Table of Contents

***Supplemental Table 1*: Ineligibility summary of screen failures**

Inclusion/ Exclusion Category	Inclusion/Exclusion Criterion	# Times Marked Ineligible
Inclusion and Exclusion	Number of participants failing any eligibility criterion	32
Inclusion	Any inclusion criterion	24
	Ability and willingness to provide informed consent	6
	Available for the duration of the trial	14
	Good general health as shown by medical history, physical exam, and screening laboratory tests.	2
	Meets laboratory parameters for hematology, chemistry, and urinalysis	2
Exclusion	Any exclusion criterion	10
	Angioedema within the last 3 years if episodes are considered serious or have required medication within the last 2 years	1
	Any condition for which participation would not be in the best interest of the subject (e.g., compromise the well-being) or that could prevent, limit, or confound the study endpoints.	4
	Known autoimmune disease	1
	Malignancy	1
	Planned travel to areas with active Zika virus transmission during the study period	1
	Positive serology for HIV-1, Hepatitis B surface antigen, or anti-Hepatitis C virus antibodies prior to enrollment.	1
	Psychiatric condition that compromises safety of the participant or precludes compliance with the protocol, specifically excluding persons with psychoses within the past 3 years, ongoing risk for suicide.	1

Supplemental Table 2: **Overall safety summary table by treatment group**

		Schedule 1 (N=10)		**Schedule 2 (N=10)**		**Schedule 3 (N=10)**		**All ZPIV (N=30)**		**Placebo (N=6)**	
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Solicited Symptoms	Any Solicited Symptom	10	100	8	80	10	100	28	93·3	5	83·3
	Any Systemic Symptom	8	80	7	70	9	90	24	80	4	66·7
	Any Local Symptom	10	100	8	80	8	80	26	86·7	3	50
Unsolicited Adverse Events	Any AE	6	60	2	20	5	50	13	43·3	4	66·7
	Any Related AE	0	0	0	0	0	0	0	0	0	0
	Any SAE	0	0	0	0	0	0	0	0	0	0
	Any Related SAE	0	0	0	0	0	0	0	0	0	0
Withdrawals	Any Early Withdrawal	2	20	2	20	0	0	4	13·3	1	16·7
	Early Withdrawal Related to AE	0	0	0	0	0	0	0	0	0	0

Supplemental Table 3: **Number and Percentage of Participants Experiencing Solicited Events Following Dose 1 by Symptom, Maximum Severity, and Treatment Group – Systemic Symptoms**

		Schedule 1 (N=10)			Schedule 2 (N=10)			Schedule 3 (N=10)			All ZPIV (N=30)			Placebo (N=6)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Any Systemic Symptom	None	3	30	6.7, 65.2	3	30	6.7, 65.2	1	10	0.3, 44.5	7	23	9.9, 42.3	2	33	4.3, 77.7
	Mild	5	50	18.7, 81.3	6	60	26.2, 87.8	6	60	26.2, 87.8	17	57	37.4, 74.5	4	67	22.3, 95.7
	Moderate	2	20	2.5, 55.6	1	10	0.3, 44.5	3	30	6.7, 65.2	6	20	7.7, 38.6	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Abdominal Pain	None	9	90	55.5, 99.7	10	100	69.2, 100.0	8	80	44.4, 97.5	27	90	73.5, 97.9	5	83	35.9, 99.6
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	2	20	2.5, 55.6	2	7	0.8, 22.1	1	17	0.4, 64.1
	Moderate	1	10	0.3, 44.5	0	0	0.0, 30.8	0	0	0.0, 30.8	1	3	0.1, 17.2	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Arthralgia/Joint Pain	None	9	90	55.5, 99.7	10	100	69.2, 100.0	9	90	55.5, 99.7	28	93	77.9, 99.2	6	100	54.1, 100.0
	Mild	1	10	0.3, 44.5	0	0	0.0, 30.8	1	10	0.3, 44.5	2	7	0.8, 22.1	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Diarrhea	None	8	80	44.4, 97.5	10	100	69.2, 100.0	7	70	34.8, 93.3	25	83	65.3, 94.4	6	100	54.1, 100.0
	Mild	2	20	2.5, 55.6	0	0	0.0, 30.8	3	30	6.7, 65.2	5	17	5.6, 34.7	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Fatigue	None	5	50	18.7, 81.3	6	60	26.2, 87.8	5	50	18.7, 81.3	16	53	34.3, 71.7	6	100	54.1, 100.0
	Mild	4	40	12.2, 73.8	3	30	6.7, 65.2	3	30	6.7, 65.2	10	33	17.3, 52.8	0	0	0.0, 45.9
	Moderate	1	10	0.3, 44.5	1	10	0.3, 44.5	2	20	2.5, 55.6	4	13	3.8, 30.7	0	0	0.0, 45.9

		Schedule 1 (N=10)			Schedule 2 (N=10)			Schedule 3 (N=10)			All ZPIV (N=30)			Placebo (N=6)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Feverishness	None	10	100	69.2, 100.0	9	90	55.5, 99.7	8	80	44.4, 97.5	27	90	73.5, 97.9	5	83	35.9, 99.6
	Mild	0	0	0.0, 30.8	1	10	0.3, 44.5	1	10	0.3, 44.5	2	7	0.8, 22.1	1	17	0.4, 64.1
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	1	10	0.3, 44.5	1	3	0.1, 17.2	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Headache	None	5	50	18.7, 81.3	7	70	34.8, 93.3	5	50	18.7, 81.3	17	57	37.4, 74.5	5	83	35.9, 99.6
	Mild	4	40	12.2, 73.8	3	30	6.7, 65.2	3	30	6.7, 65.2	10	33	17.3, 52.8	1	17	0.4, 64.1
	Moderate	1	10	0.3, 44.5	0	0	0.0, 30.8	2	20	2.5, 55.6	3	10	2.1, 26.5	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Malaise	None	8	80	44.4, 97.5	8	80	44.4, 97.5	7	70	34.8, 93.3	23	77	57.7, 90.1	5	83	35.9, 99.6
	Mild	2	20	2.5, 55.6	2	20	2.5, 55.6	1	10	0.3, 44.5	5	17	5.6, 34.7	1	17	0.4, 64.1
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	2	20	2.5, 55.6	2	7	0.8, 22.1	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Myalgia/Body Aches	None	9	90	55.5, 99.7	4	40	12.2, 73.8	7	70	34.8, 93.3	20	67	47.2, 82.7	5	83	35.9, 99.6
	Mild	0	0	0.0, 30.8	5	50	18.7, 81.3	3	30	6.7, 65.2	8	27	12.3, 45.9	1	17	0.4, 64.1
	Moderate	1	10	0.3, 44.5	1	10	0.3, 44.5	0	0	0.0, 30.8	2	7	0.8, 22.1	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Nausea	None	9	90	55.5, 99.7	9	90	55.5, 99.7	7	70	34.8, 93.3	25	83	65.3, 94.4	6	100	54.1, 100.0
	Mild	1	10	0.3, 44.5	1	10	0.3, 44.5	3	30	6.7, 65.2	5	17	5.6, 34.7	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9

		Schedule 1 (N=10)			Schedule 2 (N=10)			Schedule 3 (N=10)			All ZPIV (N=30)			Placebo (N=6)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Rash	None	10	100	69.2, 100.0	10	100	69.2, 100.0	9	90	55.5, 99.7	29	97	82.8, 99.9	6	100	54.1, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	1	10	0.3, 44.5	1	3	0.1, 17.2	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Temperature Elevation	None	10	100	69.2, 100.0	10	100	69.2, 100.0	9	90	55.5, 99.7	29	97	82.8, 99.9	6	100	54.1, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	1	10	0.3, 44.5	1	3	0.1, 17.2	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Vomiting	None	10	100	69.2, 100.0	10	100	69.2, 100.0	9	90	55.5, 99.7	29	97	82.8, 99.9	6	100	54.1, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	1	10	0.3, 44.5	1	3	0.1, 17.2	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9

N = Number of participants in the Safety Population who received the specified dose. Severity is the maximum severity reported over all solicited symptoms post dosing for each subject.

Supplemental Table 4: **Number and Percentage of Participants Experiencing Solicited Events Following Dose 1 by Symptom, Maximum Severity, and Treatment Group – Local Symptoms**

		Schedule 1 (N=10)			Schedule 2 (N=10)			Schedule 3 (N=10)			All ZPIV (N=30)			Placebo (N=6)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Any Local Symptoms	None	3	30	6.7, 65.2	2	20	2.5, 55.6	2	20	2.5, 55.6	7	23	9.9, 42.3	4	67	22.3, 95.7
	Mild	6	60	26.2, 87.8	7	70	34.8, 93.3	7	70	34.8, 93.3	20	67	47.2, 82.7	2	33	4.3, 77.7
	Moderate	1	10	0.3, 44.5	1	10	0.3, 44.5	1	10	0.3, 44.5	3	10	2.1, 26.5	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Erythema/Redness Measurement	None	8	80	44.4, 97.5	7	70	34.8, 93.3	8	80	44.4, 97.5	23	77	57.7, 90.1	6	100	54.1, 100.0
	Mild	2	20	2.5, 55.6	3	30	6.7, 65.2	1	10	0.3, 44.5	6	20	7.7, 38.6	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	1	10	0.3, 44.5	1	3	0.1, 17.2	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Induration/Swelling Severity	None	10	100	69.2, 100.0	7	70	34.8, 93.3	10	100	69.2, 100.0	27	90	73.5, 97.9	6	100	54.1, 100.0
	Mild	0	0	0.0, 30.8	3	30	6.7, 65.2	0	0	0.0, 30.8	3	10	2.1, 26.5	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Induration/Swelling Measurement	None	10	100	69.2, 100.0	7	70	34.8, 93.3	10	100	69.2, 100.0	27	90	73.5, 97.9	6	100	54.1, 100.0
	Mild	0	0	0.0, 30.8	3	30	6.7, 65.2	0	0	0.0, 30.8	3	10	2.1, 26.5	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Itching/Pruritus	None	10	100	69.2, 100.0	10	100	69.2, 100.0	9	90	55.5, 99.7	29	97	82.8, 99.9	6	100	54.1, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	1	10	0.3, 44.5	1	3	0.1, 17.2	0	0	0.0, 45.9
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9

		Schedule 1 (N=10)			Schedule 2 (N=10)			Schedule 3 (N=10)			All ZPIV (N=30)			Placebo (N=6)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9
Pain at Injection Site	None	3	30	6.7, 65.2	3	30	6.7, 65.2	3	30	6.7, 65.2	9	30	14.7, 49.4	4	67	22.3, 95.7
	Mild	6	60	26.2, 87.8	6	60	26.2, 87.8	6	60	26.2, 87.8	18	60	40.6, 77.3	2	33	4.3, 77.7
	Moderate	1	10	0.3, 44.5	1	10	0.3, 44.5	1	10	0.3, 44.5	3	10	2.1, 26.5	0	0	0.0, 45.9
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 11.6	0	0	0.0, 45.9

N = Number of participants in the Safety Population who received the specified dose. Severity is the maximum severity reported over all solicited symptoms post dosing for each subject.

Supplemental Table 5: **Number and Percentage of Participants Experiencing Solicited Events Following Dose 2 by Symptom, Maximum Severity, and Treatment Group – Systemic Symptoms**

		Schedule 1 (N=10)			Schedule 2 (N=10)			All ZPIV (N=20)			Placebo (N=4)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Any Systemic Symptom	None	4	40	12.2, 73.8	6	60	26.2, 87.8	10	50	27.2, 72.8	2	50	6.8, 93.2
	Mild	4	40	12.2, 73.8	3	30	6.7, 65.2	7	35	15.4, 59.2	1	25	0.6, 80.6
	Moderate	2	20	2.5, 55.6	1	10	0.3, 44.5	3	15	3.2, 37.9	1	25	0.6, 80.6
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Abdominal Pain	None	10	100	69.2, 100.0	10	100	69.2, 100.0	20	100	83.2, 100.0	3	75	19.4, 99.4
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	1	25	0.6, 80.6
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Arthralgia/Joint Pain	None	10	100	69.2, 100.0	10	100	69.2, 100.0	20	100	83.2, 100.0	4	100	39.8, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Diarrhea	None	10	100	69.2, 100.0	10	100	69.2, 100.0	20	100	83.2, 100.0	4	100	39.8, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Fatigue	None	7	70	34.8, 93.3	8	80	44.4, 97.5	15	75	50.9, 91.3	3	75	19.4, 99.4
	Mild	2	20	2.5, 55.6	1	10	0.3, 44.5	3	15	3.2, 37.9	1	25	0.6, 80.6
	Moderate	1	10	0.3, 44.5	1	10	0.3, 44.5	2	10	1.2, 31.7	0	0	0.0, 60.2

		Schedule 1 (N=10)			Schedule 2 (N=10)			All ZPIV (N=20)			Placebo (N=4)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Feverishness	None	10	100	69.2, 100.0	9	90	55.5, 99.7	19	95	75.1, 99.9	4	100	39.8, 100.0
	Mild	0	0	0.0, 30.8	1	10	0.3, 44.5	1	5	0.1, 24.9	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Headache	None	7	70	34.8, 93.3	9	90	55.5, 99.7	16	80	56.3, 94.3	3	75	19.4, 99.4
	Mild	2	20	2.5, 55.6	1	10	0.3, 44.5	3	15	3.2, 37.9	1	25	0.6, 80.6
	Moderate	1	10	0.3, 44.5	0	0	0.0, 30.8	1	5	0.1, 24.9	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Malaise	None	7	70	34.8, 93.3	8	80	44.4, 97.5	15	75	50.9, 91.3	4	100	39.8, 100.0
	Mild	3	30	6.7, 65.2	2	20	2.5, 55.6	5	25	8.7, 49.1	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Myalgia/Body Aches	None	7	70	34.8, 93.3	6	60	26.2, 87.8	13	65	40.8, 84.6	4	100	39.8, 100.0
	Mild	3	30	6.7, 65.2	3	30	6.7, 65.2	6	30	11.9, 54.3	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	1	10	0.3, 44.5	1	5	0.1, 24.9	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Nausea	None	9	90	55.5, 99.7	9	90	55.5, 99.7	18	90	68.3, 98.8	3	75	19.4, 99.4
	Mild	1	10	0.3, 44.5	0	0	0.0, 30.8	1	5	0.1, 24.9	1	25	0.6, 80.6
	Moderate	0	0	0.0, 30.8	1	10	0.3, 44.5	1	5	0.1, 24.9	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2

		Schedule 1 (N=10)			Schedule 2 (N=10)			All ZPIV (N=20)			Placebo (N=4)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Rash	None	10	100	69.2, 100.0	10	100	69.2, 100.0	20	100	83.2, 100.0	4	100	39.8, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Temperature Elevation	None	10	100	69.2, 100.0	10	100	69.2, 100.0	20	100	83.2, 100.0	4	100	39.8, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Vomiting	None	9	90	55.5, 99.7	10	100	69.2, 100.0	19	95	75.1, 99.9	4	100	39.8, 100.0
	Mild	1	10	0.3, 44.5	0	0	0.0, 30.8	1	5	0.1, 24.9	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2

N = Number of participants in the Safety Population who received the specified dose. Severity is the maximum severity reported over all solicited symptoms post dosing for each subject.

Supplemental Table 6: **Number and Percentage of Participants Experiencing Solicited Events Following Dose 2 by Symptom, Maximum Severity, and Treatment Group – Local Symptoms**

		Schedule 1 (N=10)			Schedule 2 (N=10)			All ZPIV (N=20)			Placebo (N=4)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Any Local Symptoms	None	2	20	2.5, 55.6	3	30	6.7, 65.2	5	25	8.7, 49.1	3	75	19.4, 99.4
	Mild	7	70	34.8, 93.3	6	60	26.2, 87.8	13	65	40.8, 84.6	1	25	0.6, 80.6
	Moderate	1	10	0.3, 44.5	1	10	0.3, 44.5	2	10	1.2, 31.7	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Erythema/Redness Measurement	None	8	80	44.4, 97.5	8	80	44.4, 97.5	16	80	56.3, 94.3	3	75	19.4, 99.4
	Mild	2	20	2.5, 55.6	2	20	2.5, 55.6	4	20	5.7, 43.7	1	25	0.6, 80.6
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Induration/Swelling Severity	None	9	90	55.5, 99.7	8	80	44.4, 97.5	17	85	62.1, 96.8	3	75	19.4, 99.4
	Mild	1	10	0.3, 44.5	2	20	2.5, 55.6	3	15	3.2, 37.9	1	25	0.6, 80.6
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Induration/Swelling Measurement	None	9	90	55.5, 99.7	8	80	44.4, 97.5	17	85	62.1, 96.8	3	75	19.4, 99.4
	Mild	1	10	0.3, 44.5	2	20	2.5, 55.6	3	15	3.2, 37.9	1	25	0.6, 80.6
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Itching/Pruritus	None	10	100	69.2, 100.0	10	100	69.2, 100.0	20	100	83.2, 100.0	4	100	39.8, 100.0
	Mild	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
	Moderate	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2

		Schedule 1 (N=10)			Schedule 2 (N=10)			All ZPIV (N=20)			Placebo (N=4)		
	Severity	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2
Pain at Injection Site	None	2	20	2.5, 55.6	4	40	12.2, 73.8	6	30	11.9, 54.3	4	100	39.8, 100.0
	Mild	7	70	34.8, 93.3	5	50	18.7, 81.3	12	60	36.1, 80.9	0	0	0.0, 60.2
	Moderate	1	10	0.3, 44.5	1	10	0.3, 44.5	2	10	1.2, 31.7	0	0	0.0, 60.2
	Severe	0	0	0.0, 30.8	0	0	0.0, 30.8	0	0	0.0, 16.8	0	0	0.0, 60.2

N = Number of participants in the Safety Population who received the specified dose. Severity is the maximum severity reported over all solicited symptoms post dosing for each subject.

Supplemental Table 7: **Percent Seroconversion for MN50 titer by Vaccination Day and Treatment Group, Immunogenicity Population (MN50 titer ≥ 100)**

Time Point	Statistic	Schedule 1 (N=10)	Schedule 2 (N=10)	Schedule 3 (N=10)	All ZPIV (N=30)	Placebo (N=6)
Pre-Vaccination	n/N (%)	0/10 (0.0%)	0/10 (0.0%)	0/10 (0.0%)	0/30 (0.0%)	0/6 (0.0%)
	95% CI	(0.00, 30.85)	(0.00, 30.85)	(0.00, 30.85)	(0.00, 11.57)	(0.00, 45.93)
2 Weeks Post-Final Vaccine	n/N (%)	10/10 (100.0%)	7/9 (77.8%)	0/10 (0.0%)	17/29 (58.6%)	0/5 (0.0%)
	95% CI	(69.15, 100.00)	(39.99, 97.19)	(0.00, 30.85)	(38.94, 76.48)	(0.00, 52.18)
4 Weeks Post-Final Vaccine	n/N (%)	9/9 (100.0%)	6/10 (60.0%)	0/10 (0.0%)	15/29 (51.7%)	0/5 (0.0%)
	95% CI	(66.37, 100.00)	(26.24, 87.84)	(0.00, 30.85)	(32.53, 70.55)	(0.00, 52.18)
16 Weeks Post-First Vaccine	n/N (%)	3/9 (33.3%)	0/9 (0.0%)	0/10 (0.0%)	3/28 (10.7%)	0/5 (0.0%)
	95% CI	(7.49, 70.07)	(0.00, 33.63)	(0.00, 30.85)	(2.27, 28.23)	(0.00, 52.18)
28 Weeks Post-First Vaccine	n/N (%)	1/8 (12.5%)	0/7 (0.0%)	0/10 (0.0%)	1/25 (4.0%)	0/5 (0.0%)
	95% CI	(0.32, 52.65)	(0.00, 40.96)	(0.00, 30.85)	(0.10, 20.35)	(0.00, 52.18)
40 Weeks Post-First Vaccine	n/N (%)	1/7 (14.3%)	0/6 (0.0%)	0/10 (0.0%)	1/23 (4.3%)	0/5 (0.0%)
	95% CI	(0.36, 57.87)	(0.00, 45.93)	(0.00, 30.85)	(0.11, 21.95)	(0.00, 52.18)
52 Weeks Post-First Vaccine	n/N (%)	1/8 (12.5%)	1/8 (12.5%)	0/10 (0.0%)	2/26 (7.7%)	0/5 (0.0%)
	95% CI	(0.32, 52.65)	(0.32, 52.65)	(0.00, 30.85)	(0.95, 25.13)	(0.00, 52.18)
Observed Peak*	n/N (%)	10/10 (100.0%)	8/10 (80.0%)	0/10 (0.0%)	18/30 (60.0%)	0/5 (0.0%)
	95% CI	(69.15, 100.00)	(44.39, 97.48)	(0.00, 30.85)	(40.60, 77.34)	(0.00, 52.18)

N=Number of participants in immunogenicity population.

Clopper-Pearson method used for calculating confidence intervals.

*Peak result of a participant observed across all visits.

Supplemental Table 8: **MN Geometric Mean Titer (GMT) Results with 95% Confidence Intervals by Vaccination Day and Treatment Group, Immunogenicity Population**

Time Point	Statistic	Schedule 1 (N=10)	Schedule 2 (N=10)	Schedule 3 (N=10)	All ZPIV (N=30)	Placebo (N=6)
Pre-Vaccination	N	10	10	10	30	6
	GMT	5.0	5.0	5.0	5.0	5.0
	95% CI	--	--	--	--	--
2 Weeks Post-Final Vaccine	N	10	9	10	29	5
	GMT	983.3	477.4	6.3	138.0	5.0
	95% CI	[425.5, 2272.5]	[111.7, 2039.8]	[3.7, 10.8]	[50.8, 374.8]	--
4 Weeks Post-Final Vaccine	N	9	10	10	29	5
	GMT	820.5	187.2	5.9	90.1	5.0
	95% CI	[357.0, 1885.8]	[54.6, 641.4]	[4.0, 8.8]	[36.1, 224.9]	--
16 Weeks Days Post-First Vaccine	N	9	9	10	28	5
	GMT	96.1	13.7	5.0	17.9	5.0
	95% CI	[23.1, 399.6]	[7.3, 25.9]	--	[9.4, 34.2]	--
28 Weeks Post-First Vaccine	N	8	7	10	25	5
	GMT	13.9	6.9	5.0	7.6	5.0
	95% CI	[3.5, 55.1]	[4.0, 11.9]	--	[4.9, 11.6]	--
40 Weeks Post-First Vaccine	N	7	6	10	23	5
	GMT	15.4	7.4	5.0	7.8	5.0
	95% CI	[2.2, 105.5]	[3.9, 14.2]	--	[4.6, 13.3]	--
52 Weeks Post-First Vaccine	N	8	8	10	26	5
	GMT	12.4	9.4	5.0	8.0	5.0
	95% CI	[2.8, 54.2]	[3.1, 28.7]	--	[4.9, 13.2]	--
Observed Peak**	N	10	10	10	30	5
	GMT	1153.9	517.7	6.3	155.8	5.0
	95% CI	[455.2, 2925.2]	[142.9, 1875.6]	[3.7, 10.8]	[57.5, 422.0]	--

N=Number of participants in immunogenicity population.

