

relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the investigator and the participant are co-located.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to the participant) or on the electronic document. The process for documenting signatures on an electronic document is described below.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the iMedConsent electronic platform to obtain the required signatures. This platform is compliant with 21 CFR 11.

Both the investigator and the participant will sign the electronic document using a finger, stylus, or mouse. Electronic signatures (ie, the "signature" is digitally generated) will not be used.

10.1.1 Considerations for Consent of NIH Staff or Family of Study Team Members

Consent for NIH staff will be obtained as detailed above with following additional protections:

Consent from staff members for whom this research is taking place within their own work unit or is conducted by any of their supervisors will, when possible, be obtained by an individual in a nonsupervisory relationship with that staff member. Similarly, for family of the study team, a study team member unrelated to the participant will obtain their informed consent. When consent of that staff member or family member is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on them.

10.1.2 Participation of Individuals Who Are/Become Decisionally Impaired

Participants who become permanently decisionally impaired during their participation in this study, and can therefore no longer provide their ongoing informed consent, will be withdrawn from the study (section 8.5.3). Per HRPP Policy 403, if the loss of capacity to consent is temporary and the participant is expected to regain it, then they may continue on the study without reconsent of a legally authorized representative.

10.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the IND sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform study participants, the IRB, and sponsor, and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Insufficient compliance to protocol requirements.

- Data that are not sufficiently complete and/or evaluable.

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB, and, as applicable, the FDA.

10.3 Confidentiality and Privacy

All records will be kept confidential to the extent provided by federal, state, and local law. Authorized representatives of the NIAID may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NIAID, Office for Human Research Protections (OHRP), the FDA, or the sponsor's designee.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 Future Use of Stored Specimens and Data

Coded specimens and data will be stored at the NIH indefinitely for future research after the study is complete. Human genetic testing may be performed. Plans for future use of specimens and data will be described in the informed consent document. Specimens will be stored at the NIH CC in a locked facility with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the code key.

Other investigators (at NIH and elsewhere) may wish to study these specimens and data. If the planned research falls within the category of "human subjects research" on the part of the investigators, NIH IRB review and approval will be obtained. This includes the investigators sending out coded and linked specimens or data and getting results that they can link back to their participants.

10.5 Safety Oversight

Safety oversight for this study is described in section 8.5.4.

10.6 Clinical Monitoring

According to the ICH E6(R2) GCP guidelines, section 5.18, and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol.

The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the consent process for each monitored participant; 2) to verify the prompt and accurate recording of all monitored data points in CRIMSON and prompt reporting of all SAEs; 3) to compare abstracted information entered into CRIMSON with individual participants' records and source documents (participants' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original participant information); and 4) 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 (OHRP, FDA) and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (eg, consent forms, CRIMSON data abstracts) and pertinent hospital or clinical records, including CRIMSON, 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 principal investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

10.7 Quality Assurance and Quality Control

To help ensure that NIH Office of Research Support and Compliance procedures and GCP are being carried out, a CTM designee within OCRPRO, Regulatory Compliance and Human Subjects Protection Program will conduct a study initiation visit before study enrollment begins. The purpose of this meeting is to review with the principal investigator and study team designees the roles and responsibilities concerning their commitment to adhere to the requirements of the protocol, especially in terms of NIH Office of Human Subjects Research Protections reporting requirements for reportable events. In addition, the quality management and data management plan for the study will be reviewed.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Questionnaire data will be collected in REDcap. Study data will be maintained in CRIMSON and collected directly from participants during study visits and telephone calls, or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data, including cumulative participant accrual numbers, should be generated via the chosen data capture method and submitted to the IRB as needed.

10.8.2 Study Records Retention

Study documents will be retained in accordance with regulatory and institutional requirements, ICH GCP guidelines, and the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the principal investigator and sponsor, as applicable.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will 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. Relocation of research records will not proceed without written permission from OCRPRO/NIAID.

10.9 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH IRB as per Policy 801. All deviations must be addressed in study source documents and reported as specified in the protocol quality management plan and/or monitoring plan. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

The definition of a protocol deviation is provided in section 8.5.1.

10.10 Reporting to the NIAID Clinical Director

Major protocol deviations, UP's, and deaths will be reported to the NIAID clinical director according to institutional timelines.

10.11 Reporting to the NIH Institutional Biosafety Committee

The NIH Institutional Biosafety Committee (IBC) has the responsibility to review research using recombinant DNA for compliance with NIH guidelines. In keeping with IBC requirements, any SAE reports sent to the IRB will be concurrently or subsequently provided to the IBC by the principal investigator.