*Peak result of a participant observed across all visits

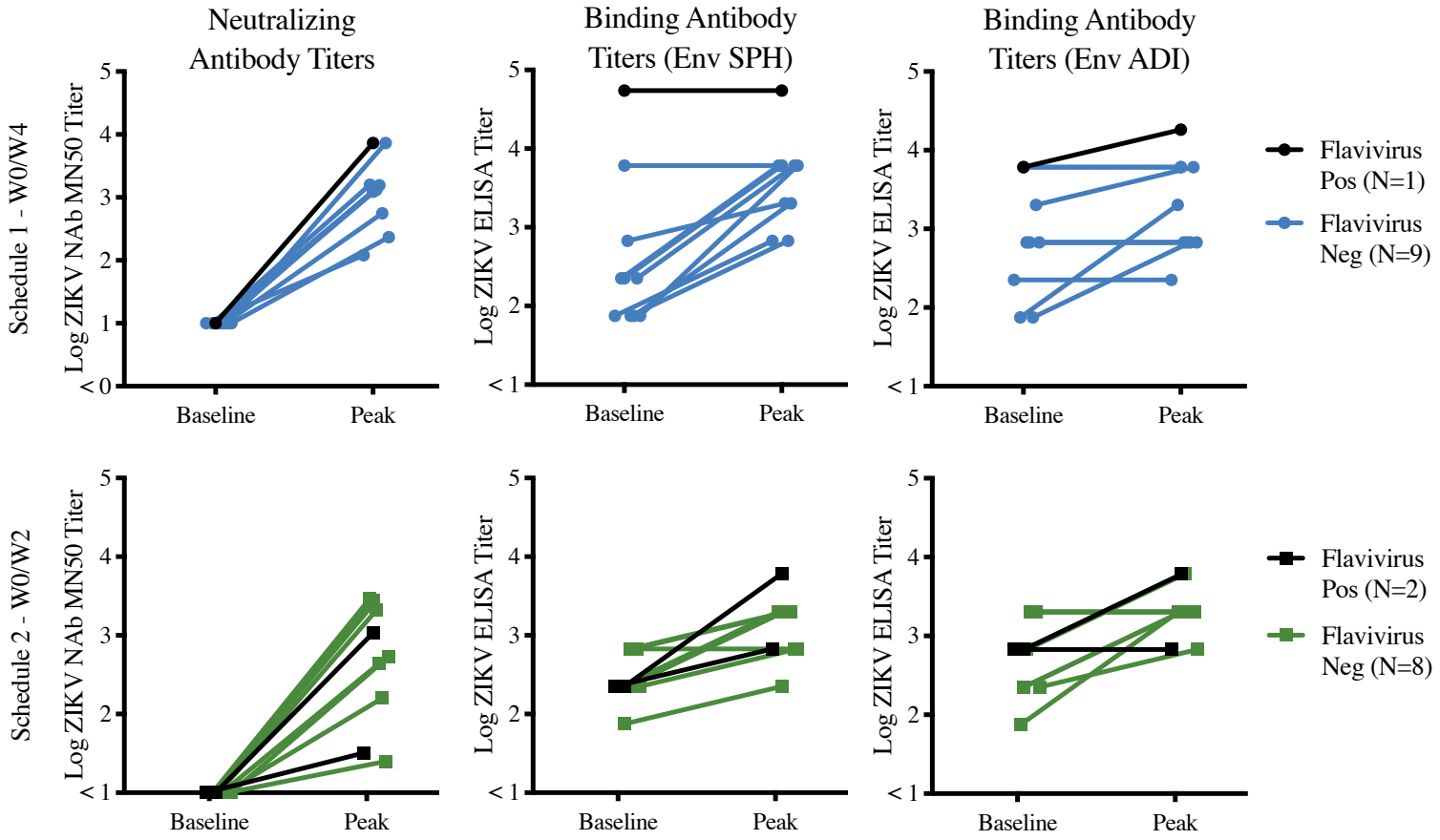
Supplemental Table 9: **Comparison of MN Geometric Mean Titer (GMT) by Treatment Group – Pairwise Comparison**

Time Point	Comparison	P-value**
Observed Peak*	ZPIV Schedule 1 vs. 2	0·4494
	ZPIV Schedule 1 vs. 3	<0·0001
	ZPIV Schedule 1 vs. Placebo	0·0018
	ZPIV Schedule 2 vs. 3	0·0001
	ZPIV Schedule 2 vs. Placebo	0·0018
	ZPIV Schedule 3 vs. Placebo	0·4795

N=Number of participants in immunogenicity population.

*Peak result of a participant observed across all visits

**p-value is calculated via Wilcoxon rank sum test without multiple comparison adjustment



Supplemental Figure 1: Humoral immune responses against Zika Virus (ZIKV) following vaccination by pre-existing flavivirus immune status. Log MN50 and ELISA titers by treatment group (Schedule 1 and 2 only) stratified by pre-existing flavivirus immunity status. Binding antibody titers are against ZIKV Env antigens from SPH2015 strain and ADI kit.

Protocol Title:	A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Flavivirus-Naïve, Healthy Adults
Short Title:	Zika Virus Purified Inactivated Vaccine (ZPIV) Accelerated Vaccination Schedule Study
Protocol Number:	Z001
Phase:	Phase 1
Sponsor:	Beth Israel Deaconess Medical Center Boston, MA
Sponsor Status:	Not for-Profit Organization
Study Location:	Center for Virology and Vaccine Research Clinical Trials Unit, Beth Israel Deaconess Medical Center
Study Period:	October 2016 – November 2017
Collaborating Institutions:	Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA, Vaccine Research Center/National Institute of Allergy and Infectious Diseases (VRC/NIAID), Bethesda, MD, USA
Date of Protocol Version:	August 3, 2016 1.0

TABLE OF CONTENTS

List of Abbreviations

AE	Adverse event
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCVI	Antibody-dependent cell-mediated virus inhibition
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotranferase
AST	Aspartate aminotransferase
BIDMC	Beth Israel Deaconess Medical Center
CBC	Complete blood count
$CD4^+$	A functional subclass of T cells, helper T lymphocytes (Th), that are necessary for augmentation and coordination of innate and adaptive effector responses, humoral and cellular
$CD8^+$	Cytotoxic T-cells that destroy host cells that have become infected by viruses or other intracellular pathogens
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CRF	Case report form
CTL	Cytotoxic T lymphocyte
CVVR	Center for Virology and Vaccine Research
DNA	Deoxyribonucleic acid
DMC	Data Management Center
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FDA	US Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMS	Global Medical Safety
HIV-1	Human immunodeficiency virus, type 1
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICF	Informed Consent Form
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
mL	Mililiters
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PI	Principal investigator
PO	Per Oral
PSRT	Protocol Safety Review Team
RBC	Red blood cell count
rtPCR	Real-time PCR
SAE	Serious adverse event
SMC	Safety Monitoring Committee
SST	Serum separating tubes
SUSAR	Suspected unexpected serious adverse reaction
$TCID_{50}$	50% Tissue Culture Infective Dose
VP	Viral particles

WBC	White blood cell count
ZPIV	Zika Virus Purified Inactivated Vaccine

1 OVERVIEW

Title

A Phase 1, Randomized, Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine in Flavivirus-Naïve, Healthy Adults

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 90, 180, 270, and 365 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

Study Products and Routes of Administration

- **Zika Virus Purified Inactivated Vaccine (ZPIV) with Alum Adjuvant:** Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at the manufacturing facility prior to shipment to clinic.
- **Placebo**: The placebo product is saline.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV	ZPIV		
	B	2		Placebo	Placebo		
4	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		48					

Participants

Healthy, flavivirus-naive participants aged 18-50 years:

- 40 vaccinees
- 8 placebo recipients
- 48 total participants

Design

Single center, randomized, controlled, double-blind phase 1 trial

Duration per participant

12 months active follow-up per participant

Estimated total study duration

18 months (includes screening and active follow-up)

Investigational New Drug (IND) Sponsor

Beth Israel Deaconess Medical Center, Boston, MA, USA

Vaccine Manufacturer

Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA, Vaccine Research Center

Data Management Center (DMC)

The EMMES Corporation, Rockville, MD, USA

Endpoint Assay Laboratories

Center for Virology and Vaccine Research (CVVR), Beth Israel Deaconess Medical Center (BIDMC), Boston, MA, USA

Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA

Vaccine Research Center, National Institute of Allergy and Infectious Diseases (VRC/NIAID), Bethesda, MD, USA

Study Site

Center for Virology and Vaccine Research Clinical Trials Unit, Beth Israel Deaconess Medical Center, Boston, MA, USA

Safety Monitoring

Protocol Safety Review Team (PSRT)
Safety Monitoring Committee (SMC)

2 BACKGROUND AND RATIONALE

2.1 Introduction

Zika virus (ZIKV) is an emerging vector-borne RNA virus that, since early 2015, has caused an increased incidence of systemic disease and neurologic complications in an expanded region of the Western Hemisphere. ZIKV, of the family *Flaviviridae,* is related to other pathogens of global importance to humans, including dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV) and tick-borne encephalitic virus (TBEV). ZIKV was first isolated in Uganda in 1947 from a sentinel rhesus macaque and has since diverged along two lineages: African and Asian. The virus is almost exclusively transmitted by the *Aedes aegypti* mosquito but has been isolated from several other species of the genus *Aedes,* including *A. albopictus, A. africanus* and *A. luteocephalus*. The majority of human ZIKV infections result in an asymptomatic infection or a benign, self-limited acute febrile illness. ZIKV disease typically manifests as a rash, fever, conjunctivitis and arthralgia. Until 2007 when several outbreak clusters erupted in the South Pacific, ZIKV disease was relatively rare and geographically limited. Between 2007 and 2016, however, Zika has been documented in 60 countries and territories, 46 of which recorded their first case in either 2015 or 2016.

A clinical pattern has emerged from the most recent outbreaks that was never observed before. Nine months after local ZIKV transmission was first documented in Brazil, public health officials detected an increase in neonates born with microcephaly in the northeastern part of the country (WHO 2016). Epidemiologic studies, together with *in vitro* and *in vivo* experiments, have confirmed a causal association between ZIKV infections during pregnancy and the consequent occurrence of serious birth defects including microcephaly, brain malformations and ocular defects (Rasmussen SA, 2016). An increase in Guillain-Barré syndrome (GBS), meningitis and encephalitis have also been found in people with primary ZIKV infections (Cao-Lormeau, 2016) (Carteaux G, 2016), (Broutet N, 2016). The neuro-invasiveness of Zika virus infection and its complications ultimately prompted the World Health Organization (WHO) to declare, in February 2016, the emerging ZIKV epidemic as Public Health Emergency of International Concern.

2.2 Rationale for developing and testing a Zika Virus Purified Inactivated Vaccine (ZPIV)

There is no specific treatment or prophylactic currently available for ZIKV disease, other than supportive care and mosquito control. Given the expanding distribution and significant morbidity associated with ZIKV disease, the development of a safe and effective vaccine against ZIKV is a high global public health priority. The ZIKV vaccine to be used in this trial is a formalin-inactivated whole virus vaccine that was produced by the Walter Reed Army Institute of Research (WRAIR). There has been extensive experience with formalin-inactivated vaccines for other viruses, including other flaviviruses, such as the safe and immunogenic experimental dengue purified inactivated vaccine (Martinez 2015) and the licensed IXIARO® Japanese encephalitis vaccine. Experience with these other flavivirus vaccines has informed the design and development of the current ZIKV vaccine. However, the safety, tolerability and immunogenicity of a ZIKV vaccine, including the inactivated vaccine, is still to be determined. Because the safety and immunogenicity profile of this vaccine is still unknown, this Phase I trial, in concert with three others, will address a critical gap necessary to complete an early and rapid assessment of the viability of this ZIKV vaccine candidate. The first two clinical trials will explore the safety and immune response to the vaccine in flavivirus naïve individuals (National

Institutes of Health (NIH) and in participants who have been previously vaccinated with JEV or YFV vaccines (WRAIR). The third trial (NIH) will be conducted in Puerto Rico, whose population has background exposure to dengue virus and potentially yellow fever and West Nile virus as well. The dose and regimen that will be utilized in these initial trials was selected based on past experience with other inactivated flavivirus vaccines. Potential risks related to ZIKV vaccine administration may be similar to those of other inactivated flavivirus vaccines. Two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine developed by WRAIR and a JEV vaccine (IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated.

In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting and fever with the last three AEs occurring after the second administration of the 5 mcg dose. Unsolicited AEs detected among subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

2.3 Rationale for studying an accelerated vaccine schedule

The Phase 1 testing of a one- or two-dose ZPIV vaccine for Zika virus along a compressed schedule will complement the three other Phase 1 trials that are testing a standard prime-boost schedule of 0 and 4 weeks. This trial will test the WRAIR-developed vaccine in 48 healthy adults according to either a one-dose vaccination regimen or two-dose schedules of 0/2 weeks and 0/4 weeks. Investigation into the immunogenicity of a single dose or accelerated schedule of vaccination is salient to vaccine deployment in the setting of an emergent outbreak. The more rapidly a vaccine schedule confers immunity, the better chance an individual has of being protected from disease during the outbreak and the higher likelihood that transmission can be interrupted within populations. An accelerated schedule could not only benefit general populations living in Zika-endemic areas but first responders who need to be rapidly deployed to those areas. Emergency health responders and military personnel would potentially benefit from a shorter vaccination schedule that confers protection equivalent to that afforded by a standard, but more prolonged 1-month regimen. This study will evaluate those abbreviated schedules.

3 PRECLINICAL EXPERIENCE

3.1. *In vitro* characterization of ZPIV

The ZIKV vaccine is formalin-inactivated whole virus vaccine using a 2015 isolate from Puerto Rico (PRVABC59 V3 strain). The vaccine was produced in VERO cells and manufactured via a process that has been used successfully for other flavivirus vaccines (*e.g.* JEV vaccine). The clinical material used to develop the ZPIV was manufactured in Vero cells cultured in medium containing heat inactivated fetal bovine serum, Neomycin, and Streptomycin. Following infection with ZIKV (Puerto Rico PRVABC59 strain), culture supernatants were collected, and clarified by centrifugation and filtration (0.45 µm followed by 0.22 µm). The clarified viral fluids were treated with Benzonase to remove cellular DNA, and then concentrated by ultrafiltration followed filtration (0.45 µm). The concentrated virus was purified by column chromatography using a Capto™Core 700 column. The virus-containing fractions were pooled. The purified pool was filtered (0.22 µm). Sucrose was added and the virus was inactivated for 7 days at 22 °C with 0.05% formalin (1:2000 dilution of 37% formaldehyde). On day 2 of the inactivation, the virus was filtered (0.22 µm). Following inactivation, the formalin-treated virus pool was filtered (0.22 µm), concentrated to 50 µg/mL, and diafiltered to remove residual formaldehyde. Sucrose in PBS was added to a final concentration of 3% sucrose. The bulk was sterile filtered with a 0.22 sterile in-line filter. Inactivation was confirmed by inoculation of Vero cells. The purified inactivated virus (PIV) was then adsorbed to aluminum hydroxide (Alhydrogel®), which will be used to further dilute the adjuvanted vaccine to attain the moderate and low doses (i.e., 2.5 mcg and 1.25 mcg, respectively). Use of alum as an adjuvant is well established and generally well tolerated.

3.2 *In vivo* characterization of ZPIV

ZPIV has been tested in both mouse and rhesus monkey models. A single dose of purified inactivated virus vaccine induced high titers of ZIKV neutralizing antibodies and conferred complete protection, as measured by viremia, in both susceptible mice and nonhuman primates against challenge with a ZIKV strains from Brazil and Puerto Rico. Purified IgG from vaccinated animals also conferred passive protection in adoptive transfer studies in mice. CD4 and CD8 T lymphocyte depletion in vaccinated mice did not abrogate protective efficacy. These data demonstrate that protection against ZIKV challenge can be achieved by single-dose ZPIV and that humoral immunity alone is sufficient for that protection. These studies were carried out with non-GLP vaccine material. Overall, no clinical adverse events or clear changes in hematologies or chemistries were observed in rhesus monkeys following ZIKV PIV vaccination at weeks 0 and 4 (Larocca 2016) (Abbink 2016).

4 CLINICAL EXPERIENCE

4.1 Clinical experience with ZPIV in humans

While there is no human clinical experience with the ZIKV vaccine, two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine (WRAIR) and a JEV vaccine (manufactured as IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated. In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting, and fever with the last three AEs occurring after the second administration of the 5 mcg dose. Unsolicited AEs detected among subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

A formaldehyde-inactivated JEV vaccine (IXIARO®) has been licensed for use in the US since 2009. The package insert indicates that the most common adverse reactions reported were headache, myalgia, influenza-like illness, and fatigue (Valnera Austria GmBH, 2015). No effect was seen on the safety profile of IXIARO® compared to placebo (aluminum hydroxide) when examined by age, sex, or ethnic origin. In a Phase 3 clinical trial involving 2675 subjects who received either two doses of IXIARO® (6 mcg) or placebo (phosphate buffered saline [PBS] plus aluminum hydroxide), there was little difference between the active and placebo groups (Valnera Austria GmBH, 2015). The overall percentage of subjects who experienced at least one AE was 59% in the active group versus 57% in the placebo. Injection site reactions were mild to moderate in severity, and consisted of (in descending order of occurrence) pain, tenderness, erythema, induration, edema and pruritus. The most common systemic AEs post first dose and second dose were headache, myalgia, fatigue, influenza-like illness, nausea, nasopharygnitis, and fever. Sixteen SAEs were reported in 10 subjects who received vaccine and 6 subjects who received placebo. The SAEs that occurred among subjects in the IXIARO® group were dermatomyositis, appendicitis, rectal hemorrhage, limb abscess involving contralateral arm, chest pain, ovarian torsion, ruptured corpus luteal cyst, and three orthopedic injuries. No deaths occurred in the trial. IXIARO® is also licensed at one half the dose (3 mcg) for infants and children aged 2 months to <3 years; the full strength is recommended for use in older children. Fever was the most commonly observed AEs up to 12 years of age.

In summary, inactivated flavivirus vaccines appear to be reasonably well tolerated. However, typical safety considerations with inactivated flavivirus vaccines include local reactions of pain, tenderness, erythema, induration, edema and pruritus, and mild to moderate systemic reactions including headache, myalgia, fatigue, influenza-like illness, nausea, and fever.

5 OBJECTIVES

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 90, 180, 270, and 365 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

6 STUDY DESIGN

This is a phase 1 trial of one or more administrations of Zika Virus Purified Inactivated Vaccine (ZPIV). The trial will be conducted under a placebo controlled, double-blind, randomized allocation of study product. This design is intended to reduce the likelihood of observer bias, provide control for confounding variables of intercurrent illness, and aid in the interpretation of laboratory data. There are four groups in the study and blinding will be maintained to vaccine or placebo within each group as noted in the table below.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV	ZPIV		
	B	2		Placebo	Placebo		
4	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		48					

It is anticipated that enrollment will be completed within a 6-month period, and that participants will be actively followed for 12 months. Groups 1 through 4 will be enrolled in sequence to accelerate the collection of peak immunogenicity time-points from the groups with longer schedules (peak time-points are D56, D42, D35, and D28 for Groups 1-4, respectively).

6.1 Safety Assessment

To assess the safety of the administered vaccine, participants will document local and systemic reactogenicity on the first day of each vaccine administration period (days 0, 7, 14, and/or 28 depending on the group assignment) and for the subsequent 6 days following each vaccine administration. The investigator will interview each participant about AEs at each visit throughout the study. Two to 3 days after each vaccine administration, a member of the site staff will have a remote safety follow-up communication with the participant by telephone. The participant will be questioned about occurrence of reactogenicity. The participant will be brought in for a clinic visit based on this assessment, if deemed necessary by the investigator/sub-investigator or upon request of the participant. All participants will also be seen in clinic at the end of each reactogenicity period (days 7, 14, 21, and/or 35 depending on the group assignment). All reactogenic events and AEs will be recorded on case report forms (CRFs) from the signing of the informed consent form (ICF) until the last study visit. To assess the participants' cellular and humoral immune response, blood samples will be taken at the clinic visits. Safety and immunogenicity visits will be conducted as outlined in the Schedule of Procedures (SOP).

6.2 Immunogenicity Assessment

Blood samples will be obtained at various time points after immunization (Section 18.3). These will be assayed for the magnitude of neutralizing and binding antibody titers to Zika virus at 14 and 28 days following each administration of ZPIV, as well as at 90, 180, 270 and 365 days following the first dose of ZPIV. The primary readout for neutralizing antibody assays will be the results of pseudovirus reporter and microneutralization assays. The secondary readout will be the result of plaque reduction neutralization assays. The third readout will be binding antibody titers by ELISA. Exploratory assessments may include epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, and Zika-specific T cell responses.

6.3 Monitoring

The Protocol Safety Review Team (PSRT) will review all adverse events (AEs) on a regular and expedited basis as needed. In addition, the PSRT will review safety data reports on a weekly basis until 12 participants have been enrolled after which reports will be reviewed biweekly. The PSRT will include the Study Chair(s), co-investigators, and representatives from WRAIR and VRC/NIH. Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs should also be reviewed by the Safety Monitoring Committee (SMC). The SMC will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC will be completely independent from the protocol team and sponsor. The SMC will evaluate safety and tolerability data on a regular basis. The SMC may review an individual SAE or it may choose to review AEs, SAEs, and laboratory and vital sign data. The SMC may unblind any amount of safety information needed to conduct their assessment. The conclusions of the SMC will be communicated to the investigators and the IRB/Ethics Committees and the national regulatory authorities as appropriate. The sponsor agrees to abide by the decision of the SMC and any directives issued by the national regulatory authorities, the Institutional Review Boards or Ethics Committees.

6.4 Randomization Procedures and Enrollment

Participants will be enrolled in the study after ascertainment by one of the study investigators that all of the inclusion and none of the exclusion criteria have been met. Once confirmation of eligibility for the trial has been performed, the participant will be randomized. Randomized treatment assignments will be generated by the Data Management Center (DMC). After successful randomization, an allocation number is provided. The DMC will create the randomization table and the sponsor will monitor the implementation of this process. The allocation number is referenced against a confidential list provided to an unblinded on-site pharmacist to determine the assignment/treatment allocation.

The 12 participants in each group will be randomized in a ratio of 5 vaccinees to 1 placebo recipient.

6.7 Method of Blinding and Unblinding

The participants, clinical staff, investigator, and sponsor personnel will be blinded to treatment allocation throughout the study. The pharmacist with primary responsibility for vaccine

dispensing will not be blinded to the treatment and will maintain the randomization code and complete assignments of participants according to the randomization allocation. Routine unblinding of treatment allocations may occur only after all participants have had their last study visit and the database is locked.

7 STUDY POPULATION

The study population will include healthy men or women aged 18-50 years old who are able and willing to provide written informed consent. Participants will be enrolled in the study once eligibility criteria are met.

7.1 Participant Inclusion Criteria

1. Age 18-50 years old.

2. Ability and willingness to provide informed consent.

3. Assessment of understanding: completion of a questionnaire prior to first screening procedure; verbally demonstrate understanding of all questionnaire items answered incorrectly.

4. Available for the duration of the trial.

5. Good general health as shown by medical history, physical exam, and screening laboratory tests.

6. The following laboratory parameters:

    - Hematology
        - Hemoglobin ≥10.5 g/dL for women; ≥11 g/dL for men
        - Absolute Neutrophil Count (ANC): ≥1000/mm^3
        - Platelets: 125,000 to 550,000/mm^3
    - Chemistry
        - Creatinine: <1.1 x upper limit of normal (ULN)
        - AST: <1.25 x ULN
        - ALT: <1.25 x ULN
    - Normal urinalysis
        - Negative urine glucose.
        - Negative or trace urine protein.
        - Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis within institutional range).

7. All female participants must be willing to undergo serum or urine beta human chorionic gonadotropin pregnancy tests at time points indicated in the Schedule of Procedures and must test negative prior to vaccination.

8. All sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until Week 12.

9. If a woman of child-bearing potential, committed to use an effective method of contraception when sexually active with men until Week 12, including:

- Condoms (male or female) with or without spermicide.
- Diaphragm or cervical cap with spermicide.
- Intrauterine device.
- Hormonal contraception.
- Successful vasectomy in the male partner (considered successful if a woman reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity post-vasectomy).
- Not be of reproductive potential, such as having undergone hysterectomy, bilateral oophorectomy, or tubal ligation.

Participant Exclusion Criteria:

1. History of flavivirus infection or previous receipt of flavivirus vaccine.

2. Positive serology for flaviviruses (all four dengue virus serotypes, Japanese encephalitis, Yellow fever virus, and West Nile virus), HIV-1, Hepatitis B surface antigen, or anti-hepatitis C virus antibodies prior to enrollment.

3. Planned travel to areas with circulating Zika virus during the study period.

4. Recent (within 3 weeks) travel to a Zika endemic area.

5. Current or planned participation in another clinical trial of an experimental agent during the study period.

6. Pregnant or lactating.

7. Any condition, including any clinically significant acute or chronic medical condition, for which, in the opinion of the investigator, participation would not be in the best interest of the subject (eg, compromise the well-being) or that could prevent, limit, or confound the protocol-specified assessments.

8. Use of anticancer, antituberculosis or other medications considered significant by the investigator within the previous 6 months.

9. Receipt of live-attenuated vaccine within the previous 60 days or planned receipt within 60 days after vaccination with Investigational Product (within 14 days for live attenuated influenza vaccine [LAIV]); or receipt of other vaccine (e.g., influenza, pneumococcal), allergy treatment with antigen injections or tuberculin skin test within the previous 14 days or planned receipt within 14 days after vaccination with Investigational Product

10. Receipt of blood transfusion or blood-derived products within the previous 3 months.

11. Previous severe local or systemic reactions to vaccination.

12. History of splenectomy

13. History of seizure in the last 3 years (participants with a history of seizures who have neither required medications nor had a seizure for 3 years are not excluded)

14. Known autoimmune disease

15. Asthma other than mild, well-controlled asthma. Exclude participants who:
    a. Use a bronchodilator (beta 2 agonist) daily, or
    b. In the past year have (any of the following):
        i. Had > 1 exacerbation of symptoms treated with oral steroids
        ii. Routinely used moderate to high dose inhaled corticosteroids (e.g., more than the equivalent of 250 mcg fluticasone; 400 mcg budesonide; 500 mcg beclomethasone; or 1000 mcg triamcinolone/flunisolide, as a daily dose) or theophylline
        iii. Needed emergency care, urgent care, hospitalization, or intubation for asthma
    c. Prophylactic bronchodilator use prior to exercise is not exclusionary

16. Diabetes mellitus type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

17. Thyroidectomy, or thyroid disease requiring medication during the last 12 months

18. Angioedema within the last 3 years if episodes are considered serious or have required medication within the last 2 years

19. Uncontrolled Hypertension:
    a. If a person has been diagnosed with hypertension during screening or previously, exclude for hypertension that is not well controlled. Well- controlled hypertension is defined as blood pressure consistently ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm
    b. If a person has NOT been diagnosed with hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 90 mm Hg at enrolment

20. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

21. Malignancy (Not excluded: a participant with a surgical excision and subsequent observation period that in the investigator's estimation has a reasonable assurance of sustained cure or is unlikely to recur during the study period)

22. Psychiatric condition that compromises safety of the participant or precludes compliance with the protocol, specifically excluding persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years

8 STUDY PRODUCTS

8.1 Study Products

Detailed instructions for study products including preparation, storage and documentation are provided under separate cover in the Study Operations Manual. Additional information is also provided in the Investigator's Brochure for the ZPIV product.

8.1.1 Zika Virus Purified Inactivated Vaccine (ZPIV)

Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at manufacturing facility prior to shipment to clinic.

8.1.2 Placebo

The placebo is saline.

8.2 Handling of Study Treatments

8.2.1 Packaging and Labeling

ZPIV and placebo will be provided as a refridgerated liquid. The vaccine will be provided in individual dosage vials and labeled in accordance with Good Manufacturing Practice (GMP).

8.2.2 Shipment and Storage

ZPIV should be stored at 4 °C (± 5 °C).

8.2.3 Dose preparation and administration

The unblinded pharmacist (or other unblinded staff member qualified to handle and dispense medication) will dispense ZPIV or placebo in a pre-filled capped syringe with the participant's study ID and allocation number.

9 CONDUCT OF THE STUDY

9.1 Informed Consent

The informed consent form documents that a participant (1) understands the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in this study. Informed consent encompasses all written or verbal study information staff provide to the participant before and during the trial.

The informed consent process continues throughout the study. At each study encounter, staff should consider reviewing the procedures and requirements for that encounter and for the remaining encounters. Additionally, if any new information is learned that might affect the participant's decision to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign a revised informed consent form.

Participants must sign a screening or protocol-specific consent before any procedures to determine eligibility are performed. All recruitment and prescreening materials will be approved by the Institutional Review Board.

9.1.1 Screening Consent Form

An IRB approved general vaccine screening protocol and consent may be used as part of the initial screening procedure for this trial. Results from this IRB approved general screening or prescreening may be used to determine eligibility in this protocol, provided the tests are conducted within the time period specified in the eligibility criteria.

9.1.2 Protocol-Specific Consent Form

The protocol-specific consent form describes the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. The consent form is developed in accordance with IRB requirements and the principles of informed consent as described in Title 45, Code of Federal Regulations (CFR) Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonization (ICH) Guideline 4.8.10. It must be approved by all responsible ethical review bodies before any participants can be consented for the study.

9.1.3 Test of Understanding

Study staff should ensure that participants understand the study before screening them. This process involves reviewing the informed consent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely. A Test of Understanding is used to document the participant's understanding of key concepts in this Zika vaccine trial. The participant must complete the Test of Understanding—with the assistance of staff, if necessary, in reading and understanding the questions and responses—before screening. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

9.2 Clinical and Laboratory Evaluations – Participants

Please see Section 18.3 for a concise outline of study procedures. Screening should occur within 56 days of enrollment. For visit windows, refer to the Schedule of Procedures (Section 18.3).

9.3 Unscheduled Visits

Unscheduled Visits/Contacts are visits/contact that are not described in the Schedule of Procedures (Section 18.3). Unscheduled visits may occur any time during the study:

- For administrative reasons, e.g., the participant or household contact may have questions for study staff or may need to re-schedule a follow-up visit.
- To obtain laboratory test results from a previous visit.
- For other reasons as requested by the participant or site investigator.

All unscheduled visits will be documented in the participant's study records on applicable source documents and entered into the Case Report Form (CRF).

9.4 Unblinding Visit

After all participants (from all 4 groups) have completed the active follow-up part of the study, and the database is locked, unblinding will occur. Participants will be informed as to whether or not they received study product or placebo. The unblinding visit may be performed in person or by phone.

9.5 HIV Counseling and Testing

HIV testing will be performed as part of screening evaluations. Therefore, HIV counseling will also be performed in compliance with the CDC's guidelines and local guidelines for HIV counseling, testing, and referral. All participants who become HIV-infected during the study will be terminated from the study. Potential and enrolled participants identified as HIV-infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.6 Prior and Concomitant Treatment

All concomitant medications will be recorded on CRFs from the signing of the ICF until the last study visit. Study participants can receive medications such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or antihistamines as needed although they must be documented. Use of these medications as routine prophylaxis prior to study vaccination is discouraged.

9.7 Study discontinuation

A participant will be taken out of the study in the event of:

- Repeated failure to comply with protocol requirements
- Decision by the study sponsor of PI to stop or cancel the study
- Decision by local regulatory authorities or IRB to stop or cancel the study
- Participant's request

9.7.1 Early discontinuation or withdrawal of participants

Each participant has the right to withdraw from the study at any time for any reason without affecting the right to treatment by the investigator/designee. The investigator should make an attempt to contact participants who did not return for scheduled visits or follow-up. Although the participant is not obliged to give reason(s) for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights.

The investigator also has the right to withdraw a participant, e.g. because of worsening health status, intercurrent illness, AEs, or pregnancy (for pregnancy follow-up see Section 10.4). The sponsor reserves the right to request the withdrawal of a participant due to safety issues, protocol violations, or administrative or other reasons.

Any unnecessary withdrawal should be avoided. Should a participant be withdrawn, all efforts should be made to complete and report the observations as thoroughly as possible. Whenever a participant is withdrawn from the study, independent of the reason, a final evaluation must be completed for that participant and the primary reason for which the participant was withdrawn must be stated. All documentation concerning the participant must be as complete as possible.

For those participants who are unable to continue participation in the study, but who do not withdraw consent, an early termination visit will be conducted. Any participant who withdraws consent will not have any further data collected after consent has been withdrawn.

9.7.2 Premature termination of the study

The sponsor reserves the right to discontinue the study for safety, ethical, or administrative reasons. Should the study be discontinued, no further vaccinations will be administered but participants who are still actively participating at the time of discontinuation will be followed through the remainder of their follow up visits.

9.7.3 Study pausing rules

If the trial is placed on safety pause, all enrollment and vaccinations will be suspended pending review. The AEs that may lead to a safety pause or prompt PSRT AE review are summarized below in Table 2. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the PI or PSRT, or participant may be threatened.

Table 2: AE notification and safety pause/AE review rules

Event and relationship to study product	Severity	Occurrence	Site PI action	PSRT or SMC action
SAE, related[1]	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
SAE, not related[2]	Grade 5	Any	Phone, email or fax forms to PSRT chair within 24 hours	PSRT review within 2 business days to consider pause
AE[3], related	Grade 3 or 4[4]	Second[5]	Phone, email or fax notification to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AE[3], related	Grade 3 or 4[4]	First	Phone, email or fax notification to PSRT chair within 24 hours	PSRT review within 2 business days to consider pause

[1] Related SAE refers to SAE deemed to be definitely, probably, or possibly related to study vaccine.

[2] Not related SAE refers to SAE deemed to be probably not related or not related to the study vaccine.

[3] Does not include the following reactogenicity symptoms (fever, fatigue/malaise, myalgia, arthralgia, chills, headache, nausea, vomiting).

[4] If no evidence of disease is present other than an abnormal laboratory value, the test must be repeated (entailing blood re-draw) at least one time. The verification period will be a maximum of 72 hours after initial awareness of the abnormal laboratory value. When signs and symptoms are present, repeat test will not be needed.

[5] PSRT will determine whether the reported related AE (Grade 3 or 4) is a second occurrence of a previously reported AE (Grade 3 or 4).

For events in the table above, the Site Principal Investigator (PI) notifies the PSRT (within 24 hours). The PSRT will convene within two business days to review these AEs. The PSRT will review and determine disposition (including whether the SMC needs to review the event).

If a study pause is triggered, all enrollment and vaccinations will be held until review by the SMC. Resumption of enrollment and study treatment may be determined by the SMC (in consultation with the FDA, if required) following a cumulative review of the available safety data. If a decision to resume study enrollment and study treatment administration is made, the SMC will record its judgment in a memorandum to the study file and notify the sponsor, who will then forward the memorandum to the principal investigators. The clinical site will be allowed to resume activities upon receipt of written notification from the sponsor or its designee. As needed, the appropriate regulatory authorities will be informed in writing of the decision by the SMC to resume or discontinue study activities. The site is responsible for notifying the IRB according to local standards and regulations. The sponsor is responsible for notifying the FDA.

9.8 Laboratory evaluations

The total approximate volume of blood that will be collected from each participant is presented in Section 18.3. Total blood volume drawn from each participant will not exceed the American Association of Blood Banks (AABB), and US Food and Drug Administration (FDA) guidelines of 550 mL in any eight-week period.

All biological samples must be collected in the appropriate manner. The investigator will ensure that the personnel and laboratory under his/her supervision comply with these requirements.

Further details on shipment, handling, and storage of the samples are provided in the Specimen Handling Guidelines.

Any residual samples will be stored indefinitely following completion of the study at the sponsor-designated laboratories. Any future analyses conducted on the samples will maintain participant anonymity. Participants will have to provide their approval for long-term storage of their biological specimens. Participants will have the right to opt out of having their biological specimens stored once all analyses in the Informed Consent Form are completed. Opting out of this procedure does not impact the participant's ability to participate in the study.

9.9 Potential risks and benefits

9.9.1 Risks related to vaccines

Participants may exhibit general signs and symptoms associated with the intramuscular administration of a vaccine or placebo. Side effects, if observed, are expected to be short term, mild, and not requiring treatment.

Participants may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, hives or even difficulty breathing. Severe reactions are rare. Medications are available at the clinical site to treat serious allergic reactions.

The effect of this vaccine on a fetus or nursing baby is unknown, as well as the effect on semen. Therefore, sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until 12 weeks after final immunization (Week 16, 14, 13, and 12 for Groups 1-4, respectively). Female participants of child-bearing potential will be required to agree to use birth control for sexual intercourse beginning prior to the vaccination and continuing through Week 12 weeks after final immunization (Week 16, 14, 13, and 12 for Groups 1-4, respectively). Women who are pregnant or nursing will be excluded from the study.

9.9.2 Blood draws

Blood drawing may cause pain, bruising, and, rarely, infection at the site where the blood is taken.

9.9.3 Unknown risks

There may be other serious risks that are not known. Participants may believe that this vaccine provides protection against acquiring Zika infection, and therefore not use appropriate precautions to avoid Zika infection. They will receive extensive counseling throughout the study to address this potential problem. It is not known if the study vaccine increases, decreases, or has no effect on the chance of becoming Zika infected when exposed, or if upon becoming Zika infected, the person's disease course will be more or less severe.

9.9.4 Potential benefits

There is no direct benefit to the participant for participation in this clinical trial. Although study participants may benefit from clinical testing and physical examination, they may receive no direct benefit from participation. Society may benefit from knowledge gained in this study that may aid in the development of a Zika vaccine.

10 SAFETY ASSESSMENTS

10.1 Adverse events

10.1.1 Definitions

Adverse events
An AE is any untoward medical occurrence in a patient or participant, and does not necessarily have a causal relationship with a medical treatment. This includes any noxious, pathological, or unintended change in anatomical, physiological, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory-detected changes. These might occur in any phase of the clinical study whether associated with the study vaccine or not. This includes exacerbations of pre-existing conditions or events, intercurrent illness, or vaccine or drug interactions. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation are not considered AEs. Discrete episodes of chronic conditions occurring during a study period should be reported as AEs in order to assess changes in frequency or severity.

Serious adverse events
Seriousness refers to the outcome of an AE. Seriousness is determined by both the investigator and medical monitor, and can also be determined by the sponsor (*FDA 2010 Guidance for Industry and Investigators; Safety Reporting Requirements for INDs and BA/BE Studies*). An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in **death.**
- Is **life-threatening**: i.e. the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Results in persistent or significant **disability/incapacity**: i.e. results in a substantial disruption of the participant's ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as headache, nausea, vomiting, diarrhea, influenza, infusion site reactions, or accidental trauma (e.g. sprained ankle).
- Requires in-patient **hospitalization** or prolongation of existing hospitalization: i.e. the participant is detained (usually at least an overnight stay) at the hospital or emergency ward for treatment that would not have been appropriate in the physician's office or in an out-patient setting. Hospitalization for either elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures[1] (including hospitalization for 'social' reasons) that are not the result of an AE, are **not** considered as SAEs.
- Is a **congenital anomaly or birth defect** in the offspring of a study participant
- Is an **important medical event** that may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above: e.g. interventions such as intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization, or development of drug

[1]A procedure that may take place during the study period and should not interfere with the study drug administration or any of the ongoing protocol-specific procedures.

dependency or drug abuse. Based on medical and scientific judgment, these events should usually be considered serious.

A suspected transmission of any infectious agent via a medicinal product is always considered as an important medical event, i.e. an SAE.

Although **not** considered as an SAE, cancer should be reported in the same way as SAEs.

Note: If anything untoward is reported during an elective procedure, that occurrence must be reported as an AE, either serious or non-serious according to the criteria defined above.

When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious.

Adverse reactions
An adverse reaction is an AE judged to be related to study vaccine.

Related AEs (adverse reactions) are defined as those judged by the investigator, sponsor or independent safety monitor to be possibly, probably, or definitely related to study vaccine.

When an AE is judged to be related to study vaccine and also is judged to be serious and unexpected, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting. All SAEs in this study will be considered unexpected as there are no expected AEs associated with this vaccine product.

Adverse reactions will be reported in accordance with FDA 2009 Guidance for Clinical Investigators, Sponsors and IRBs; Adverse Events Reporting to IRBs – Improving Human Participant Protection.

10.1.2 Surveillance, reporting, and documentation of adverse events

The recording of AEs is an essential part of study documentation. The investigator is responsible for documenting all AEs as set out in the following sections.

10.1.2.1 Surveillance of adverse events

At each visit through 28 days following last vaccination, all AEs, either observed by the investigator or one of his/her clinical collaborators, or reported by the participants spontaneously or in response to a direct question, will be evaluated by the investigator or designee. All SAEs will be reported through the final visit (Study Day 365).

Participants will be instructed to contact the investigator immediately should he/she experience any signs or symptoms he/she perceives as severe from the time of vaccination through a period of 12 months.

10.1.2.2 Documentation of adverse events

All AEs will be collected on case report forms (CRFs) from the signing of the ICF until 28 days following last vaccination. SAEs will be collected through Study Day 365.

AEs and SAEs should be documented in terms of signs and symptoms observed by the investigator or reported by the participants. Whenever possible, a medical diagnosis should be made. The nature of each event, date and (where appropriate) time of onset, outcome, severity, and causal relationship should be established. Details of any symptomatic/corrective treatment should be recorded on the appropriate page of the CRF.

Hospitalization for routine clinical procedures (or other reason) that is not the result of an AE, are not considered AEs but will be recorded on the AE page of the CRF ('Hospitalization, Not an AE'). The same applies for hospitalization for elective procedures related to a pre-existing condition that did not increase in severity or frequency during the study. If hospitalization was planned before administration of the study vaccine, it will be documented in the Medical History page of the CRF (see below).

The following events will be documented in the Medical History page of the CRF:

- Pre-existing conditions or signs and/or symptoms present in a participant before study start. This includes conditions that were not recognized at study entry but later during the study period.
- Hospitalization arising from a pre-existing condition and planned before the administration of the vaccine.

10.1.2.3 Post-vaccination reactions occurring immediately after each vaccine administration

Participants will be observed for minimum of 30 minutes following vaccine administration. Vital signs (temperature, blood pressure, pulse and respiratory rate) will be taken and qualified study personnel will evaluate for any signs or symptoms of reactions.

Possible reactions may include fever (≥37.7 ºC), fatigue/malaise, headache, myalgia, arthralgia, chills, nausea, vomiting, and arm pain. For life-threatening allergic reactions that occur immediately post-vaccination, the site has specific procedures developed for handling such emergencies.

10.1.2.4 Reactions occurring within seven days following each vaccine administration

Participants will be instructed to notify specified study personnel immediately if any unusual or severe sign or symptom appears after vaccine administration. Participants will maintain a study diary during this period to record potential reactions.

10.1.3 SAE Reporting

The sponsor has a regulatory obligation to report SAEs to the FDA according to established timetables for reporting based on specific criteria. The investigator will report SAEs to the PSRT and IRB within 24 hours from the time they first learn of the event. The SAE form will be completed as thoroughly as possible and signed by the investigator or his/her designee before

transmittal to the PSRT and IRB. The investigator will provide his/her assessment of causality to study treatment at the time of the initial SAE report. The investigator will not delay in the reporting of any SAE in order to obtain additional information. Any additional information, if collected, will be reported to the PSRT and IRB as a follow-up to the initial report.

All SAEs must be reported immediately (within 24 hours of discovery) by email or fax to the IRB at the following contact information:

Email: Jessica Ripton, jripton@bidmc.harvard.edu
Fax: (617) 975-8501

The PSRT will perform a clinical review of the information provided to identify any missing data. The PSRT will also contact the study site to clarify any discrepant or missing information, to answer questions and to provide guidance to the site, if needed. The investigator will report the SAE as an acceptable medical diagnosis. If a preliminary diagnosis has not yet been made, then each symptom will be listed separately. A follow-up report will be issued when a diagnosis is made.

The investigator must report SAEs to the appropriate IRB as requested by the board according to local legal requirements.

10.2 Reporting requirements to the local IRB

The PI/designee will be responsible for providing all safety reports and reporting all SAEs, study pauses, social harms, and major deviations to the local regulatory authorities, such as a local IRB, and any regulatory agencies, in a timely manner according to the institution's guidelines and to local regulations.

10.3 Causality assessment of study vaccine to adverse events

Every effort should be made by the investigator to explain any AE and assess its potential causal relationship, i.e. to administration of the study treatment or to alternative causes (e.g. natural history of the underlying diseases, concomitant therapy). This applies to all AEs, whether serious or non-serious.

The investigator will use the following guidelines to assess the causal relationship of an AE to study injection:

Not related:	Adverse experiences felt to be due to extraneous causes that neither follows a known pattern of response nor a reasonable temporal relationship to study vaccine.
Remote (probably not related):	Adverse experiences that are unlikely to be related to study vaccine but which follow a reasonable temporal relationship, such that this cannot be completely excluded or events that could be associated with study vaccine but which are unrelated in time.
Possibly related:	Adverse experiences that may be due to extraneous causes but which follow a known pattern of response and/or a reasonable

temporal relationship to study vaccine.

Probably related:	Adverse experiences that cannot be explained by extraneous causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.
Definitely related:	Adverse experiences that have a definite relationship to the study vaccine (e.g. anaphylactic reaction after vaccination) without any other explained causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.

In the final analyses, events categorized as “not related” and “remote” will be considered as not related to study vaccine; events categorized as “possibly related”, “probably related” and “definitely related” will be considered as related to study vaccine.

10.3.1 Severity of adverse events

All AE and lab data will be coded for severity using the Division of Microbiology and Infectious Disease Revised 2013 Toxicity Table included in Section 18.1, and also located on the website: https://www.niaid.nih.gov/labsandresources/resources/dmidclinrsrch/Pages/pharma.aspx.

For AEs not identified in the grading table, the following guidelines will be applied:

Mild	Grade 1	Symptoms causing no or minimal interference with usual social and functional activities
Moderate	Grade 2	Symptoms causing greater than minimal interference with usual social and functional activities
Severe	Grade 3	Symptoms causing inability to perform usual social and functional activities
Potentially life-threatening	Grade 4	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability
Fatal	Grade 5	For any AE where the outcome is death, the severity of the AE is classified as Grade 5

The clinical research site team will ascertain accurate recording of all AEs during the study. AE CRFs will be completed by the clinical research site staff on a daily basis as the data become available from the isolation unit, clinic or laboratory.