10.12 Publication and Data Sharing Policy

10.12.1 Human Data Sharing Plan

We will comply with NIH policies on data access, sharing and dissemination, and clinical trials registration, as applicable. Human data generated in this study may be shared for future research as follows:

- De-identified data in an NIH-funded or supported public repository.
- De-identified data in another public repository.
- Identified data in the Biomedical Translational Research Information System (automatic for activities in the CC) and/or the NIAID Genomic Research Integration System.
- De-identified data with approved outside collaborators under appropriate agreements.
- De-identified data in publications and/or public presentations.

Data will be shared at the time of or shortly after publication.

10.12.2 Genomic Data Sharing Compliance

Although the NIH Genomic Data Sharing Policy is not required for this research, we may want to share genomic data with an NIH-designated repository, such as the Database of Genotypes and Phenotypes. We will comply with all requirements in order to do so, including creating a Data Sharing Plan and completing an Institutional Certification and seeking approval prior to

beginning the research. Prior to sharing the data, we will de-identify it, code it, and maintain the code key linked to the data.

10.13 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIAID have an established mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

ADE	Antibody-dependent enhancement
AE	Adverse event
AIM	Activation-induced marker
ALT	Alanine transaminase
ANC	Absolute neutrophil count
AR	Adverse reaction
CBC	Complete blood count
CC	Clinical Center
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CITE-seq	Cellular indexing of transcriptomes and epitopes by sequencing
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case report form
CRIMSON	Clinical Research Information System of the NIAID
CRL	Charles River Laboratories
CSO	Clinical Safety Office
CTM	Clinical Trials Management
DENV	Dengue virus
DNA	Deoxyribonucleic acid
DSMB	Data safety monitoring board
E	Envelope (protein)
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FNA	Fine needle aspiration
GCP	Good Clinical Practice
GMT	Geometric mean titer
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRPP	Human Research Protection Program
IBC	Institutional Biosafety Committee
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IGHV	Immunoglobulin heavy chain
IND	Investigational New Drug application
INR	International normalized ratio
IRB	Institutional review board
LID	Laboratory of Infectious Diseases
LLOD	Lower limit of detection
LN	Lymph node
MAI	Medical advisory investigator
MBC	Memory B cell
MedDRA	Medical Dictionary for Regulatory Activities
NHP	Non-human primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NS3	Non-structural protein 3
NSAID	Nonsteroidal anti-inflammatory drug
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
PBMC	Peripheral blood mononuclear cell
PFU	Plaque-forming unit(s)
$PRNT_{50}$	50% plaque reduction neutralization test
PT	Prothrombin time
PTT	Partial thromboplastin time
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
REDCap	Research Electronic Data Capture
RNA-seq	Ribonucleic acid sequencing
SAE	Serious adverse event
SAR	Suspected adverse reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SCID	Severe combined immunodeficiency
SE	Standard error
SERF	Safety expedited report form
SMM	Sponsor medical monitor
SNP	Single nucleotide polymorphism
SOA	Schedule of activities
SRCP	Safety review and communications plan
std	Standard deviation
SUSAR	Serious and unexpected suspected adverse reaction
Tfh	T follicular helper
UP	Unanticipated problem
UPnonAE	Unanticipated problem that is not an adverse event
US	United States
UTR	Untranslated region
VEIU	Viral Epidemiology and Immunity Unit
YFV	Yellow fever virus

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Appendix 1. Grading the Severity of Adverse Events

The FDA Guidance for Industry (September 2007), "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" is the basis for the severity grading of AEs in this protocol. Several modifications to the table have been made:

- "Emergency room visit" is not automatically considered a life-threatening event; these words have been removed from any "grade 4' definition where they appear in the table copied from the guidance document.
- Any laboratory value shown as a "graded" value in the table, that is within the institutional normal range, will not be severity graded or recorded as an adverse event.
- Severity grading for hemoglobin decrease, on the basis of the magnitude of decrease from baseline is not applicable at the grade 1 level; only absolute hemoglobin will be used to define grade 1.
- Severity grading definition for grade 4 local reaction to injectable product (Erythema/Redness and Induration/Swelling) includes added text "requiring medical attention."
- Bruising is added to the table of local reactions to injectable product with the same grading criteria as erythema/redness except for a grade 4 event, which is a hematoma requiring medical attention.
- Chills, arthralgias, retro-orbital pain, photophobia, dengue rash are added to the systemic events section with the same grading as fatigue and myalgia.

When not otherwise specified in the table, the following guidance will be used to assign a severity grade:

Grade 1 (Mild): No effect on activities of daily living

Grade 2 (Moderate): Some interference with activity not requiring medical intervention

Grade 3 (Severe): Prevents daily activity and requires medical intervention

Grade 4 (Life-threatening): Hospitalization; immediate medical intervention or therapy required to prevent death.

Grade 5 (Death): Death is assigned a Grade 5 severity.

Only the single AE that is assessed as the primary cause of death should be assigned a "grade 5" severity.