The investigators will monitor and analyze study data including all AE and lab data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study vaccine. AEs will be deemed either related to study vaccine or not related to study vaccine. To ensure that all AEs are captured in a timely manner, CRFs will be entered in real-time entry, and participanted to analysis to identify AEs that may invoke study pausing rules.

The PI or designee must review AE CRFs to insure prompt and complete identification of all events that require expedited reporting as SAEs, study pausing rules or other serious and unexpected events.

Study vaccine related AEs will be followed by the clinical research site team through resolution or until study completion. Non-study vaccine related AEs will be followed to resolution or study completion, whichever occurs first.

10.3.2 Follow-up of ongoing adverse events and assessment of outcome

10.3.2.1 Follow-up of non-serious adverse events

Non-serious AEs already documented in the CRF at a previous assessment and designated as 'ongoing' should be reviewed at subsequent visits. If the event has resolved, the documentation in the CRF should be completed. If the frequency or severity of a non-serious AE changes significantly, a new record of the AE has to be started. If the AE becomes serious, the procedures for reporting of SAEs have to be followed (see Section 10.1.3).

New non-serious AEs will be recorded through 28 days post-vaccination. Ongoing non-serious AEs will be monitored until the Day 365 visit (or until the last study visit for participants who withdraw early (see Section 18.3)).

Outcomes will be assessed as:

1. Resolved
2. Resolved with sequelae
3. Resolving
4. Not resolved
5. Fatal
6. Unknown

Clinically significant abnormal laboratory values will be followed up until they have returned to normal, stabilized, or a satisfactory explanation has been provided.

10.3.2.2 Follow-up of serious adverse events

All SAEs must be followed-up until the event has either resolved, subsided, stabilized, disappeared, or is otherwise explained, or the study participant is lost to follow-up, but no longer than 12 months after the administration of the study vaccine. Outcomes are assessed as above.

All follow-up activities must be reported in a timely manner to the PSRT (if necessary on one or several consecutive SAE report forms). All form fields with additional or changed information must be completed and the SAE Report Form should be forwarded to the PSRT as soon as possible but at the latest within 7 calendar days after receipt of the new information.

Clinically significant laboratory abnormalities reported as SAEs will be followed-up until they have returned to normal, or a satisfactory explanation has been provided.

Reports related to the subsequent course of any SAE reported for any participant must be submitted to the sponsor.

10.3.3 Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to currently available best treatment. The applied measures should be recorded in the CRF.

10.4 Handling of pregnancy cases

Pregnancy events will be reported through 3 months following final vaccination. All initial reports of pregnancy must be reported to the PSRT by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, stillbirth, and congenital anomaly) are considered SAEs and must be reported using the Serious Adverse Event Form.

Because the effect of the study vaccine on sperm is unknown, pregnancies in partners of male participants included in the study will be reported by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

10.5 Physical examination

Physical examinations will be performed by the investigator or designated medically-trained clinician. The time points of these examinations are specified in Section 18.3. Any abnormalities or changes should be documented in the source document and recorded on the CRF.

A new, clinically significant finding (in the opinion of the investigator) not noted at screening must be captured as an AE. In addition, resolution of any abnormal findings during the study will be noted in the source document and in the CRF.

10.6 Routine safety laboratory

Samples for routine safety laboratory parameters will be collected at the time points specified in Section 18.3.

The following routine safety laboratory parameters will be determined:

- **Serum chemistry:** AST, ALT, and Creatinine
- **Complete blood count:** hemoglobin, hematocrit, white blood cells (WBC), WBC differentiation, red blood cell count (RBC), and platelet count

Laboratory values will be graded according to the DMID toxicity scale (Section 18.1) and, if clinically significant, reported as AEs.

10.7 Vital signs

Vital sign measurements will be performed at time points specified in Section 18.3.

The following measurements will be performed:

- Heart rate (bpm)
- Systolic and diastolic blood pressure (mmHg)
- Respiratory rate
- Body temperature (oral)

A confirmatory vital signs measurement can be performed if inconsistent with a prior measurement. If any clinically significant changes in vital signs are noted, they will be reported as AEs and followed to resolution, or until reaching a clinically stable endpoint.

10.9 Protocol Safety Review Team and Safety Monitoring Committee

The Protocol Safety Review Team (PSRT) will include the Protocol Chair, Protocol Co-Chairs, Site Principal Investigator, co-investigators and partners at WRAIR and VRC/NIH. Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs not requiring a study pause should also be reviewed by the SMC.

The Safety Monitoring Committee (SMC) will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC is an independent multidisciplinary group consisting of experts in flavivirus infection (e.g., dengue, West Nile Virus, etc.) that, collectively, has experience in the management, conduct, and monitoring of vaccine trials. Members of the SMC are not directly affiliated with this protocol and are not situated at the study site. The SMC also will review individual Expedited Adverse Event (EAE) reports. The SMC will conduct additional special reviews at the request of the PSRT.

11 IMMUNOGENICITY ASSAYS

11.1 Zika-associated immunogenicity

Blood samples for the determination of cellular and humoral responses will be collected as specified in Section 18.3.

Humoral response assays to Zika will likely include, but are not limited to: microneutralization assays, ELISA, and plaque reduction neutralization assays. Table 3 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 3: Humoral immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Principle Timepoint(s)
Humoral responses	Secondary	Serum	ZIKV microneutralization (MN) assay	Log10 MN50 titers	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 * Peak
	Secondary	Serum	ZIKV Env ELISA	Log10 endpoint titers	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 * Peak
	Exploratory	Serum	ZIKV plaque reduction assays	Log10 titer	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 * Peak

Evaluations of cellular immune responses to Zika will likely include, but are not limited to, interferon gamma producing cells (ELISPOT) assays. Table 4 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 4: Cellular immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Primary Timepoint(s)
T-cell responses	Exploratory	PBMC	Interferon gamma producing cells (ELISPOT)	Number of spot forming cells per 10^6 PBMC	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 *Peak

Immunologic assays may be performed and analyzed in a blinded fashion after all participants of a given group reach the peak immunogenicity time-point for their group (D56, D42, D35, and D28 for Groups 1-4, respectively). This will allow more rapid accrual of data to facilitate product

development strategies. The optimal choice of immunogenicity assays will take into consideration the latest technological advances.

11.2 DNA/RNA Assays

Blood samples collected for cellular immunogenicity may also be used for exploratory DNA and RNA micro-array and deep-sequencing assays.

11.4 Additional Immunogenicity Assays

Additional immunogenicity assessments of systemic and mucosal responses may potentially include the following: epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, and Zika epitope-specific T cell responses.

12 DATA EVALUATION AND STATISTICS

All data entry will be performed by qualified and trained study staff. When all data have been entered and validated, the final database will be locked. An interim blinded analysis will be performed 28 days following the final immunization (group assignments will be known). A second interim blinded analysis will be performed at the 6 month time-point. Final analysis will be performed when D365 data has been collected.

12.1 Analysis populations and data sets

12.1.1 Safety population

All participants who received ZPIV or placebo, and for whom any post-dose data is available, will be included in the safety population.

12.1.2 Immunogenicity population

The immunogenicity population will consist of all participants who received ZPIV or placebo, and who have at least one measured post-dose blood sample collected.

12.2 Endpoints

Safety and tolerability:

- Incidence, intensity, and relationship to vaccination of solicited local and systemic adverse events (AEs) during the 7-day follow-up period (Days 0-6) after each ZPIV dose.
- Incidence, intensity, and relationship to vaccination of unsolicited AEs during the 28-day follow-up period (Days 0-27 after each ZPIV dose)
- Grade 2 and Grade 3 laboratory abnormalities at Day 7 after each ZPIV dose.
- Incidence of serious adverse events (SAEs) and related AEs from Day 0 through Day 365.

Immunogenicity:

- Principle Endpoints: Proportion (95% CI) of participants per dose group with positive responses and mean response (e.g. GMT) per group with 95% CI for the following 2 parameters:
    - ZIKV microneutralization Log10 MN50 titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D0.
    - Zika Env-specific Log10 endpoint ELISA titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D0.

- Additional Endpoints:
    - Plaque reduction neutralization test titer at 14 and 28 days following each vaccination, and at 3, 6, 9, and 12 months from D0.

- Durability and kinetics of humoral immune responses: Log10 MN50 and Log10 endpoint ELISA titers at 14 and 28 days following *first* (and second) vaccination, and at 3, 9 and 12 months.
- Cellular immunogenicity: IFN-γ ELISPOT responses to prM, Env, Cap, and NS1 peptides at 28 days following final vaccination and at 6 months from D0.

12.3 Sample size consideration

The number of participants chosen for this study will provide a preliminary safety and immunogenicity assessment. The sample size is not based on formal hypothesis testing considerations, but within the range of participants (i.e., 20-80) recommended in the Code of Federal Regulations (CFR 312.21) for first-in-human product Phase 1 evaluations. Placebo recipients are included for blinding and safety purposes and will provide additional control specimens for immunogenicity assays.

13 ETHICAL AND LEGAL REQUIREMENTS

13.1 General requirements

The study will be performed according to this Study Protocol and in compliance with the Declaration of Helsinki, the guidelines of the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) and the respective local legal requirements including the following: US Code of Federal Regulations 45CFR Pt 46; 21CFR Pt 50, 21CFR Pt 56 and 21CFR Pt 312.

13.2 Institutional Review Board/Ethics Committee

Before the start of the study, the investigator will submit the Study Protocol, Informed Consent Form, and other study-related documents as required by applicable laws and regulations to the responsible IRB for written approval.

The investigator will inform the IRB according to applicable laws and regulations about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

Unless otherwise instructed by the IRB or local regulation the investigator must submit to the IRB:

- All subsequent Amendments to the Study Protocol, changes to the Informed Consent Form or revisions of other documents originally submitted for review
- New or revised participant recruiting materials approved by the sponsor, if applicable
- All subsequent changes of logistical or administrative aspects (for information)
- Serious and/or unexpected AEs occurring during the study, where required
- New information that may affect adversely the safety of the participants or the conduct of the study
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Annual update and/or request for re-approval, where required
- Date of study completion, where required

13.3 Regulatory authorities

Before initiating the study, the sponsor will submit any required application to the regulatory authorities and obtain approval according to applicable laws and regulations. The sponsor will also inform the regulatory authorities about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

13.4 Participant information and informed consent

The study informed consent describes the investigational products to be used and all aspects involved in protocol participation. A properly executed written informed consent, in compliance with the Declaration of Helsinki, guidelines of the Council of International Organization of Medical Sciences (CIOMS), the Belmont Report, the US Code of Federal Regulations 21 CFR 50, must be obtained from each participant prior to entry into the trial or prior to performing any unusual or non-routine procedure that involves risk to the participant. The investigator must

provide a copy of the approved informed consent to the participant and a signed copy must be maintained in the participant's record file. Before a participant's participation in the study, it is the investigator's responsibility to obtain this written informed consent from the participant, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or study medications are administered.

All items must be explained by the investigator or designee in a language that is easy to understand. Participants will also be informed that the participation is voluntary and that they have the right to withdraw at any time without giving reasons and without any disadvantages for their subsequent care. Participants will confirm their consent in writing before study start and any study-specific procedure.

Participants must be given enough time to consider participation in the study.

13.5 Indemnity

The Sponsor and Institution are responsible to have appropriate liability insurance. For research-related injuries and/or medical problems determined to result from receiving the Investigational Product, treatment including necessary emergency treatment and proper follow-up care will be made available to the participant free of charge at the expense of the Sponsor.

13.6 Data access and protection

Confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

The collection and processing of personal data from participants enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of participants confidential.

The informed consent includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to the participant's source data/documents for study-related monitoring, audit, IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The participant has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

13.7 Future use and storage and blood samples

Each study participant will be asked to consent voluntarily for their blood samples to be stored for other research studies that may be done after this study is completed. Future testing may involve DNA/RNA tests. For participants unwilling to have their blood samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed after all the tests specified for this study have been concluded.

All samples for which consent has been obtained, and for which additional material is available after study-specified testing is complete, will be stored for future testing. All applicable approvals will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB.

13.8 Reimbursement

Participants and household contacts will be reimbursed for time and inconvenience in accordance with the standards and legal obligations for compensation required by each site. Any applicable guidelines by IRBs/ECs for compensation of research participants and household contacts will be sought and followed.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

Monitoring of clinic activities will be performed regularly to ensure that the study is carried out according to this Study Protocol and in compliance with GCP and applicable legal requirements.

Source documents will be reviewed for verification of consistency with CRF data. The investigator guarantees direct access to source documents for monitoring purposes. Source data verification will be performed in accordance with data protection regulations and guidelines. All information reviewed will be handled according to these rules and regulations.

The monitor will review each participant's data as outlined in the study specific Clinical Monitoring Plan.

14.2 Audit and inspections

Audits and inspections may be carried out by qualified delegates authorized by the sponsor or by authorities. The investigator consents to cooperate and to allow direct access to all source documents and other study-related data during an audit or inspection. All information disclosed will be handled in accordance with applicable data protection rules and regulations.

14.3 Data quality assurance

All CRF data will be entered into a validated, 21CFR Part 11 compliant, computerized clinical data management system.

The site is required to have a plan in place for assuring the quality of the research being conducted.

15 DOCUMENTATION, ARCHIVING, AND PUBLICATIONS

15.1 Documentation

Source documents are original documents, data, and records from which the participant's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, radiographs, and correspondence.

The Investigator and staff are responsible for ensuring maintenance of a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the sponsor, FDA, and/or applicable regulatory authorities.

All CRF entries have to be verifiable by the source data in the participant file. This does not apply to CRF entries that are defined as source data.

15.2 Archiving

The Investigator is responsible for the archiving of the Investigator's file, the participant's file, and the source data according to national and international legal requirements.

Any records related to the conduct of the study may not be destroyed without written authorization by the sponsor. According to GCP requirements, study records should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

15.3 Clinical Study Reports and Publications

The results of the clinical study will be documented in the Clinical Study Report.
The study results may be published and/or presented at scientific meetings. Terms of publication will be addressed in an agreement between the sponsor and participating partners.

16 REFERENCES

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17 PROTOCOL SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the appendices and provide the necessary assurances that this study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirement(s).

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18 APPENDICIES

18.1 Toxicity Tables

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007

Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal R_x = Therapy
Req = Required	Mod = Moderate
IV = Intravenous	ADL = Activities of Daily Living
Dec = Decreased	

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1	**Mild** Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	**Moderate** Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	**Severe** Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible
GRADE 4	**Life-threatening** Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.
- For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.
- Some protocols may have additional protocol specific grading criteria, which will supercede the use of these tables for specified criteria.

HEMATOLOGY				
	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	9.5 - 10.5 gm/dL	8.0 - 9.4gm/dL	6.5 - 7.9 gm/dL	< 6.5 gm/dL
Absolute Neutrophil Count	1000-1500/ mm^3	750-999/ mm^3	500-749/ mm^3	<500/ mm^3
Platelets	75,000-99,999/ mm^3	50,000-74,999/ mm^3	20,000-49,999/ mm^3	<20,000/ mm^3
WBCs	11,000-13,000/ mm^3	13,000-15,000 / mm^3	15,000-30,000/ mm^3	>30,000 or <1,000 / mm^3
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%	----------
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: <100 mg/dL High: >600 mg/dL	Low: < 50 mg/dL ----------	Fibrinogen associated with gross bleeding or with disseminated coagulation
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	51-60 mcg/ml	> 60 mcg/ml
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 -3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Methemoglobin	5.0 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9%	> 20.0 %

CHEMISTRIES				
	Grade 1	Grade 2	Grade 3	Grade 4
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required	< 2.0 mEq/L or abnormal potassium *with* paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	> 7.0 mEq/L or abnormal potassium *with* life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany

CHEMISTRIES (continued)				
	Grade 1	Grade 2	Grade 3	Grade 4
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL or abnormal calcium *with* life threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	1.0 -1.4 mg/dL intensive therapy or hospitalization required	< 1.0 mg/dL or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	1.5 – 1.75 x ULN	> 1.75 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/dL	12.1 – 15.0 mg/dL	>15.0 mg/dL
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or dialysis required

ENZYMES				
	Grade 1	Grade 2	Grade 3	Grade 4
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN

URINALYSIS				
	Grade 1	Grade 2	Grade 3	Grade 4
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts	obstructive or required transfusion

CARDIOVASCULAR				
	Grade 1	Grade 2	Grade 3	Grade 4
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrythmia; hospitalization and treatment required
Hypertension	transient increase > 20 mm/Hg; no treatment	recurrent, chronic increase > 20mm/Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible	end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required	mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment
Pericarditis	minimal effusion	mild/ moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

RESPIRATORY				
	Grade 1	Grade 2	Grade 3	Grade 4
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment	----------
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present	cyanosis: FEV_1 < 25% of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring Oxygen therapy

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids	hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids	physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/day or mild diarrhea last < 1 week	moderate or persistent; 5-7 loose stools/day or diarrhea lasting >1 week	>7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required	hypotensive shock or physiologic consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods	unable to drink fluids; requires IV fluids

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement	incapacitating; or not responsive to narcotic analgesia
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities)	sensory loss involves limbs and trunk; paralysis; or seizures

MUSCULOSKELETAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living	disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living	permanent and/or disabling joint distruction
Myalgia	myalgia with no limitation of activity	muscle tenderness (at other than injection site) or with moderate impairment of activity	severe muscle tenderness with marked impairment of activity	frank myonecrosis

SKIN				
	Grade 1	Grade 2	Grade 3	Grade 4
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15mm	15-30 mm	>30mm	
Erythema	< 15mm	15-30 mm	>30mm	
Edema	< 15mm	15-30 mm	>30mm	
Rash at Injection Site	< 15mm	15-30 mm	>30mm	
Pruritus	slight itching at injection site	moderate itching at injection extremity	itching over entire body	

SYSTEMIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy	intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	39.6 - 40.5 C or 103 - 105 F	> 40 C or > 105 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25-50% > 48 hours	normal activity decreased > 50% can't work	unable to care for self

18.2 Key Study Roles

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18.3 Schedule Of Procedures

Table 2a

Group 1 Schedule:

***Visits 2 and 6 are phone calls**

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7)	D14 (14)	D28 (28) (0)	D31 (3)	D35 (7)	D42 (14)	D56 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Clinical Assessments														
Consent, Demographics	X													
Medical History Review	X	X				X								X
Inclusion and Exclusion	X	X												
Medications[a]	X	X		X	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X		X	X	X	X	X	X	X
Laboratory Tests														
Screening Labs (30 mL)	X													
Other Flavivirus Serologies[e] (20 mL)	X													
CBC w/diff and Chemistry Panel (15 mL)		X		X	X	X		X	X	X	X	X	X	X
Pregnancy[f]	X	X				X								
Humoral (30 mL)		X			X	X			X	X	X	X	X	X
CMI (40 ml)		X			X	X			X	X	X	X	X	X
Study Related Procedures														
Vaccination		**X**				**X**								
Distribute Diary		X				X								
Review Diary[g]				X				X						
Phone AEs [h]			X				X							
Blood Volume (mL)	50	85	0	15	85	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at 0 and 28 day visits

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Screening for all serologic evidence of all flavivirus infection/vaccination

[f] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Table 2b

Group 2 Schedule:

***Visits 2 and 5 are phone calls**

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7)	D14 (14) (0)	D17 (3)	D21 (7)	D28 (28) (14)	D42 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Clinical Assessments													
Consent, Demographics	X												
Medical History Review	X	X			X								X
Inclusion and Exclusion	X	X											
Medications[a]	X	X		X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X		X	X	X	X	X	X	X
Laboratory Assessments													
Screening Labs (30 mL)	X												
Other Flavivirus Serologies[e] (20 mL)	X												
CBC w/diff and Chemistry Panel (15 mL)		X		X	X		X	X	X	X	X	X	X
Pregnancy[f]	X	X			X								
Humoral (30 mL)		X			X			X	X	X	X	X	X
CMI (40 ml)		X			X			X	X	X	X	X	X
Study Related Procedures													
Vaccination		**X**			**X**								
Distribute Diary		X			X								
Review Diary[g]				X			X						
Phone AEs [h]			X			X							
Blood Volume (mL)	50	85	0	15	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at 0 and 14 day visits

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Screening for all serologic evidence of all flavivirus infection/vaccination

[f] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Table 2c

Group 3 Schedule:

***Visits 2 and 4 are phone calls**

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7) (0)	D10 (10) (3)	D14 (14) (7)	D21 (21) (14)	D28 (28) (21)	D35 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Clinical Assessments													
Consent, Demographics	X												
Medical History Review	X	X		X									X
Inclusion and Exclusion	X	X											
Medications[a]	X	X		X		X	X	X	X	X	X	X	X
Physical Exam[b]	X	X		X		X	X	X	X	X	X	X	X
Vital signs[c]	X	X		X		X	X	X	X	X	X	X	X
Laboratory Assessments													
Screening Labs (30 mL)	X												
Other Flavivirus Serologies[e] (20 mL)	X												
CBC w/diff and Chemistry Panel (15 mL)		X		X		X	X	X	X	X	X	X	X
Pregnancy[f]	X	X		X									
Humoral (30 mL)		X		X		X	X	X	X	X	X	X	X
CMI (40 ml)		X				X	X	X	X	X	X	X	X
Study Related Procedures													
Vaccination		**X**		**X**									
Distribute Diary		X		X									
Review Diary[g]				X		X							
Phone AEs [h]			X		X								
Blood Volume (mL)	50	85	0	85	0	85	85	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at 0 and 7 day visits

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Screening for all serologic evidence of all flavivirus infection/vaccination

[f] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Table 2d

Group 4 Schedule:

***Visit 2 is a phone call**

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7)	D14 (14)	D28 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Clinical Assessments										
Consent, Demographics	X									
Medical History Review	X	X								X
Inclusion and Exclusion	X	X								
Medications[a]	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X	X	X	X	X
Laboratory Assessments										
Screening Labs (30 mL)	X									
Other Flavivirus Serologies[e] (20 mL)	X									
CBC w/diff and Chemistry Panel (15 mL)		X		X	X	X	X	X	X	X
Pregnancy[f]	X	X								
Humoral (30 mL)		X			X	X	X	X	X	X
CMI (40 ml)		X			X	X	X	X	X	X
Study Related Procedures										
Vaccination		**X**								
Distribute Diary		X								
Review Diary[g]				X						
Phone AEs [h]			X							
Blood Volume (mL)	50	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at day 0

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Screening for all serologic evidence of all flavivirus infection/vaccination

[f] Serum pregnancy test at screening and urine pregnancy test at vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Protocol Title:	A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
Short Title:	Zika Virus Purified Inactivated Vaccine (ZPIV) Accelerated Vaccination Schedule Study
Protocol Number:	Z001
Phase:	Phase 1
Sponsor:	Kathryn E. Stephenson Boston, MA
Sponsor Status:	Sponsor-Investigator
Study Location:	Center for Virology and Vaccine Research Clinical Trials Unit, Beth Israel Deaconess Medical Center
Study Period:	October 2016 – November 2017
Collaborating Institutions:	Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA, Vaccine Research Center/National Institute of Allergy and Infectious Diseases (VRC/NIAID), Bethesda, MD, USA
Date of Protocol Version:	August 10, 2016 2.0

TABLE OF CONTENTS

List of Abbreviations

AE	Adverse event
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCVI	Antibody-dependent cell-mediated virus inhibition
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotranferase
AST	Aspartate aminotransferase
BIDMC	Beth Israel Deaconess Medical Center
CBC	Complete blood count
$CD4^+$	A functional subclass of T cells, helper T lymphocytes (Th), that are necessary for augmentation and coordination of innate and adaptive effector responses, humoral and cellular
$CD8^+$	Cytotoxic T-cells that destroy host cells that have become infected by viruses or other intracellular pathogens
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CRF	Case report form
CTL	Cytotoxic T lymphocyte
CVVR	Center for Virology and Vaccine Research
DNA	Deoxyribonucleic acid
DMC	Data Management Center
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FDA	US Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMS	Global Medical Safety
HIV-1	Human immunodeficiency virus, type 1
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICF	Informed Consent Form
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
mL	Mililiters
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PI	Principal investigator
PO	Per Oral
PSRT	Protocol Safety Review Team
RBC	Red blood cell count
rtPCR	Real-time PCR
SAE	Serious adverse event
SMC	Safety Monitoring Committee
SST	Serum separating tubes
SUSAR	Suspected unexpected serious adverse reaction
$TCID_{50}$	50% Tissue Culture Infective Dose
VP	Viral particles

WBC	White blood cell count
ZPIV	Zika Virus Purified Inactivated Vaccine

1 OVERVIEW

Title

A Phase 1, Randomized, Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine in Healthy Adults

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 90, 180, 270, and 365 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

Study Products and Routes of Administration

- **Zika Virus Purified Inactivated Vaccine (ZPIV) with Alum Adjuvant:** Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at the manufacturing facility prior to shipment to clinic.
- **Placebo**: The placebo product is saline.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV	ZPIV		
	B	2		Placebo	Placebo		
4	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		48					

Participants

Healthy participants aged 18-50 years:

- 40 vaccinees
- 8 placebo recipients
- 48 total participants

Design

Single center, randomized, controlled, double-blind phase 1 trial

Duration per participant

12 months active follow-up per participant

Estimated total study duration

18 months (includes screening and active follow-up)

Investigational New Drug (IND) Sponsor

Beth Israel Deaconess Medical Center, Boston, MA, USA

Vaccine Manufacturer

Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA

Data Management Center (DMC)

The EMMES Corporation, Rockville, MD, USA

Endpoint Assay Laboratories

Center for Virology and Vaccine Research (CVVR), Beth Israel Deaconess Medical Center (BIDMC), Boston, MA, USA

Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA

Vaccine Research Center, National Institute of Allergy and Infectious Diseases (VRC/NIAID), Bethesda, MD, USA

Study Site

Center for Virology and Vaccine Research Clinical Trials Unit, Beth Israel Deaconess Medical Center, Boston, MA, USA

Safety Monitoring

Protocol Safety Review Team (PSRT)
Safety Monitoring Committee (SMC)

2 BACKGROUND AND RATIONALE

2.1 Introduction

Zika virus (ZIKV) is an emerging vector-borne RNA virus that, since early 2015, has caused an increased incidence of systemic disease and neurologic complications in an expanded region of the Western Hemisphere. ZIKV, of the family *Flaviviridae,* is related to other pathogens of global importance to humans, including dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV) and tick-borne encephalitic virus (TBEV). ZIKV was first isolated in Uganda in 1947 from a sentinel rhesus macaque and has since diverged along two lineages: African and Asian. The virus is almost exclusively transmitted by the *Aedes aegypti* mosquito but has been isolated from several other species of the genus *Aedes,* including *A. albopictus, A. africanus* and *A. luteocephalus*. The majority of human ZIKV infections result in an asymptomatic infection or a benign, self-limited acute febrile illness. ZIKV disease typically manifests as a rash, fever, conjunctivitis and arthralgia. Until 2007 when several outbreak clusters erupted in the South Pacific, ZIKV disease was relatively rare and geographically limited. Between 2007 and 2016, however, Zika has been documented in 60 countries and territories, 46 of which recorded their first case in either 2015 or 2016.

A clinical pattern has emerged from the most recent outbreaks that was never observed before. Nine months after local ZIKV transmission was first documented in Brazil, public health officials detected an increase in neonates born with microcephaly in the northeastern part of the country (WHO 2016). Epidemiologic studies, together with *in vitro* and *in vivo* experiments, have confirmed a causal association between ZIKV infections during pregnancy and the consequent occurrence of serious birth defects including microcephaly, brain malformations and ocular defects (Rasmussen SA, 2016). An increase in Guillain-Barré syndrome (GBS), meningitis and encephalitis have also been found in people with primary ZIKV infections (Cao-Lormeau, 2016) (Carteaux G, 2016), (Broutet N, 2016). The neuro-invasiveness of Zika virus infection and its complications ultimately prompted the World Health Organization (WHO) to declare, in February 2016, the emerging ZIKV epidemic as Public Health Emergency of International Concern.

2.2 Rationale for developing and testing a Zika Virus Purified Inactivated Vaccine (ZPIV)

There is no specific treatment or prophylactic currently available for ZIKV disease, other than supportive care and mosquito control. Given the expanding distribution and significant morbidity associated with ZIKV disease, the development of a safe and effective vaccine against ZIKV is a high global public health priority. The ZIKV vaccine to be used in this trial is a formalin-inactivated whole virus vaccine that was produced by the Walter Reed Army Institute of Research (WRAIR). There has been extensive experience with formalin-inactivated vaccines for other viruses, including other flaviviruses, such as the safe and immunogenic experimental dengue purified inactivated vaccine (Martinez 2015) and the licensed IXIARO® Japanese encephalitis vaccine. Experience with these other flavivirus vaccines has informed the design and development of the current ZIKV vaccine. However, the safety, tolerability and immunogenicity of a ZIKV vaccine, including the inactivated vaccine, is still to be determined. Because the safety and immunogenicity profile of this vaccine is still unknown, this Phase I trial, in concert with three others, will address a critical gap necessary to complete an early and rapid assessment of the viability of this ZIKV vaccine candidate. The first two clinical trials will explore the safety and immune response to the vaccine in flavivirus naïve individuals (National

Institutes of Health (NIH) and in participants who have been previously vaccinated with JEV or YFV vaccines (WRAIR). The third trial (NIH) will be conducted in Puerto Rico, whose population has background exposure to dengue virus and potentially yellow fever and West Nile virus as well. The dose and regimen that will be utilized in these initial trials was selected based on past experience with other inactivated flavivirus vaccines. Potential risks related to ZIKV vaccine administration may be similar to those of other inactivated flavivirus vaccines. Two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine developed by WRAIR and a JEV vaccine (IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated.

In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting and fever with the last three AEs occurring after the second administration of the 5 mcg dose. Unsolicited AEs detected among subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

2.3 Rationale for studying an accelerated vaccine schedule

The Phase 1 testing of a one- or two-dose ZPIV vaccine for Zika virus along a compressed schedule will complement the three other Phase 1 trials that are testing a standard prime-boost schedule of 0 and 4 weeks. This trial will test the WRAIR-developed vaccine in 48 healthy adults according to either a one-dose vaccination regimen or two-dose schedules of 0/2 weeks and 0/4 weeks. Investigation into the immunogenicity of a single dose or accelerated schedule of vaccination is salient to vaccine deployment in the setting of an emergent outbreak. The more rapidly a vaccine schedule confers immunity, the better chance an individual has of being protected from disease during the outbreak and the higher likelihood that transmission can be interrupted within populations. An accelerated schedule could not only benefit general populations living in Zika-endemic areas but first responders who need to be rapidly deployed to those areas. Emergency health responders and military personnel would potentially benefit from a shorter vaccination schedule that confers protection equivalent to that afforded by a standard, but more prolonged 1-month regimen. This study will evaluate those abbreviated schedules.

3 PRECLINICAL EXPERIENCE

3.1. *In vitro* characterization of ZPIV

The ZIKV vaccine is formalin-inactivated whole virus vaccine using a 2015 isolate from Puerto Rico (PRVABC59 V3 strain). The vaccine was produced in VERO cells and manufactured via a process that has been used successfully for other flavivirus vaccines (*e.g.* JEV vaccine). The clinical material used to develop the ZPIV was manufactured in Vero cells cultured in medium containing heat inactivated fetal bovine serum, Neomycin, and Streptomycin. Following infection with ZIKV (Puerto Rico PRVABC59 strain), culture supernatants were collected, and clarified by centrifugation and filtration (0.45 µm followed by 0.22 µm). The clarified viral fluids were treated with Benzonase to remove cellular DNA, and then concentrated by ultrafiltration followed filtration (0.45 µm). The concentrated virus was purified by column chromatography using a Capto™Core 700 column. The virus-containing fractions were pooled. The purified pool was filtered (0.22 µm). Sucrose was added and the virus was inactivated for 7 days at 22 °C with 0.05% formalin (1:2000 dilution of 37% formaldehyde). On day 2 of the inactivation, the virus was filtered (0.22 µm). Following inactivation, the formalin-treated virus pool was filtered (0.22 µm), concentrated to 50 µg/mL, and diafiltered to remove residual formaldehyde. Sucrose in PBS was added to a final concentration of 3% sucrose. The bulk was sterile filtered with a 0.22 sterile in-line filter. Inactivation was confirmed by inoculation of Vero cells. The purified inactivated virus (PIV) was then adsorbed to aluminum hydroxide (Alhydrogel®), which will be used to further dilute the adjuvanted vaccine to attain the moderate and low doses (i.e., 2.5 mcg and 1.25 mcg, respectively). Use of alum as an adjuvant is well established and generally well tolerated.

3.2 *In vivo* characterization of ZPIV

ZPIV has been tested in both mouse and rhesus monkey models. A single dose of purified inactivated virus vaccine induced high titers of ZIKV neutralizing antibodies and conferred complete protection, as measured by viremia, in both susceptible mice and nonhuman primates against challenge with a ZIKV strains from Brazil and Puerto Rico. Purified IgG from vaccinated animals also conferred passive protection in adoptive transfer studies in mice. CD4 and CD8 T lymphocyte depletion in vaccinated mice did not abrogate protective efficacy. These data demonstrate that protection against ZIKV challenge can be achieved by single-dose ZPIV and that humoral immunity alone is sufficient for that protection. These studies were carried out with non-GLP vaccine material. Overall, no clinical adverse events or clear changes in hematologies or chemistries were observed in rhesus monkeys following ZIKV PIV vaccination at weeks 0 and 4 (Larocca 2016) (Abbink 2016).

4 CLINICAL EXPERIENCE

4.1 Clinical experience with ZPIV in humans

While there is no human clinical experience with ZPIV, two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine (WRAIR) and a JEV vaccine (manufactured as IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated. In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting, and fever with the last three AEs occurring after the 2nd administration of the 5 mcg dose. Unsolicited AEs detected in subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

A formaldehyde-inactivated JEV vaccine (IXIARO®) has been licensed for use in the US since 2009. The package insert indicates that the most common adverse reactions reported were headache, myalgia, influenza-like illness, and fatigue (Valnera Austria GmBH, 2015). No effect was seen on the safety profile of IXIARO® compared to placebo (aluminum hydroxide) when examined by age, sex, or ethnic origin. In a Phase 3 clinical trial involving 2675 subjects who received either two doses of IXIARO® (6 mcg) or placebo (phosphate buffered saline [PBS] plus aluminum hydroxide), there was little difference between the active and placebo groups (Valnera Austria GmBH, 2015). The overall percentage of subjects who experienced at least one AE was 59% in the active group versus 57% in the placebo. Injection site reactions were mild to moderate in severity, and consisted of (in descending order of occurrence) pain, tenderness, erythema, induration, edema and pruritus. The most common systemic AEs post first dose and second dose were headache, myalgia, fatigue, influenza-like illness, nausea, nasopharygnitis, and fever. Sixteen SAEs were reported in 10 subjects who received vaccine and 6 subjects who received placebo. The SAEs that occurred among subjects in the IXIARO® group were dermatomyositis, appendicitis, rectal hemorrhage, limb abscess involving contralateral arm, chest pain, ovarian torsion, ruptured corpus luteal cyst, and three orthopedic injuries. No deaths occurred in the trial. IXIARO® is also licensed at one half the dose (3 mcg) for infants and children aged 2 months to <3 years; the full strength is recommended for use in older children. Fever was the most commonly observed AEs up to 12 years of age.

The association of ZIKV infections with the rare autoimmune disease, Guillain-Barre syndrome (GBS) and acute disseminated encephalomyelitis (ADEM) is a concern for ZIKV vaccines in general. In natural ZIKV infections, it is not yet clear whether the increased incidence of GBS is the result of an autoimmune reaction or is the result of neurotropism of the virus. Meningoencephalitis from ZIKV infection has also recently been reported.

In summary, inactivated flavivirus vaccines appear to be reasonably well tolerated. However, typical safety considerations with inactivated flavivirus vaccines include local reactions of pain, tenderness, erythema, induration, edema and pruritus, and mild to moderate systemic reactions including headache, myalgia, fatigue, influenza-like illness, nausea, and fever. The potential for a vaccine to induce GBS is not known.

5 OBJECTIVES

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 90, 180, 270, and 365 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

6 STUDY DESIGN

This is a phase 1 trial of one or more administrations of Zika Virus Purified Inactivated Vaccine (ZPIV). The trial will be conducted under a placebo controlled, double-blind, randomized allocation of study product. This design is intended to reduce the likelihood of observer bias, provide control for confounding variables of intercurrent illness, and aid in the interpretation of laboratory data. There are four groups in the study and blinding will be maintained to vaccine or placebo within each group as noted in the table below.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV	ZPIV		
	B	2		Placebo	Placebo		
4	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		48					

It is anticipated that enrollment will be completed within a 6-month period, and that participants will be actively followed for 12 months. Groups 1 through 4 will be enrolled in sequence to accelerate the collection of peak immunogenicity time-points from the groups with longer schedules (peak time-points are D56, D42, D35, and D28 for Groups 1-4, respectively).

6.1 Safety Assessment

To assess the safety of the administered vaccine, participants will document local and systemic reactogenicity on the first day of each vaccine administration period (days 0, 7, 14, and/or 28 depending on the group assignment) and for the subsequent 6 days following each vaccine administration. The investigator will interview each participant about AEs at each visit throughout the study. Two to 3 days after each vaccine administration, a member of the site staff will have a remote safety follow-up communication with the participant by telephone. The participant will be questioned about occurrence of reactogenicity. The participant will be brought in for a clinic visit based on this assessment, if deemed necessary by the investigator/sub-investigator or upon request of the participant. All participants will also be seen in clinic at the end of each reactogenicity period (days 7, 14, 21, and/or 35 depending on the group assignment). All reactogenic events and AEs will be recorded on case report forms (CRFs) from the signing of the informed consent form (ICF) until the last study visit. To assess the participants' cellular and humoral immune response, blood samples will be taken at the clinic visits. Safety and immunogenicity visits will be conducted as outlined in the Schedule of Procedures (SOP).

6.2 Immunogenicity Assessment

Blood samples will be obtained at various time points after immunization (Section 18.3). These will be assayed for the magnitude of neutralizing and binding antibody titers to Zika virus at 14 and 28 days following each administration of ZPIV, as well as at 90, 180, 270 and 365 days following the first dose of ZPIV. The primary readout for neutralizing antibody assays will be the results of pseudovirus reporter and microneutralization assays. The secondary readout will be the result of plaque reduction neutralization assays. The third readout will be binding antibody titers by ELISA. Exploratory assessments may include epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, and Zika-specific T cell responses.

6.3 Monitoring

The Protocol Safety Review Team (PSRT) will review all adverse events (AEs) on a regular and expedited basis as needed. In addition, the PSRT will review safety data reports on a weekly basis until 12 participants have been enrolled after which reports will be reviewed biweekly. The PSRT will include the Study Chair(s), co-investigators, and representatives from WRAIR and VRC/NIH. Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs should also be reviewed by the Safety Monitoring Committee (SMC). The SMC will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC will be completely independent from the protocol team and sponsor. The SMC will evaluate safety and tolerability data on a regular basis. The SMC may review an individual SAE or it may choose to review AEs, SAEs, and laboratory and vital sign data. The SMC may unblind any amount of safety information needed to conduct their assessment. The conclusions of the SMC will be communicated to the investigators and the IRB/Ethics Committees and the national regulatory authorities as appropriate. The sponsor agrees to abide by the decision of the SMC and any directives issued by the national regulatory authorities, the Institutional Review Boards or Ethics Committees.

6.4 Randomization Procedures and Enrollment

Participants will be enrolled in the study after ascertainment by one of the study investigators that all of the inclusion and none of the exclusion criteria have been met. Once confirmation of eligibility for the trial has been performed, the participant will be randomized. Randomized treatment assignments will be generated by the Data Management Center (DMC). After successful randomization, an allocation number is provided. The DMC will create the randomization table and the sponsor will monitor the implementation of this process. The allocation number is referenced against a confidential list provided to an unblinded on-site pharmacist to determine the assignment/treatment allocation.

The 12 participants in each group will be randomized in a ratio of 5 vaccinees to 1 placebo recipient.

6.7 Method of Blinding and Unblinding

The participants, clinical staff, investigator, and sponsor personnel will be blinded to treatment allocation throughout the study. The pharmacist with primary responsibility for vaccine

dispensing will not be blinded to the treatment and will maintain the randomization code and complete assignments of participants according to the randomization allocation. Routine unblinding of treatment allocations may occur only after all participants have had their last study visit and the database is locked.

7 STUDY POPULATION

The study population will include healthy men or women aged 18-50 years old who are able and willing to provide written informed consent. Participants will be enrolled in the study once eligibility criteria are met.

7.1 Participant Inclusion Criteria

1. Age 18-50 years old.

2. Ability and willingness to provide informed consent.

3. Assessment of understanding: completion of a questionnaire prior to first screening procedure; verbally demonstrate understanding of all questionnaire items answered incorrectly.

4. Available for the duration of the trial.

5. Good general health as shown by medical history, physical exam, and screening laboratory tests.

6. The following laboratory parameters:

    - Hematology
        - Hemoglobin ≥10.5 g/dL for women; ≥11 g/dL for men
        - Absolute Neutrophil Count (ANC): ≥1000/mm^3
        - Platelets: 125,000 to 550,000/mm^3
    - Chemistry
        - Creatinine: <1.1 x upper limit of normal (ULN)
        - AST: <1.25 x ULN
        - ALT: <1.25 x ULN
    - Normal urinalysis
        - Negative urine glucose.
        - Negative or trace urine protein.
        - Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis within institutional range).

7. All female participants must be willing to undergo serum or urine beta human chorionic gonadotropin pregnancy tests at time points indicated in the Schedule of Procedures and must test negative prior to vaccination.

8. All sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until Week 12.

9. If a woman of child-bearing potential, committed to use an effective method of contraception when sexually active with men until Week 12, including:

- Condoms (male or female) with or without spermicide.
- Diaphragm or cervical cap with spermicide.
- Intrauterine device.
- Hormonal contraception.
- Successful vasectomy in the male partner (considered successful if a woman reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity post-vasectomy).
- Not be of reproductive potential, such as having undergone hysterectomy, bilateral oophorectomy, or tubal ligation.

Participant Exclusion Criteria:

1. History of known flavivirus infection or previous receipt of flavivirus vaccine.

2. Positive serology for HIV-1, Hepatitis B surface antigen, or anti-hepatitis C virus antibodies prior to enrollment.

3. Planned travel to areas with active Zika virus transmission during the study period.

4. Recent (within 3 weeks) travel to an area with active Zika virus transmission.

5. Current or planned participation in another clinical trial of an experimental agent during the study period.

6. Pregnant or lactating.

7. Any condition, including any clinically significant acute or chronic medical condition, for which, in the opinion of the investigator, participation would not be in the best interest of the subject (eg, compromise the well-being) or that could prevent, limit, or confound the protocol-specified assessments.

8. Use of anticancer, antituberculosis or other medications considered significant by the investigator within the previous 6 months.

9. Receipt of live-attenuated vaccine within the previous 60 days or planned receipt within 60 days after vaccination with Investigational Product (within 14 days for live attenuated influenza vaccine [LAIV]); or receipt of other vaccine (e.g., influenza, pneumococcal), allergy treatment with antigen injections or tuberculin skin test within the previous 14 days or planned receipt within 14 days after vaccination with Investigational Product

10. Receipt of blood transfusion or blood-derived products within the previous 3 months.

11. Previous severe local or systemic reactions to vaccination.

12. History of splenectomy

13. History of seizure in the last 3 years (participants with a history of seizures who have neither required medications nor had a seizure for 3 years are not excluded)

14. Known autoimmune disease

15. Asthma other than mild, well-controlled asthma. Exclude participants who:
    a. Use a bronchodilator (beta 2 agonist) daily, or
    b. In the past year have (any of the following):
        i. Had > 1 exacerbation of symptoms treated with oral steroids
        ii. Routinely used moderate to high dose inhaled corticosteroids (e.g., more than the equivalent of 250 mcg fluticasone; 400 mcg budesonide; 500 mcg beclomethasone; or 1000 mcg triamcinolone/flunisolide, as a daily dose) or theophylline
        iii. Needed emergency care, urgent care, hospitalization, or intubation for asthma
    c. Prophylactic bronchodilator use prior to exercise is not exclusionary

16. Diabetes mellitus type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

17. Thyroidectomy, or thyroid disease requiring medication during the last 12 months

18. Angioedema within the last 3 years if episodes are considered serious or have required medication within the last 2 years

19. Uncontrolled Hypertension:
    a. If a person has been diagnosed with hypertension during screening or previously, exclude for hypertension that is not well controlled. Well- controlled hypertension is defined as blood pressure consistently ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm
    b. If a person has NOT been diagnosed with hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 90 mm Hg at enrolment

20. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

21. Malignancy (Not excluded: a participant with a surgical excision and subsequent observation period that in the investigator's estimation has a reasonable assurance of sustained cure or is unlikely to recur during the study period)

22. Psychiatric condition that compromises safety of the participant or precludes compliance with the protocol, specifically excluding persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years

8 STUDY PRODUCTS

8.1 Study Products

Detailed instructions for study products including preparation, storage and documentation are provided under separate cover in the Study Operations Manual. Additional information is also provided in the Investigator's Brochure for the ZPIV product.

8.1.1 Zika Virus Purified Inactivated Vaccine (ZPIV)

Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at manufacturing facility prior to shipment to clinic.

8.1.2 Placebo

The placebo is saline.

8.2 Handling of Study Treatments

8.2.1 Packaging and Labeling

Each single-use 2 mL vial of ZPIV vaccine contains 10 µg/mL ZPIV antigen and 1 mg/mL aluminum hydroxide adjuvant with a fill volume of 0.7 ± 0.07 mL of a sterile, preservative-free, PBS solution, for 5 µg PIV protein and 500 µg aluminum hydroxide adjuvant per 0.5 mL dose and for intramuscular injection only.

8.2.2 Shipment and Storage

ZPIV vials should be stored at 2-8 °C. During storage, a clear liquid with a white precipitate will be observed; this is the alum adjuvant and this appearance is to be expected following refrigerated storage. **Do Not Freeze.**

8.2.3 Dose preparation and administration

The unblinded pharmacist (or other unblinded staff member qualified to handle and dispense medication) will dispense ZPIV or placebo in a pre-filled capped syringe with the participant's study ID and allocation number. The vaccine vial should be shaken well to disperse and fully resuspend the alum-adsorbed vaccine, after which it should appear as a turbid white to slightly yellow suspension with white particulates.