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials

Modified from FDA Guidance - September 2007

A. Tables of Clinical Abnormalities

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life - Threatening (Grade 4)**
Injection site pain	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Injection site tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Hospitalization
[1] Injection site erythema/Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis requiring medical attention
[1] Injection site bruising	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Hematoma requiring medical attention
[2] Injection site induration/swelling	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis requiring medical attention
Injection site pruritus	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Pruritis requiring medical attention
[3]Vital Signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life - Threatening (Grade 4)**
[4] Fever (°C) (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104

Tachycardia - beats per minute	101 – 115	116 – 130	> 130	Hospitalization for arrhythmia
[5] Bradycardia - beats per Minute	50 – 54	45 – 49	< 45	Hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	Hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	Hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89 and	80 – 84 and	< 80 and	Hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

[1]In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[2]Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

[3]Participant should be at rest for all vital sign measurements.

[4]Oral temperature: no recent hot or cold beverages or smoking.

[5]When resting heart rate is between 60-100 beats per minute, use clinical judgement when characterizing bradycardia among some healthy participant populations, for example, conditioned athletes or those who engage in regular aerobic exercise.

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life - Threatening (Grade 4)**
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	Hospitalization
Dyspnea	Dyspnea on exertion	Dyspnea with normal activity	Dyspnea at rest	Hospitalization
Nausea	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Vomiting	No interference with activity or 1 – 2 episodes/24 hours, may require 1 dose of medication/treatment	Some interference with activity or > 2 episodes/24 hours or requires >1 dose of medication/treatment	Prevents daily activity, requires outpatient IV hydration	Hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gm/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	Hospitalization
Headache	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Retro-Orbital Pain (ROP)	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization

Photophobia	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Chills	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Fatigue	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Myalgia	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Arthralgia	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization
Rash	Rash is present but asymptomatic	Rash is symptomatic (pruritus/pain) but does not interfere with function	Rash is symptomatic and interferes with function	Hospitalization
Hemorrhage, blood loss	Mildly symptomatic, no therapy required	Gross blood loss or 1-2 units transfused	Massive blood loss or > 2 units transfused	Hospitalization
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity, may require 1 dose of medication/treatment	Some interference with activity or requires >1 dose of medication/treatment	Prevents daily activity and requires medical intervention	Hospitalization

B. Tables for Laboratory Abnormalities

Serum*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life - Threatening (Grade 4)*** **
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting mg/dL Random mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Elevated Creatinine mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – Hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – Hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – Hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – Hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK mg/dL	1.25–1.5 x	>1.5 – 3.0 x	>3.0 –10 x	> 10 x ULN

	ULN**	ULN	ULN	
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate, increase by factor	1.1 – 2.0 x ULN	>2.0 – 3.0 x ULN	>3.0 – 10 x ULN	> 10 x ULN
Elevated Liver Function Tests – ALT, AST, increase by factor	1.1 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10 x ULN	> 10 x ULN
Serum*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life - Threatening (Grade 4)*****
Bilirubin – when accompanied by any increase in Liver Function Test, increase by factor (hyperbilirubinemia)	1.1 – 1.25 x ULN	>1.25 – 1.5 x ULN	>1.5 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor (hyperbilirubinemia)	1.1 – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Any laboratory value shown as a "graded" value in the table that is within the institutional normal range will not be severity graded or recorded as an AE.

**ULN, upper limit of the normal range

***The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within the Grade 3 parameter should be recorded as Grade 4 hyponatremia event if the participant had a new seizure associated with the low sodium value.

Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life –Threatening (Grade 4)**
Anemia - hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Change in hemoglobin (Female) decrease from baseline value - gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
Anemia - Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Change in hemoglobin (Male) decrease from baseline value – gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
Leukocytosis – WBC cell/mm3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
Leukopenia- WBC cell/mm3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphopenia- Lymphocytes cell/mm3	750 – 1,000	500 – 749	250 – 499	< 250
Neutropenia- Neutrophils Decrease - cell/mm3	1,000 – 1,499	750 - 999	500 – 749	< 500
Eosinophilia- Eosinophils - cell/mm3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life - Threatening (Grade 4)**
Thrombocytopenia - Platelets - cell/mm3	100,000 – 120,000	75,000 – 99,999	25,000 – 74,999	< 25,000

PT – increase by factor (prothrombin time)	1.10 x ULN**	>1.1 – 1.25 x ULN	>1.25 – 1.5 x ULN	> 1.5 ULN
PTT – increase by factor (partial thromboplast in time)	1.10 – 1.4 x ULN	>1.4 – 1.6 x ULN	>1.6 – 2.33 x ULN	> 2.33 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)
*The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Any laboratory value shown as a "graded" value in the table that is within the institutional normal range will not be severity graded or recorded as an AE. **ULN, upper limit of the normal range				