9 CONDUCT OF THE STUDY

9.1 Informed Consent

The informed consent form documents that a participant (1) understands the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in this study. Informed consent encompasses all written or verbal study information staff provide to the participant before and during the trial.

The informed consent process continues throughout the study. At each study encounter, staff should consider reviewing the procedures and requirements for that encounter and for the remaining encounters. Additionally, if any new information is learned that might affect the participant's decision to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign a revised informed consent form.

Participants must sign a screening or protocol-specific consent before any procedures to determine eligibility are performed. All recruitment and prescreening materials will be approved by the Institutional Review Board.

9.1.1 Screening Consent Form

An IRB approved general vaccine screening protocol and consent may be used as part of the initial screening procedure for this trial. Results from this IRB approved general screening or prescreening may be used to determine eligibility in this protocol, provided the tests are conducted within the time period specified in the eligibility criteria.

9.1.2 Protocol-Specific Consent Form

The protocol-specific consent form describes the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. The consent form is developed in accordance with IRB requirements and the principles of informed consent as described in Title 45, Code of Federal Regulations (CFR) Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonization (ICH) Guideline 4.8.10. It must be approved by all responsible ethical review bodies before any participants can be consented for the study.

9.1.3 Test of Understanding

Study staff should ensure that participants understand the study before screening them. This process involves reviewing the informed consent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely. A Test of Understanding is used to document the participant's understanding of key concepts in this Zika vaccine trial. The participant must complete the Test of Understanding—with the assistance of staff, if necessary, in reading and understanding the questions and responses—before screening. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

9.2 Clinical and Laboratory Evaluations – Participants

Please see Section 18.3 for a concise outline of study procedures. Screening should occur within 56 days of enrollment. For visit windows, refer to the Schedule of Procedures (Section 18.3).

9.3 Unscheduled Visits

Unscheduled Visits/Contacts are visits/contact that are not described in the Schedule of Procedures (Section 18.3). Unscheduled visits may occur any time during the study:

- For administrative reasons, e.g., the participant or household contact may have questions for study staff or may need to re-schedule a follow-up visit.
- To obtain laboratory test results from a previous visit.
- For other reasons as requested by the participant or site investigator.

All unscheduled visits will be documented in the participant's study records on applicable source documents and entered into the Case Report Form (CRF).

9.4 Unblinding Visit

After all participants (from all 4 groups) have completed the active follow-up part of the study, and the database is locked, unblinding will occur. Participants will be informed as to whether or not they received study product or placebo. The unblinding visit may be performed in person or by phone.

9.5 HIV Counseling and Testing

HIV testing will be performed as part of screening evaluations. Therefore, HIV counseling will also be performed in compliance with the CDC's guidelines and local guidelines for HIV counseling, testing, and referral. All participants who become HIV-infected during the study will be terminated from the study. Potential and enrolled participants identified as HIV-infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.6 Prior and Concomitant Treatment

All concomitant medications will be recorded on CRFs from the signing of the ICF until the last study visit. Study participants can receive medications such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or antihistamines as needed although they must be documented. Use of these medications as routine prophylaxis prior to study vaccination is discouraged.

9.7 Study discontinuation

A participant will be taken out of the study in the event of:

- Repeated failure to comply with protocol requirements
- Decision by the study sponsor of PI to stop or cancel the study
- Decision by local regulatory authorities or IRB to stop or cancel the study
- Participant's request

9.7.1 Early discontinuation or withdrawal of participants

Each participant has the right to withdraw from the study at any time for any reason without affecting the right to treatment by the investigator/designee. The investigator should make an attempt to contact participants who did not return for scheduled visits or follow-up. Although the participant is not obliged to give reason(s) for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights.

The investigator also has the right to withdraw a participant, e.g. because of worsening health status, intercurrent illness, AEs, or pregnancy (for pregnancy follow-up see Section 10.4). The sponsor reserves the right to request the withdrawal of a participant due to safety issues, protocol violations, or administrative or other reasons.

Any unnecessary withdrawal should be avoided. Should a participant be withdrawn, all efforts should be made to complete and report the observations as thoroughly as possible. Whenever a participant is withdrawn from the study, independent of the reason, a final evaluation must be completed for that participant and the primary reason for which the participant was withdrawn must be stated. All documentation concerning the participant must be as complete as possible.

For those participants who are unable to continue participation in the study, but who do not withdraw consent, an early termination visit will be conducted. Any participant who withdraws consent will not have any further data collected after consent has been withdrawn.

9.7.2 Premature termination of the study

The sponsor reserves the right to discontinue the study for safety, ethical, or administrative reasons. Should the study be discontinued, no further vaccinations will be administered but participants who are still actively participating at the time of discontinuation will be followed through the remainder of their follow up visits.

9.7.3 Study pausing rules

If the trial is placed on safety pause, all enrollment and vaccinations will be suspended pending review. The AEs that may lead to a safety pause or prompt PSRT AE review are summarized below in Table 2. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the PI or PSRT, or participant may be threatened.

Table 2: AE notification and safety pause/AE review rules

Event and relationship to study product	Severity	Occurrence	Site PI action	PSRT or SMC action
SAE, related[1]	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
SAE, not related[2]	Grade 5	Any	Phone, email or fax forms to PSRT chair within 24 hours	PSRT review within 2 business days to consider pause
AE[3], related	Grade 3 or 4[4]	Second[5]	Phone, email or fax notification to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AE[3], related	Grade 3 or 4[4]	First	Phone, email or fax notification to PSRT chair within 24 hours	PSRT review within 2 business days to consider pause

[1] Related SAE refers to SAE deemed to be definitely, probably, or possibly related to study vaccine.

[2] Not related SAE refers to SAE deemed to be probably not related or not related to the study vaccine.

[3] Does not include the following reactogenicity symptoms (fever, fatigue/malaise, myalgia, arthralgia, chills, headache, nausea, vomiting).

[4] If no evidence of disease is present other than an abnormal laboratory value, the test must be repeated (entailing blood re-draw) at least one time. The verification period will be a maximum of 72 hours after initial awareness of the abnormal laboratory value. When signs and symptoms are present, repeat test will not be needed.

[5] PSRT will determine whether the reported related AE (Grade 3 or 4) is a second occurrence of a previously reported AE (Grade 3 or 4).

For events in the table above, the Site Principal Investigator (PI) notifies the PSRT (within 24 hours). The PSRT will convene within two business days to review these AEs. The PSRT will review and determine disposition (including whether the SMC needs to review the event).

If a study pause is triggered, all enrollment and vaccinations will be held until review by the SMC. Resumption of enrollment and study treatment may be determined by the SMC (in consultation with the FDA, if required) following a cumulative review of the available safety data. If a decision to resume study enrollment and study treatment administration is made, the SMC will record its judgment in a memorandum to the study file and notify the sponsor, who will then forward the memorandum to the principal investigators. The clinical site will be allowed to resume activities upon receipt of written notification from the sponsor or its designee. As needed, the appropriate regulatory authorities will be informed in writing of the decision by the SMC to resume or discontinue study activities. The site is responsible for notifying the IRB according to local standards and regulations. The sponsor is responsible for notifying the FDA.

9.8 Laboratory evaluations

The total approximate volume of blood that will be collected from each participant is presented in Section 18.3. Total blood volume drawn from each participant will not exceed the American Association of Blood Banks (AABB), and US Food and Drug Administration (FDA) guidelines of 550 mL in any eight-week period.

All biological samples must be collected in the appropriate manner. The investigator will ensure that the personnel and laboratory under his/her supervision comply with these requirements.

Further details on shipment, handling, and storage of the samples are provided in the Specimen Handling Guidelines.

Any residual samples will be stored indefinitely following completion of the study at the sponsor-designated laboratories. Any future analyses conducted on the samples will maintain participant anonymity. Participants will have to provide their approval for long-term storage of their biological specimens. Participants will have the right to opt out of having their biological specimens stored once all analyses in the Informed Consent Form are completed. Opting out of this procedure does not impact the participant's ability to participate in the study.

9.9 Potential risks and benefits

9.9.1 Risks related to vaccines

Participants may exhibit general signs and symptoms associated with the intramuscular administration of a vaccine or placebo. Side effects, if observed, are expected to be short term, mild, and not requiring treatment.

Participants may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, hives or even difficulty breathing. Severe reactions are rare. Medications are available at the clinical site to treat serious allergic reactions.

The effect of this vaccine on a fetus or nursing baby is unknown, as well as the effect on semen. Therefore, sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until 12 weeks after final immunization (Week 16, 14, 13, and 12 for Groups 1-4, respectively). Female participants of child-bearing potential will be required to agree to use birth control for sexual intercourse beginning prior to the vaccination and continuing through Week 12 weeks after final immunization (Week 16, 14, 13, and 12 for Groups 1-4, respectively). Women who are pregnant or nursing will be excluded from the study.

9.9.2 Blood draws

Blood drawing may cause pain, bruising, and, rarely, infection at the site where the blood is taken.

9.9.3 Unknown risks

There may be other serious risks that are not known. Participants may believe that this vaccine provides protection against acquiring Zika infection, and therefore not use appropriate precautions to avoid Zika infection. They will receive extensive counseling throughout the study to address this potential problem. It is not known if the study vaccine increases, decreases, or has no effect on the chance of becoming Zika infected when exposed, or if upon becoming Zika infected, the person's disease course will be more or less severe.

9.9.4 Potential benefits

There is no direct benefit to the participant for participation in this clinical trial. Although study participants may benefit from clinical testing and physical examination, they may receive no direct benefit from participation. Society may benefit from knowledge gained in this study that may aid in the development of a Zika vaccine.

10 SAFETY ASSESSMENTS

10.1 Adverse events

10.1.1 Definitions

Adverse events

An AE is any untoward medical occurrence in a patient or participant, and does not necessarily have a causal relationship with a medical treatment. This includes any noxious, pathological, or unintended change in anatomical, physiological, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory-detected changes. These might occur in any phase of the clinical study whether associated with the study vaccine or not. This includes exacerbations of pre-existing conditions or events, intercurrent illness, or vaccine or drug interactions. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation are not considered AEs. Discrete episodes of chronic conditions occurring during a study period should be reported as AEs in order to assess changes in frequency or severity.

Serious adverse events

Seriousness refers to the outcome of an AE. Seriousness is determined by both the investigator and medical monitor, and can also be determined by the sponsor (*FDA 2010 Guidance for Industry and Investigators; Safety Reporting Requirements for INDs and BA/BE Studies*). An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in **death.**
- Is **life-threatening**: i.e. the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Results in persistent or significant **disability/incapacity**: i.e. results in a substantial disruption of the participant's ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as headache, nausea, vomiting, diarrhea, influenza, infusion site reactions, or accidental trauma (e.g. sprained ankle).
- Requires in-patient **hospitalization** or prolongation of existing hospitalization: i.e. the participant is detained (usually at least an overnight stay) at the hospital or emergency ward for treatment that would not have been appropriate in the physician's office or in an out-patient setting. Hospitalization for either elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures[1] (including hospitalization for 'social' reasons) that are not the result of an AE, are **not** considered as SAEs.
- Is a **congenital anomaly or birth defect** in the offspring of a study participant
- Is an **important medical event** that may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above: e.g. interventions such as intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization, or development of drug

[1]A procedure that may take place during the study period and should not interfere with the study drug administration or any of the ongoing protocol-specific procedures.

dependency or drug abuse. Based on medical and scientific judgment, these events should usually be considered serious.

A suspected transmission of any infectious agent via a medicinal product is always considered as an important medical event, i.e. an SAE.

Although **not** considered as an SAE, cancer should be reported in the same way as SAEs.

Note: If anything untoward is reported during an elective procedure, that occurrence must be reported as an AE, either serious or non-serious according to the criteria defined above.

When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious.

Adverse reactions
An adverse reaction is an AE judged to be related to study vaccine.

Related AEs (adverse reactions) are defined as those judged by the investigator, sponsor or independent safety monitor to be possibly, probably, or definitely related to study vaccine.

When an AE is judged to be related to study vaccine and also is judged to be serious and unexpected, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting. All SAEs in this study will be considered unexpected as there are no expected AEs associated with this vaccine product.

Adverse reactions will be reported in accordance with FDA 2009 Guidance for Clinical Investigators, Sponsors and IRBs; Adverse Events Reporting to IRBs – Improving Human Participant Protection.

10.1.2 Surveillance, reporting, and documentation of adverse events

The recording of AEs is an essential part of study documentation. The investigator is responsible for documenting all AEs as set out in the following sections.

10.1.2.1 Surveillance of adverse events

At each visit through 28 days following last vaccination, all AEs, either observed by the investigator or one of his/her clinical collaborators, or reported by the participants spontaneously or in response to a direct question, will be evaluated by the investigator or designee. All SAEs will be reported through the final visit (Study Day 365).

Participants will be instructed to contact the investigator immediately should he/she experience any signs or symptoms he/she perceives as severe from the time of vaccination through a period of 12 months.

10.1.2.2 Documentation of adverse events

All AEs will be collected on case report forms (CRFs) from the signing of the ICF until 28 days following last vaccination. SAEs will be collected through Study Day 365.

AEs and SAEs should be documented in terms of signs and symptoms observed by the investigator or reported by the participants. Whenever possible, a medical diagnosis should be made. The nature of each event, date and (where appropriate) time of onset, outcome, severity, and causal relationship should be established. Details of any symptomatic/corrective treatment should be recorded on the appropriate page of the CRF.

Hospitalization for routine clinical procedures (or other reason) that is not the result of an AE, are not considered AEs but will be recorded on the AE page of the CRF ('Hospitalization, Not an AE'). The same applies for hospitalization for elective procedures related to a pre-existing condition that did not increase in severity or frequency during the study. If hospitalization was planned before administration of the study vaccine, it will be documented in the Medical History page of the CRF (see below).

The following events will be documented in the Medical History page of the CRF:

- Pre-existing conditions or signs and/or symptoms present in a participant before study start. This includes conditions that were not recognized at study entry but later during the study period.
- Hospitalization arising from a pre-existing condition and planned before the administration of the vaccine.

10.1.2.3 Post-vaccination reactions occurring immediately after each vaccine administration

Participants will be observed for minimum of 30 minutes following vaccine administration. Vital signs (temperature, blood pressure, pulse and respiratory rate) will be taken and qualified study personnel will evaluate for any signs or symptoms of reactions.

Possible reactions may include fever (≥37.7 ºC), fatigue/malaise, headache, myalgia, arthralgia, chills, nausea, vomiting, and arm pain. For life-threatening allergic reactions that occur immediately post-vaccination, the site has specific procedures developed for handling such emergencies.

10.1.2.4 Reactions occurring within seven days following each vaccine administration

Participants will be instructed to notify specified study personnel immediately if any unusual or severe sign or symptom appears after vaccine administration. Participants will maintain a study diary during this period to record potential reactions.

10.1.3 SAE Reporting

The sponsor has a regulatory obligation to report SAEs to the FDA according to established timetables for reporting based on specific criteria. The investigator will report SAEs to the PSRT and IRB within 24 hours from the time they first learn of the event. The SAE form will be completed as thoroughly as possible and signed by the investigator or his/her designee before

transmittal to the PSRT and IRB. The investigator will provide his/her assessment of causality to study treatment at the time of the initial SAE report. The investigator will not delay in the reporting of any SAE in order to obtain additional information. Any additional information, if collected, will be reported to the PSRT and IRB as a follow-up to the initial report.

All SAEs must be reported immediately (within 24 hours of discovery) by email or fax to the IRB at the following contact information:

Email: Jessica Ripton, jripton@bidmc.harvard.edu
Fax: (617) 975-8501

The PSRT will perform a clinical review of the information provided to identify any missing data. The PSRT will also contact the study site to clarify any discrepant or missing information, to answer questions and to provide guidance to the site, if needed. The investigator will report the SAE as an acceptable medical diagnosis. If a preliminary diagnosis has not yet been made, then each symptom will be listed separately. A follow-up report will be issued when a diagnosis is made.

The investigator must report SAEs to the appropriate IRB as requested by the board according to local legal requirements.

10.2 Reporting requirements to the local IRB

The PI/designee will be responsible for providing all safety reports and reporting all SAEs, study pauses, social harms, and major deviations to the local regulatory authorities, such as a local IRB, and any regulatory agencies, in a timely manner according to the institution's guidelines and to local regulations.

10.3 Causality assessment of study vaccine to adverse events

Every effort should be made by the investigator to explain any AE and assess its potential causal relationship, i.e. to administration of the study treatment or to alternative causes (e.g. natural history of the underlying diseases, concomitant therapy). This applies to all AEs, whether serious or non-serious.

The investigator will use the following guidelines to assess the causal relationship of an AE to study injection:

Not related:	Adverse experiences felt to be due to extraneous causes that neither follows a known pattern of response nor a reasonable temporal relationship to study vaccine.
Remote (probably not related):	Adverse experiences that are unlikely to be related to study vaccine but which follow a reasonable temporal relationship, such that this cannot be completely excluded or events that could be associated with study vaccine but which are unrelated in time.
Possibly related:	Adverse experiences that may be due to extraneous causes but which follow a known pattern of response and/or a reasonable

temporal relationship to study vaccine.

Probably related:	Adverse experiences that cannot be explained by extraneous causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.
Definitely related:	Adverse experiences that have a definite relationship to the study vaccine (e.g. anaphylactic reaction after vaccination) without any other explained causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.

In the final analyses, events categorized as “not related” and “remote” will be considered as not related to study vaccine; events categorized as “possibly related”, “probably related” and “definitely related” will be considered as related to study vaccine.

10.3.1 Severity of adverse events

All AE and lab data will be coded for severity using the Division of Microbiology and Infectious Disease Revised 2013 Toxicity Table included in Section 18.1, and also located on the website: https://www.niaid.nih.gov/labsandresources/resources/dmidclinrsrch/Pages/pharma.aspx.

For AEs not identified in the grading table, the following guidelines will be applied:

Mild	Grade 1	Symptoms causing no or minimal interference with usual social and functional activities
Moderate	Grade 2	Symptoms causing greater than minimal interference with usual social and functional activities
Severe	Grade 3	Symptoms causing inability to perform usual social and functional activities
Potentially life-threatening	Grade 4	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability
Fatal	Grade 5	For any AE where the outcome is death, the severity of the AE is classified as Grade 5

The clinical research site team will ascertain accurate recording of all AEs during the study. AE CRFs will be completed by the clinical research site staff on a daily basis as the data become available from the isolation unit, clinic or laboratory.

The investigators will monitor and analyze study data including all AE and lab data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study vaccine. AEs will be deemed either related to study vaccine or not related to study vaccine. To ensure that all AEs are captured in a timely manner, CRFs will be entered in real-time entry, and participanted to analysis to identify AEs that may invoke study pausing rules.

The PI or designee must review AE CRFs to insure prompt and complete identification of all events that require expedited reporting as SAEs, study pausing rules or other serious and unexpected events.

Study vaccine related AEs will be followed by the clinical research site team through resolution or until study completion. Non-study vaccine related AEs will be followed to resolution or study completion, whichever occurs first.

10.3.2 Follow-up of ongoing adverse events and assessment of outcome

10.3.2.1 Follow-up of non-serious adverse events

Non-serious AEs already documented in the CRF at a previous assessment and designated as 'ongoing' should be reviewed at subsequent visits. If the event has resolved, the documentation in the CRF should be completed. If the frequency or severity of a non-serious AE changes significantly, a new record of the AE has to be started. If the AE becomes serious, the procedures for reporting of SAEs have to be followed (see Section 10.1.3).

New non-serious AEs will be recorded through 28 days post-vaccination. Ongoing non-serious AEs will be monitored until the Day 365 visit (or until the last study visit for participants who withdraw early (see Section 18.3)).

Outcomes will be assessed as:

1. Resolved
2. Resolved with sequelae
3. Resolving
4. Not resolved
5. Fatal
6. Unknown

Clinically significant abnormal laboratory values will be followed up until they have returned to normal, stabilized, or a satisfactory explanation has been provided.

10.3.2.2 Follow-up of serious adverse events

All SAEs must be followed-up until the event has either resolved, subsided, stabilized, disappeared, or is otherwise explained, or the study participant is lost to follow-up, but no longer than 12 months after the administration of the study vaccine. Outcomes are assessed as above.

All follow-up activities must be reported in a timely manner to the PSRT (if necessary on one or several consecutive SAE report forms). All form fields with additional or changed information must be completed and the SAE Report Form should be forwarded to the PSRT as soon as possible but at the latest within 7 calendar days after receipt of the new information.

Clinically significant laboratory abnormalities reported as SAEs will be followed-up until they have returned to normal, or a satisfactory explanation has been provided.

Reports related to the subsequent course of any SAE reported for any participant must be submitted to the sponsor.

10.3.3 Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to currently available best treatment. The applied measures should be recorded in the CRF.

10.4 Handling of pregnancy cases

Pregnancy events will be reported through 3 months following final vaccination. All initial reports of pregnancy must be reported to the PSRT by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, stillbirth, and congenital anomaly) are considered SAEs and must be reported using the Serious Adverse Event Form.

Because the effect of the study vaccine on sperm is unknown, pregnancies in partners of male participants included in the study will be reported by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

10.5 Physical examination

Physical examinations will be performed by the investigator or designated medically-trained clinician. The time points of these examinations are specified in Section 18.3. Any abnormalities or changes should be documented in the source document and recorded on the CRF.

A new, clinically significant finding (in the opinion of the investigator) not noted at screening must be captured as an AE. In addition, resolution of any abnormal findings during the study will be noted in the source document and in the CRF.

10.6 Routine safety laboratory

Samples for routine safety laboratory parameters will be collected at the time points specified in Section 18.3.

The following routine safety laboratory parameters will be determined:

- **Serum chemistry:** AST, ALT, and Creatinine
- **Complete blood count:** hemoglobin, hematocrit, white blood cells (WBC), WBC differentiation, red blood cell count (RBC), and platelet count

Laboratory values will be graded according to the DMID toxicity scale (Section 18.1) and, if clinically significant, reported as AEs.

10.7 Vital signs

Vital sign measurements will be performed at time points specified in Section 18.3.

The following measurements will be performed:

- Heart rate (bpm)
- Systolic and diastolic blood pressure (mmHg)
- Respiratory rate
- Body temperature (oral)

A confirmatory vital signs measurement can be performed if inconsistent with a prior measurement. If any clinically significant changes in vital signs are noted, they will be reported as AEs and followed to resolution, or until reaching a clinically stable endpoint.

10.9 Protocol Safety Review Team and Safety Monitoring Committee

The Protocol Safety Review Team (PSRT) will include the Protocol Chair, Protocol Co-Chairs, Site Principal Investigator, co-investigators and partners at WRAIR and VRC/NIH. Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs not requiring a study pause should also be reviewed by the SMC.

The Safety Monitoring Committee (SMC) will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC is an independent multidisciplinary group consisting of experts in flavivirus infection (e.g., dengue, West Nile Virus, etc.) that, collectively, has experience in the management, conduct, and monitoring of vaccine trials. Members of the SMC are not directly affiliated with this protocol and are not situated at the study site. The SMC also will review individual Expedited Adverse Event (EAE) reports. The SMC will conduct additional special reviews at the request of the PSRT.

11 IMMUNOGENICITY ASSAYS

11.1 Zika-associated immunogenicity

Blood samples for the determination of cellular and humoral responses will be collected as specified in Section 18.3.

Humoral response assays to Zika will likely include, but are not limited to: microneutralization assays, ELISA, and plaque reduction neutralization assays. Table 3 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 3: Humoral immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Principle Timepoint(s)
Humoral responses	Secondary	Serum	ZIKV microneutralization (MN) assay	Log10 MN50 titers	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 * Peak
	Secondary	Serum	ZIKV Env ELISA	Log10 endpoint titers	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 * Peak
	Exploratory	Serum	ZIKV plaque reduction assays	Log10 titer	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 * Peak

Evaluations of cellular immune responses to Zika will likely include, but are not limited to, interferon gamma producing cells (ELISPOT) assays. Table 4 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 4: Cellular immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Primary Timepoint(s)
T-cell responses	Exploratory	PBMC	Interferon gamma producing cells (ELISPOT)	Number of spot forming cells per 10^6 PBMC	Group 1: D0, D56*, D180 Group 2: D0, D42*, D180 Group 3: D0, D35*, D180 Group 4: D0, D28*, D180 *Peak

Immunologic assays may be performed and analyzed in a blinded fashion after all participants of a given group reach the peak immunogenicity time-point for their group (D56, D42, D35, and D28 for Groups 1-4, respectively). This will allow more rapid accrual of data to facilitate product

development strategies. The optimal choice of immunogenicity assays will take into consideration the latest technological advances.

11.2 DNA/RNA Assays

Blood samples collected for cellular immunogenicity may also be used for exploratory DNA and RNA micro-array and deep-sequencing assays.

11.4 Additional Immunogenicity Assays

Additional immunogenicity assessments of systemic and mucosal responses may potentially include the following: flavivirus serology, epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, and Zika epitope-specific T cell responses.

12 DATA EVALUATION AND STATISTICS

All data entry will be performed by qualified and trained study staff. When all data have been entered and validated, the final database will be locked. An interim blinded analysis will be performed 28 days following the final immunization (group assignments will be known). A second interim blinded analysis will be performed at the 6 month time-point. Final analysis will be performed when D365 data has been collected.

12.1 Analysis populations and data sets

12.1.1 Safety population

All participants who received ZPIV or placebo, and for whom any post-dose data is available, will be included in the safety population.

12.1.2 Immunogenicity population

The immunogenicity population will consist of all participants who received ZPIV or placebo, and who have at least one measured post-dose blood sample collected.

12.2 Endpoints

Safety and tolerability:

- Incidence, intensity, and relationship to vaccination of solicited local and systemic adverse events (AEs) during the 7-day follow-up period (Days 0-6) after each ZPIV dose.
- Incidence, intensity, and relationship to vaccination of unsolicited AEs during the 28-day follow-up period (Days 0-27 after each ZPIV dose)
- Grade 2 and Grade 3 laboratory abnormalities at Day 7 after each ZPIV dose.
- Incidence of serious adverse events (SAEs) and related AEs from Day 0 through Day 365.

Immunogenicity:

- Principle Endpoints: Proportion (95% CI) of participants per dose group with positive responses and mean response (e.g. GMT) per group with 95% CI for the following 2 parameters:
    - ZIKV microneutralization Log10 MN50 titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D0.
    - Zika Env-specific Log10 endpoint ELISA titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D0.

- Additional Endpoints:
    - Plaque reduction neutralization test titer at 14 and 28 days following each vaccination, and at 3, 6, 9, and 12 months from D0.

- Durability and kinetics of humoral immune responses: Log10 MN50 and Log10 endpoint ELISA titers at 14 and 28 days following *first* (and second) vaccination, and at 3, 9 and 12 months.
- Cellular immunogenicity: IFN-γ ELISPOT responses to prM, Env, Cap, and NS1 peptides at 28 days following final vaccination and at 6 months from D0.

12.3 Sample size consideration

The number of participants chosen for this study will provide a preliminary safety and immunogenicity assessment. The sample size is not based on formal hypothesis testing considerations, but within the range of participants (i.e., 20-80) recommended in the Code of Federal Regulations (CFR 312.21) for first-in-human product Phase 1 evaluations. Placebo recipients are included for blinding and safety purposes and will provide additional control specimens for immunogenicity assays.

13 ETHICAL AND LEGAL REQUIREMENTS

13.1 General requirements

The study will be performed according to this Study Protocol and in compliance with the Declaration of Helsinki, the guidelines of the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) and the respective local legal requirements including the following: US Code of Federal Regulations 45CFR Pt 46; 21CFR Pt 50, 21CFR Pt 56 and 21CFR Pt 312.

13.2 Institutional Review Board/Ethics Committee

Before the start of the study, the investigator will submit the Study Protocol, Informed Consent Form, and other study-related documents as required by applicable laws and regulations to the responsible IRB for written approval.

The investigator will inform the IRB according to applicable laws and regulations about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

Unless otherwise instructed by the IRB or local regulation the investigator must submit to the IRB:

- All subsequent Amendments to the Study Protocol, changes to the Informed Consent Form or revisions of other documents originally submitted for review
- New or revised participant recruiting materials approved by the sponsor, if applicable
- All subsequent changes of logistical or administrative aspects (for information)
- Serious and/or unexpected AEs occurring during the study, where required
- New information that may affect adversely the safety of the participants or the conduct of the study
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Annual update and/or request for re-approval, where required
- Date of study completion, where required

13.3 Regulatory authorities

Before initiating the study, the sponsor will submit any required application to the regulatory authorities and obtain approval according to applicable laws and regulations. The sponsor will also inform the regulatory authorities about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

13.4 Participant information and informed consent

The study informed consent describes the investigational products to be used and all aspects involved in protocol participation. A properly executed written informed consent, in compliance with the Declaration of Helsinki, guidelines of the Council of International Organization of Medical Sciences (CIOMS), the Belmont Report, the US Code of Federal Regulations 21 CFR 50, must be obtained from each participant prior to entry into the trial or prior to performing any unusual or non-routine procedure that involves risk to the participant. The investigator must

provide a copy of the approved informed consent to the participant and a signed copy must be maintained in the participant's record file. Before a participant's participation in the study, it is the investigator's responsibility to obtain this written informed consent from the participant, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or study medications are administered.

All items must be explained by the investigator or designee in a language that is easy to understand. Participants will also be informed that the participation is voluntary and that they have the right to withdraw at any time without giving reasons and without any disadvantages for their subsequent care. Participants will confirm their consent in writing before study start and any study-specific procedure.

Participants must be given enough time to consider participation in the study.

13.5 Indemnity

The Sponsor and Institution are responsible to have appropriate liability insurance. For research-related injuries and/or medical problems determined to result from receiving the Investigational Product, treatment including necessary emergency treatment and proper follow-up care will be made available to the participant free of charge at the expense of the Sponsor.

13.6 Data access and protection

Confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

The collection and processing of personal data from participants enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of participants confidential.

The informed consent includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to the participant's source data/documents for study-related monitoring, audit, IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The participant has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

13.7 Future use and storage and blood samples

Each study participant will be asked to consent voluntarily for their blood samples to be stored for other research studies that may be done after this study is completed. Future testing may involve DNA/RNA tests. For participants unwilling to have their blood samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed after all the tests specified for this study have been concluded.

All samples for which consent has been obtained, and for which additional material is available after study-specified testing is complete, will be stored for future testing. All applicable approvals will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB.

13.8 Reimbursement

Participants and household contacts will be reimbursed for time and inconvenience in accordance with the standards and legal obligations for compensation required by each site. Any applicable guidelines by IRBs/ECs for compensation of research participants and household contacts will be sought and followed.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

Monitoring of clinic activities will be performed regularly to ensure that the study is carried out according to this Study Protocol and in compliance with GCP and applicable legal requirements.

Source documents will be reviewed for verification of consistency with CRF data. The investigator guarantees direct access to source documents for monitoring purposes. Source data verification will be performed in accordance with data protection regulations and guidelines. All information reviewed will be handled according to these rules and regulations.

The monitor will review each participant's data as outlined in the study specific Clinical Monitoring Plan.

14.2 Audit and inspections

Audits and inspections may be carried out by qualified delegates authorized by the sponsor or by authorities. The investigator consents to cooperate and to allow direct access to all source documents and other study-related data during an audit or inspection. All information disclosed will be handled in accordance with applicable data protection rules and regulations.

14.3 Data quality assurance

All CRF data will be entered into a validated, 21CFR Part 11 compliant, computerized clinical data management system.

The site is required to have a plan in place for assuring the quality of the research being conducted.

15 DOCUMENTATION, ARCHIVING, AND PUBLICATIONS

15.1 Documentation

Source documents are original documents, data, and records from which the participant's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, radiographs, and correspondence.

The Investigator and staff are responsible for ensuring maintenance of a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the sponsor, FDA, and/or applicable regulatory authorities.

All CRF entries have to be verifiable by the source data in the participant file. This does not apply to CRF entries that are defined as source data.

15.2 Archiving

The Investigator is responsible for the archiving of the Investigator's file, the participant's file, and the source data according to national and international legal requirements.

Any records related to the conduct of the study may not be destroyed without written authorization by the sponsor. According to GCP requirements, study records should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

15.3 Clinical Study Reports and Publications

The results of the clinical study will be documented in the Clinical Study Report.
The study results may be published and/or presented at scientific meetings. Terms of publication will be addressed in an agreement between the sponsor and participating partners.

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17 PROTOCOL SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the appendices and provide the necessary assurances that this study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirement(s).

Protocol Co-Chairs:

Signed: _____ Date: _____

Kathryn E. Stephenson, MD, MPH
Beth Israel Deaconess Medical Center

Signed: _____ Date: _____

Kayvon Modjarrad, MD, PhD
Walter Reed Army Institute of Research

Sponsor:

Signed: _____ Date: _____

Kathryn E. Stephenson, MD, MPH
Beth Israel Deaconess Medical Center

Site Principal Investigator:

Signed: _____ Date: _____

Kathryn E. Stephenson, MD, MPH
Beth Israel Deaconess Medical Center

18 APPENDICIES

18.1 Toxicity Tables

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE NOVEMBER 2007

Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal R_X = Therapy
Req = Required	Mod = Moderate
IV = Intravenous	ADL = Activities of Daily Living
Dec = Decreased	

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1	**Mild** Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	**Moderate** Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	**Severe** Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible
GRADE 4	**Life-threatening** Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.
- For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.
- Some protocols may have additional protocol specific grading criteria, which will supercede the use of these tables for specified criteria.

HEMATOLOGY				
	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	9.5 - 10.5 gm/dL	8.0 - 9.4gm/dL	6.5 - 7.9 gm/dL	< 6.5 gm/dL
Absolute Neutrophil Count	1000-1500/ mm^3	750-999/ mm^3	500-749/ mm^3	<500/ mm^3
Platelets	75,000-99,999/ mm^3	50,000-74,999/ mm^3	20,000-49,999/ mm^3	<20,000/ mm^3
WBCs	11,000-13,000/ mm^3	13,000-15,000 / mm^3	15,000-30,000/ mm^3	>30,000 or <1,000 / mm^3
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%	----------
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: <100 mg/dL High: >600 mg/dL	Low: < 50 mg/dL ----------	Fibrinogen associated with gross bleeding or with disseminated coagulation
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	51-60 mcg/ml	> 60 mcg/ml
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 -3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Methemoglobin	5.0 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9%	> 20.0 %

CHEMISTRIES				
	Grade 1	Grade 2	Grade 3	Grade 4
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required	< 2.0 mEq/L or abnormal potassium *with* paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	> 7.0 mEq/L or abnormal potassium *with* life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany

CHEMISTRIES (continued)				
	Grade 1	Grade 2	Grade 3	Grade 4
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL or abnormal calcium *with* life threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	1.0 -1.4 mg/dL intensive therapy or hospitalization required	< 1.0 mg/dL or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	1.5 – 1.75 x ULN	> 1.75 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/dL	12.1 – 15.0 mg/dL	>15.0 mg/dL
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or dialysis required

ENZYMES				
	Grade 1	Grade 2	Grade 3	Grade 4
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN

URINALYSIS				
	Grade 1	Grade 2	Grade 3	Grade 4
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts	obstructive or required transfusion

CARDIOVASCULAR				
	Grade 1	Grade 2	Grade 3	Grade 4
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrythmia; hospitalization and treatment required
Hypertension	transient increase > 20 mm/Hg; no treatment	recurrent, chronic increase > 20mm/Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible	end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required	mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment
Pericarditis	minimal effusion	mild/ moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

RESPIRATORY				
	Grade 1	Grade 2	Grade 3	Grade 4
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment	----------
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present	cyanosis: FEV_1 < 25% of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring Oxygen therapy

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids	hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids	physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/day or mild diarrhea last < 1 week	moderate or persistent; 5-7 loose stools/day or diarrhea lasting >1 week	>7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required	hypotensive shock or physiologic consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods	unable to drink fluids; requires IV fluids

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement	incapacitating; or not responsive to narcotic analgesia
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities)	sensory loss involves limbs and trunk; paralysis; or seizures

MUSCULOSKELETAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living	disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living	permanent and/or disabling joint distruction
Myalgia	myalgia with no limitation of activity	muscle tenderness (at other than injection site) or with moderate impairment of activity	severe muscle tenderness with marked impairment of activity	frank myonecrosis

SKIN				
	Grade 1	Grade 2	Grade 3	Grade 4
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15mm	15-30 mm	>30mm	
Erythema	< 15mm	15-30 mm	>30mm	
Edema	< 15mm	15-30 mm	>30mm	
Rash at Injection Site	< 15mm	15-30 mm	>30mm	
Pruritus	slight itching at injection site	moderate itching at injection extremity	itching over entire body	

SYSTEMIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy	intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	39.6 - 40.5 C or 103 - 105 F	> 40 C or > 105 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25-50% > 48 hours	normal activity decreased > 50% can't work	unable to care for self

18.2 Key Study Roles

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18.3 Schedule Of Procedures

Table 2a

Group 1 Schedule:

***Visits 2 and 6 are phone calls**

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7)	D14 (14)	D28 (28) (0)	D31 (3)	D35 (7)	D42 (14)	D56 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Clinical Assessments														
Consent, Demographics	X													
Medical History Review	X	X				X								X
Inclusion and Exclusion	X	X												
Medications[a]	X	X		X	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X		X	X	X	X	X	X	X
Laboratory Tests														
Screening Labs (30 mL)	X													
Other Flavivirus Serologies[e] (20 mL)	X													
CBC w/diff and Chemistry Panel (15 mL)		X		X	X	X		X	X	X	X	X	X	X
Pregnancy[f]	X	X				X								
Humoral (30 mL)		X			X	X			X	X	X	X	X	X
CMI (40 ml)		X			X	X			X	X	X	X	X	X
Study Related Procedures														
Vaccination		**X**				**X**								
Distribute Diary		X				X								
Review Diary[g]				X				X						
Phone AEs [h]			X				X							
Blood Volume (mL)	50	85	0	15	85	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at 0 and 28 day visits

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Table 2b

Group 2 Schedule:

*Visits 2 and 5 are phone calls

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7)	D14 (14) (0)	D17 (3)	D21 (7)	D28 (28) (14)	D42 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Clinical Assessments													
Consent, Demographics	X												
Medical History Review	X	X			X								X
Inclusion and Exclusion	X	X											
Medications[a]	X	X		X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X		X	X	X	X	X	X	X
Laboratory Assessments													
Screening Labs (30 mL)	X												
Other Flavivirus Serologies[e] (20 mL)	X												
CBC w/diff and Chemistry Panel (15 mL)		X		X	X		X	X	X	X	X	X	X
Pregnancy[f]	X	X			X								
Humoral (30 mL)		X			X			X	X	X	X	X	X
CMI (40 ml)		X			X			X	X	X	X	X	X
Study Related Procedures													
Vaccination		**X**			**X**								
Distribute Diary		X			X								
Review Diary[g]				X			X						
Phone AEs[h]			X			X							
Blood Volume (mL)	50	85	0	15	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at 0 and 14 day visits

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Table 2c

Group 3 Schedule:

***Visits 2 and 4 are phone calls**

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7) (0)	D10 (10) (3)	D14 (14) (7)	D21 (21) (14)	D28 (28) (21)	D35 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Clinical Assessments													
Consent, Demographics	X												
Medical History Review	X	X		X									X
Inclusion and Exclusion	X	X											
Medications[a]	X	X		X		X	X	X	X	X	X	X	X
Physical Exam[b]	X	X		X		X	X	X	X	X	X	X	X
Vital signs[c]	X	X		X		X	X	X	X	X	X	X	X
Laboratory Assessments													
Screening Labs (30 mL)	X												
Other Flavivirus Serologies[e] (20 mL)	X												
CBC w/diff and Chemistry Panel (15 mL)		X		X		X	X	X	X	X	X	X	X
Pregnancy[f]	X	X		X									
Humoral (30 mL)		X		X		X	X	X	X	X	X	X	X
CMI (40 ml)		X				X	X	X	X	X	X	X	X
Study Related Procedures													
Vaccination		**X**		**X**									
Distribute Diary		X		X									
Review Diary[g]				X		X							
Phone AEs [h]			X		X								
Blood Volume (mL)	50	85	0	85	0	85	85	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at 0 and 7 day visits

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Table 2d

Group 4 Schedule:

***Visit 2 is a phone call**

Tests and Observations (Days)	D56 to -2	D0 (0)	D3 (3)	D7 (7)	D14 (14)	D28 (28)	D90	D180	D270	D365
Visit	**Screen**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Clinical Assessments										
Consent, Demographics	X									
Medical History Review	X	X								X
Inclusion and Exclusion	X	X								
Medications[a]	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X	X	X	X	X
Laboratory Assessments										
Screening Labs (30 mL)	X									
Other Flavivirus Serologies[e] (20 mL)	X									
CBC w/diff and Chemistry Panel (15 mL)		X		X	X	X	X	X	X	X
Pregnancy[f]	X	X								
Humoral (30 mL)		X			X	X	X	X	X	X
CMI (40 ml)		X			X	X	X	X	X	X
Study Related Procedures										
Vaccination		**X**								
Distribute Diary		X								
Review Diary[g]				X						
Phone AEs [h]			X							
Blood Volume (mL)	50	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 0 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[C] Vital signs will be performed pre and post vaccination at day 0

[d] Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), glucose, BUN, Cr, ALT, AST, CPK

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Serum pregnancy test at screening and urine pregnancy test at vaccination day

[g] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[h] Detail AEs including injection site reactions via telephone on these days

Protocol Title:	A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
Short Title:	Zika Virus Purified Inactivated Vaccine (ZPIV) Accelerated Vaccination Schedule Study
Protocol Number:	Z001
Phase:	Phase 1
Sponsor:	Kathryn E. Stephenson Boston, MA
Sponsor Status:	Sponsor-Investigator
Study Location:	Center for Virology and Vaccine Research Clinical Trials Unit, Beth Israel Deaconess Medical Center
Study Period:	October 2016 – November 2017
Collaborating Institutions:	Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA, Vaccine Research Center/National Institute of Allergy and Infectious Diseases (VRC/NIAID), Bethesda, MD, USA
Date of Protocol Version:	October 26, 2016 3.0

TABLE OF CONTENTS

List of Abbreviations

AE	Adverse event
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCVI	Antibody-dependent cell-mediated virus inhibition
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotranferase
AST	Aspartate aminotransferase
BIDMC	Beth Israel Deaconess Medical Center
CBC	Complete blood count
$CD4^+$	A functional subclass of T cells, helper T lymphocytes (Th), that are necessary for augmentation and coordination of innate and adaptive effector responses, humoral and cellular
$CD8^+$	Cytotoxic T-cells that destroy host cells that have become infected by viruses or other intracellular pathogens
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CRF	Case report form
CTL	Cytotoxic T lymphocyte
CVVR	Center for Virology and Vaccine Research
DNA	Deoxyribonucleic acid
DMC	Data Management Center
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FDA	US Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMS	Global Medical Safety
HIV-1	Human immunodeficiency virus, type 1
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICF	Informed Consent Form
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
mL	Mililiters
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PI	Principal investigator
PO	Per Oral
PSRT	Protocol Safety Review Team
RBC	Red blood cell count
rtPCR	Real-time PCR
SAE	Serious adverse event
SMC	Safety Monitoring Committee
SST	Serum separating tubes
SUSAR	Suspected unexpected serious adverse reaction
$TCID_{50}$	50% Tissue Culture Infective Dose
VP	Viral particles

WBC	White blood cell count
ZPIV	Zika Virus Purified Inactivated Vaccine

1 OVERVIEW

Title

A Phase 1, Randomized, Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine in Healthy Adults

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 90, 180, 270, and 365 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

Study Products and Routes of Administration

- **Zika Virus Purified Inactivated Vaccine (ZPIV) with Alum Adjuvant:** Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at the manufacturing facility prior to shipment to clinic.
- **Placebo**: The placebo product is saline.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV	ZPIV		
	B	2		Placebo	Placebo		
4	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		48					

Participants

Healthy participants aged 18-50 years:

- 40 vaccinees
- 8 placebo recipients
- 48 total participants

Design

Single center, randomized, controlled, double-blind phase 1 trial

Duration per participant

12 months active follow-up per participant

Estimated total study duration

18 months (includes screening and active follow-up)

Investigational New Drug (IND) Sponsor

Beth Israel Deaconess Medical Center, Boston, MA, USA

Vaccine Manufacturer

Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA

Data Management Center (DMC)

The EMMES Corporation, Rockville, MD, USA

Endpoint Assay Laboratories

Center for Virology and Vaccine Research (CVVR), Beth Israel Deaconess Medical Center (BIDMC), Boston, MA, USA

Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA

Vaccine Research Center, National Institute of Allergy and Infectious Diseases (VRC/NIAID), Bethesda, MD, USA

Study Site

Center for Virology and Vaccine Research Clinical Trials Unit, Beth Israel Deaconess Medical Center, Boston, MA, USA

Safety Monitoring

Protocol Safety Review Team (PSRT)
Safety Monitoring Committee (SMC)

2 BACKGROUND AND RATIONALE

2.1 Introduction

Zika virus (ZIKV) is an emerging vector-borne RNA virus that, since early 2015, has caused an increased incidence of systemic disease and neurologic complications in an expanded region of the Western Hemisphere. ZIKV, of the family *Flaviviridae,* is related to other pathogens of global importance to humans, including dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV) and tick-borne encephalitic virus (TBEV). ZIKV was first isolated in Uganda in 1947 from a sentinel rhesus macaque and has since diverged along two lineages: African and Asian. The virus is almost exclusively transmitted by the *Aedes aegypti* mosquito but has been isolated from several other species of the genus *Aedes,* including *A. albopictus, A. africanus* and *A. luteocephalus*. The majority of human ZIKV infections result in an asymptomatic infection or a benign, self-limited acute febrile illness. ZIKV disease typically manifests as a rash, fever, conjunctivitis and arthralgia. Until 2007 when several outbreak clusters erupted in the South Pacific, ZIKV disease was relatively rare and geographically limited. Between 2007 and 2016, however, Zika has been documented in 60 countries and territories, 46 of which recorded their first case in either 2015 or 2016.

A clinical pattern has emerged from the most recent outbreaks that was never observed before. Nine months after local ZIKV transmission was first documented in Brazil, public health officials detected an increase in neonates born with microcephaly in the northeastern part of the country (WHO 2016). Epidemiologic studies, together with *in vitro* and *in vivo* experiments, have confirmed a causal association between ZIKV infections during pregnancy and the consequent occurrence of serious birth defects including microcephaly, brain malformations and ocular defects (Rasmussen SA, 2016). An increase in Guillain-Barré syndrome (GBS), meningitis and encephalitis have also been found in people with primary ZIKV infections (Cao-Lormeau, 2016) (Carteaux G, 2016), (Broutet N, 2016). The neuro-invasiveness of Zika virus infection and its complications ultimately prompted the World Health Organization (WHO) to declare, in February 2016, the emerging ZIKV epidemic as Public Health Emergency of International Concern.

2.2 Rationale for developing and testing a Zika Virus Purified Inactivated Vaccine (ZPIV)

There is no specific treatment or prophylactic currently available for ZIKV disease, other than supportive care and mosquito control. Given the expanding distribution and significant morbidity associated with ZIKV disease, the development of a safe and effective vaccine against ZIKV is a high global public health priority. The ZIKV vaccine to be used in this trial is a formalin-inactivated whole virus vaccine that was produced by the Walter Reed Army Institute of Research (WRAIR). There has been extensive experience with formalin-inactivated vaccines for other viruses, including other flaviviruses, such as the safe and immunogenic experimental dengue purified inactivated vaccine (Martinez 2015) and the licensed IXIARO® Japanese encephalitis vaccine. Experience with these other flavivirus vaccines has informed the design and development of the current ZIKV vaccine. However, the safety, tolerability and immunogenicity of a ZIKV vaccine, including the inactivated vaccine, is still to be determined. Because the safety and immunogenicity profile of this vaccine is still unknown, this Phase I trial, in concert with three others, will address a critical gap necessary to complete an early and rapid assessment of the viability of this ZIKV vaccine candidate. The first two clinical trials will explore the safety and immune response to the vaccine in flavivirus naïve individuals (National

Institutes of Health (NIH) and in participants who have been previously vaccinated with JEV or YFV vaccines (WRAIR). The third trial (NIH) will be conducted in Puerto Rico, whose population has background exposure to dengue virus and potentially yellow fever and West Nile virus as well. The dose and regimen that will be utilized in these initial trials was selected based on past experience with other inactivated flavivirus vaccines. Potential risks related to ZIKV vaccine administration may be similar to those of other inactivated flavivirus vaccines. Two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine developed by WRAIR and a JEV vaccine (IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated.

In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting and fever with the last three AEs occurring after the second administration of the 5 mcg dose. Unsolicited AEs detected among subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

2.3 Rationale for studying an accelerated vaccine schedule

The Phase 1 testing of a one- or two-dose ZPIV vaccine for Zika virus along a compressed schedule will complement the three other Phase 1 trials that are testing a standard prime-boost schedule of 0 and 4 weeks. This trial will test the WRAIR-developed vaccine in 48 healthy adults according to either a one-dose vaccination regimen or two-dose schedules of 0/2 weeks and 0/4 weeks. Investigation into the immunogenicity of a single dose or accelerated schedule of vaccination is salient to vaccine deployment in the setting of an emergent outbreak. The more rapidly a vaccine schedule confers immunity, the better chance an individual has of being protected from disease during the outbreak and the higher likelihood that transmission can be interrupted within populations. An accelerated schedule could not only benefit general populations living in Zika-endemic areas but first responders who need to be rapidly deployed to those areas. Emergency health responders and military personnel would potentially benefit from a shorter vaccination schedule that confers protection equivalent to that afforded by a standard, but more prolonged 1-month regimen. This study will evaluate those abbreviated schedules.

3 PRECLINICAL EXPERIENCE

3.1. *In vitro* characterization of ZPIV

The ZIKV vaccine is formalin-inactivated whole virus vaccine using a 2015 isolate from Puerto Rico (PRVABC59 V3 strain). The vaccine was produced in VERO cells and manufactured via a process that has been used successfully for other flavivirus vaccines (*e.g.* JEV vaccine). The clinical material used to develop the ZPIV was manufactured in Vero cells cultured in medium containing heat inactivated fetal bovine serum, Neomycin, and Streptomycin. Following infection with ZIKV (Puerto Rico PRVABC59 strain), culture supernatants were collected, and clarified by centrifugation and filtration (0.45 µm followed by 0.22 µm). The clarified viral fluids were treated with Benzonase to remove cellular DNA, and then concentrated by ultrafiltration followed filtration (0.45 µm). The concentrated virus was purified by column chromatography using a Capto™Core 700 column. The virus-containing fractions were pooled. The purified pool was filtered (0.22 µm). Sucrose was added and the virus was inactivated for 7 days at 22 °C with 0.05% formalin (1:2000 dilution of 37% formaldehyde). On day 2 of the inactivation, the virus was filtered (0.22 µm). Following inactivation, the formalin-treated virus pool was filtered (0.22 µm), concentrated to 50 µg/mL, and diafiltered to remove residual formaldehyde. Sucrose in PBS was added to a final concentration of 3% sucrose. The bulk was sterile filtered with a 0.22 sterile in-line filter. Inactivation was confirmed by inoculation of Vero cells. The purified inactivated virus (PIV) was then adsorbed to aluminum hydroxide (Alhydrogel®), which will be used to further dilute the adjuvanted vaccine to attain the moderate and low doses (i.e., 2.5 mcg and 1.25 mcg, respectively). Use of alum as an adjuvant is well established and generally well tolerated.

3.2 *In vivo* characterization of ZPIV

ZPIV has been tested in both mouse and rhesus monkey models. A single dose of purified inactivated virus vaccine induced high titers of ZIKV neutralizing antibodies and conferred complete protection, as measured by viremia, in both susceptible mice and nonhuman primates against challenge with a ZIKV strains from Brazil and Puerto Rico. Purified IgG from vaccinated animals also conferred passive protection in adoptive transfer studies in mice. CD4 and CD8 T lymphocyte depletion in vaccinated mice did not abrogate protective efficacy. These data demonstrate that protection against ZIKV challenge can be achieved by single-dose ZPIV and that humoral immunity alone is sufficient for that protection. These studies were carried out with non-GLP vaccine material. Overall, no clinical adverse events or clear changes in hematologies or chemistries were observed in rhesus monkeys following ZIKV PIV vaccination at weeks 0 and 4 (Larocca 2016) (Abbink 2016).

4 CLINICAL EXPERIENCE

4.1 Clinical experience with ZPIV in humans

While there is no human clinical experience with ZPIV, two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine (WRAIR) and a JEV vaccine (manufactured as IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated. In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting, and fever with the last three AEs occurring after the 2nd administration of the 5 mcg dose. Unsolicited AEs detected in subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

A formaldehyde-inactivated JEV vaccine (IXIARO®) has been licensed for use in the US since 2009. The package insert indicates that the most common adverse reactions reported were headache, myalgia, influenza-like illness, and fatigue (Valnera Austria GmBH, 2015). No effect was seen on the safety profile of IXIARO® compared to placebo (aluminum hydroxide) when examined by age, sex, or ethnic origin. In a Phase 3 clinical trial involving 2675 subjects who received either two doses of IXIARO® (6 mcg) or placebo (phosphate buffered saline [PBS] plus aluminum hydroxide), there was little difference between the active and placebo groups (Valnera Austria GmBH, 2015). The overall percentage of subjects who experienced at least one AE was 59% in the active group versus 57% in the placebo. Injection site reactions were mild to moderate in severity, and consisted of (in descending order of occurrence) pain, tenderness, erythema, induration, edema and pruritus. The most common systemic AEs post first dose and second dose were headache, myalgia, fatigue, influenza-like illness, nausea, nasopharygnitis, and fever. Sixteen SAEs were reported in 10 subjects who received vaccine and 6 subjects who received placebo. The SAEs that occurred among subjects in the IXIARO® group were dermatomyositis, appendicitis, rectal hemorrhage, limb abscess involving contralateral arm, chest pain, ovarian torsion, ruptured corpus luteal cyst, and three orthopedic injuries. No deaths occurred in the trial. IXIARO® is also licensed at one half the dose (3 mcg) for infants and children aged 2 months to <3 years; the full strength is recommended for use in older children. Fever was the most commonly observed AEs up to 12 years of age.

The association of ZIKV infections with the rare autoimmune disease, Guillain-Barre syndrome (GBS) and acute disseminated encephalomyelitis (ADEM) is a concern for ZIKV vaccines in general. In natural ZIKV infections, it is not yet clear whether the increased incidence of GBS is the result of an autoimmune reaction or is the result of neurotropism of the virus. Meningoencephalitis from ZIKV infection has also recently been reported.

In summary, inactivated flavivirus vaccines appear to be reasonably well tolerated. However, typical safety considerations with inactivated flavivirus vaccines include local reactions of pain, tenderness, erythema, induration, edema and pruritus, and mild to moderate systemic reactions including headache, myalgia, fatigue, influenza-like illness, nausea, and fever. The potential for a vaccine to induce GBS and other neuroinflammatory diseases is not known.

5 OBJECTIVES

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 90, 180, 270, and 365 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

6 STUDY DESIGN

This is a phase 1 trial of one or more administrations of Zika Virus Purified Inactivated Vaccine (ZPIV). The trial will be conducted under a placebo controlled, double-blind, randomized allocation of study product. This design is intended to reduce the likelihood of observer bias, provide control for confounding variables of intercurrent illness, and aid in the interpretation of laboratory data. There are four groups in the study and blinding will be maintained to vaccine or placebo within each group as noted in the table below.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV	ZPIV		
	B	2		Placebo	Placebo		
4	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		48					

It is anticipated that enrollment will be completed within a 6-month period, and that participants will be actively followed for 12 months. Groups 1 through 4 will be enrolled in sequence to accelerate the collection of peak immunogenicity time-points from the groups with longer schedules (peak time-points are D57, D43, D36, and D29 for Groups 1-4, respectively).

Sentinel Participants: Currently there are plans for two additional phase 1 clinical trials to test the ZPIV study product; these studies will begin before Z001 and will thus be responsible for monitoring the First-in-Human administrations of the vaccine. However, in the event that safety data is not available from at least 3 people from other Phase 1 studies with the vaccine at the proposed dose at the time when Z001 is ready to commence dosing, then we will enroll three sentinel subjects and assess safety for 7 days following dosing of the third sentinel prior to determining whether to vaccinate the remainder of the treatment groups. The randomization blocks ensure that at least 2 of the 3 sentinel subjects will receive active product; the third sentinel may receive either active product or placebo. The decision to vaccinate the remainder of the treatment groups will be made by the Protocol Safety Review Team (see Section 6.3)

6.1 Safety Assessment

To assess the safety of the administered vaccine, participants will document local and systemic reactogenicity on the first day of each vaccine administration period (days 1, 8, 15, and/or 29 depending on the group assignment) and for the subsequent 6 days following each vaccine administration. The investigator will interview each participant about AEs at each visit

throughout the study. Two to 3 days after each vaccine administration, a member of the site staff will have a remote safety follow-up communication with the participant by telephone. The participant will be questioned about occurrence of reactogenicity. The participant will be brought in for a clinic visit based on this assessment, if deemed necessary by the investigator/sub-investigator or upon request of the participant. All participants will also be seen in clinic at the end of each reactogenicity period (days 8, 15, 22, and/or 36 depending on the group assignment). All reactogenic events and AEs will be recorded on case report forms (CRFs) from the signing of the informed consent form (ICF) until the last study visit. To assess the participants' cellular and humoral immune response, blood samples will be taken at the clinic visits. Safety and immunogenicity visits will be conducted as outlined in the Schedule of Procedures (SOP).

6.2 Immunogenicity Assessment

Blood samples will be obtained at various time points after immunization (Section 18.3). These will be assayed for the magnitude of neutralizing and binding antibody titers to Zika virus at 14 and 28 days following each administration of ZPIV, as well as at 90, 180, 270 and 365 days following the first dose of ZPIV. The primary readout for neutralizing antibody assays will be the results of pseudovirus reporter and microneutralization assays. The secondary readout will be the result of plaque reduction neutralization assays. The third readout will be binding antibody titers by ELISA. Exploratory assessments may include epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, and Zika-specific T cell responses.

6.3 Monitoring

The Protocol Safety Review Team (PSRT) will review all adverse events (AEs) on a regular and expedited basis as needed. In addition, the PSRT will review safety data reports on a weekly basis until 12 participants have been enrolled after which reports will be reviewed biweekly. If sentinel participants are required (see above), the PSRT will also review safety data from the 3 sentinel participants after the 3rd has been followed for 7 days to determine whether to vaccinate the remainder of the treatment groups. The PSRT will include the Study Chair(s), co-investigators, and representatives from WRAIR and VRC/NIH, including a Department of Defense (DoD) Research Monitor (see below). Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs should also be reviewed by the Safety Monitoring Committee (SMC). The SMC will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC will be completely independent from the protocol team and sponsor. The SMC will evaluate safety and tolerability data on a regular basis. The SMC may review an individual SAE or it may choose to review AEs, SAEs, and laboratory and vital sign data. The SMC may unblind any amount of safety information needed to conduct their assessment. The conclusions of the SMC will be communicated to the investigators and the IRB/Ethics Committees and the national regulatory authorities as appropriate. The sponsor agrees to abide by the decision of the SMC and any directives issued by the national regulatory authorities, the Institutional Review Boards or Ethics Committees.

Department of Defense (DoD) Research Monitor: This study is supported by the Department of Defense (DoD), and therefore an independent research monitor is required. As outlined in the Department of Defense Instruction Number 3216.02, Section 8, the research monitor may

perform oversight functions (e.g., observe recruitment, enrollment procedures, etc.) and report their observations and findings to the CCI / IRB or a designated official; the monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research; the monitor shall have the authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the CCI / IRB can assess the monitor's report. Research monitors shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the US Army Medical Research and Materiel Command Human Research Protection Office. The monitor for the study will have access to all safety data as a sitting member of the PSRT. The DoD research monitor has been appointed and is the following expert on vaccine development:

Trevor A. Crowell, M.D., Ph.D.
U.S. Military HIV Research Program
6720-A Rockledge Drive, Suite 400
Bethesda, MD 20817
Office Phone: 301-500-3990
Cell Phone: 301-275-6047
Email: tcrowell@hivresearch.org

6.4 Randomization Procedures and Enrollment

Participants will be enrolled in the study after ascertainment by one of the study investigators that all of the inclusion and none of the exclusion criteria have been met. Once confirmation of eligibility for the trial has been performed, the participant will be randomized in the electronic database using randomized treatment assignments generated by the Data Management Center (DMC). After successful randomization, a blinded allocation number is provided. The DMC will create the randomization table and the sponsor will monitor the implementation of this process. The blinded allocation number is referenced against a confidential list provided to an unblinded on-site pharmacist to determine the assignment/treatment allocation.

The 12 participants in each group will be randomized in a ratio of 5 vaccinees to 1 placebo recipient.

6.7 Method of Blinding and Unblinding

The participants, clinical staff, investigator, and sponsor personnel will be blinded to treatment allocation throughout the study. The pharmacist with primary responsibility for vaccine dispensing will not be blinded to the treatment and will maintain the randomization code and complete assignments of participants according to the randomization allocation. The pharmacist will also apply masking tape to the syringes to preserve the blind between vaccine and placebo. Routine unblinding of treatment allocations may occur only after all participants have had their last study visit and the database is locked.

7 STUDY POPULATION

The study population will include healthy men or women aged 18-50 years old who are able and willing to provide written informed consent. Participants will be enrolled in the study once eligibility criteria are met.

7.1 Participant Inclusion Criteria

1. Age 18-50 years old.

2. Ability and willingness to provide informed consent.

3. Assessment of understanding: completion of a questionnaire prior to first screening procedure; verbally demonstrate understanding of all questionnaire items answered incorrectly.

4. Available for the duration of the trial.

5. Good general health as shown by medical history, physical exam, and screening laboratory tests.

6. Meets laboratory parameters for hematology, chemistry, and urinalysis*

   **Criteria for hematology, chemistry, and urinalysis defined as follows:*
   - *Hematology*
     - *Hemoglobin ≥10.5 g/dL for women; ≥11 g/dL for men*
     - *Absolute Neutrophil Count (ANC): ≥1000/mm^3*
     - *Platelets: 125,000 to 550,000/mm^3*
   - *Chemistry*
     - *Creatinine: <1.1 x upper limit of normal (ULN)*
     - *AST: <1.25 x ULN*
     - *ALT: <1.25 x ULN*
   - *Normal urinalysis*
     - *Negative urine glucose (if positive urine glucose, then a microscopic urinalysis within institutional range).*
     - *Negative or trace urine protein (if greater than trace protein, then a microscopic urinalysis within institutional range).*
     - *Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis within institutional range).*

7. Female participants must be willing to undergo serum or urine beta human chorionic gonadotropin pregnancy tests at time points indicated in Section 18.3 and must test negative prior to vaccination.

8. All sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until Week 12.

9. If a woman of child-bearing potential, committed to use an effective method of contraception* when sexually active with men from the day of first vaccination until Week 12.

**Effective methods of contraception include the following:*

- *Condoms (male or female) with or without spermicide.*
- *Diaphragm or cervical cap with spermicide.*
- *Intrauterine device.*
- *Hormonal contraception.*
- *Successful vasectomy in the male partner (considered successful if a woman reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity post-vasectomy).*
- *Not be of reproductive potential, such as having undergone hysterectomy, bilateral oophorectomy, or tubal ligation.*

Participant Exclusion Criteria:

1. History of known flavivirus infection or previous receipt of flavivirus vaccine.

2. Positive serology for HIV-1, Hepatitis B surface antigen, or anti-hepatitis C virus antibodies prior to enrollment.

3. Planned travel to areas with active Zika virus transmission during the study period.

4. Recent (within 3 weeks) travel to an area with active Zika virus transmission.

5. Current or planned participation in another clinical trial of an experimental agent during the study period.

6. Pregnant or lactating.

7. Any condition* for which participation would not be in the best interest of the subject (eg, compromise the well-being) or that could prevent, limit, or confound the study endpoints.

   **,At the discretion of the investigator and Includes any clinically significant acute or chronic medical condition.*

8. Use of anticancer, antituberculosis or other medications considered significant by the investigator within the previous 6 months.

9. Receipt of live-attenuated vaccine within the previous 60 days or planned receipt within 60 days after vaccination with Investigational Product*

   **Within 14 days for live attenuated influenza vaccine [LAIV]*

10. Receipt of other vaccine (e.g., influenza, pneumococcal), allergy treatment with antigen injections or tuberculin skin test within the previous 14 days or planned receipt within 14 days after vaccination with Investigational Product

11. Receipt of blood transfusion or blood-derived products within the previous 3 months.

12. Previous severe local or systemic reactions to vaccination.

13. History of splenectomy

14. History of seizure in the last 3 years (participants with a history of seizures who have neither required medications nor had a seizure for 3 years are not excluded)

15. Known autoimmune disease

16. Asthma other than mild, well-controlled asthma*.

    **Participants should be excluded that:*
    a. *Use a bronchodilator (beta 2 agonist) daily, or*
    b. *In the past year have (any of the following):*
        i. *Had > 1 exacerbation of symptoms treated with oral steroids*
        ii. *Routinely used moderate to high dose inhaled corticosteroids (e.g., more than the equivalent of 250 mcg fluticasone; 400 mcg budesonide; 500 mcg beclomethasone; or 1000 mcg triamcinolone/flunisolide, as a daily dose) or theophylline*
        iii. *Needed emergency care, urgent care, hospitalization, or intubation for asthma*
    c. *Prophylactic bronchodilator use prior to exercise is not exclusionary*

17. Diabetes mellitus type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

18. Thyroidectomy, or thyroid disease requiring medication during the last 12 months

19. Angioedema within the last 3 years if episodes are considered serious or have required medication within the last 2 years

20. Uncontrolled Hypertension*

    **Uncontrolled hypertension is defined as:*
    a. *If a person has been diagnosed with hypertension during screening or previously, exclude for hypertension that is not well controlled. Well- controlled hypertension is defined as blood pressure consistently ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm*
    b. *If a person has NOT been diagnosed with hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 90 mm Hg at enrolment*

21. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

22. Malignancy*

    **Not excluded: a participant with a surgical excision and subsequent observation period that in the investigator's estimation has a reasonable assurance of sustained cure or is unlikely to recur during the study period*

23. Psychiatric condition* that compromises safety of the participant or precludes compliance with the protocol

    **Specifically excluding persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years*

24. History of neuroinflammatory disorder (e.g., Guillan-Barre Syndrome and Bell's Palsy)

8 STUDY PRODUCTS

8.1 Study Products

Detailed instructions for study products including preparation, storage and documentation are provided under separate cover in the Study Operations Manual. Additional information is also provided in the Investigator's Brochure for the ZPIV product.

8.1.1 Zika Virus Purified Inactivated Vaccine (ZPIV)

Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at manufacturing facility prior to shipment to clinic.

8.1.2 Placebo

The placebo is saline.

8.2 Handling of Study Treatments

8.2.1 Packaging and Labeling

Each single-use 2 mL vial of ZPIV vaccine contains 10 µg/mL ZPIV antigen and 1 mg/mL aluminum hydroxide adjuvant with a fill volume of 0.7 ± 0.07 mL of a sterile, preservative-free, PBS solution, for 5 µg PIV protein and 500 µg aluminum hydroxide adjuvant per 0.5 mL dose and for intramuscular injection only.

8.2.2 Shipment and Storage

ZPIV vials should be stored at 2-8 °C. During storage, a clear liquid with a white precipitate will be observed; this is the alum adjuvant and this appearance is to be expected following refrigerated storage. **Do Not Freeze.**

8.2.3 Dose preparation and administration

The unblinded pharmacist (or other unblinded staff member qualified to handle and dispense medication) will dispense ZPIV or placebo in a pre-filled capped syringe with the participant's study ID and allocation number. The vaccine vial should be shaken well to disperse and fully resuspend the alum-adsorbed vaccine, after which it should appear as a turbid white to slightly yellow suspension with white particulates.

9 CONDUCT OF THE STUDY

9.1 Informed Consent

The informed consent form documents that a participant (1) understands the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in this study. Informed consent encompasses all written or verbal study information staff provide to the participant before and during the trial.

The informed consent process continues throughout the study. At each study encounter, staff should consider reviewing the procedures and requirements for that encounter and for the remaining encounters. Additionally, if any new information is learned that might affect the participant's decision to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign a revised informed consent form.

Participants must sign a screening or protocol-specific consent before any procedures to determine eligibility are performed. All recruitment and prescreening materials will be approved by the Institutional Review Board.

9.1.1 Screening Consent Form

An IRB approved general vaccine screening protocol and consent may be used as part of the initial screening procedure for this trial. Results from this IRB approved general screening or prescreening may be used to determine eligibility in this protocol, provided the tests are conducted within the time period specified in the eligibility criteria.

9.1.2 Protocol-Specific Consent Form

The protocol-specific consent form describes the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. The consent form is developed in accordance with IRB requirements and the principles of informed consent as described in Title 45, Code of Federal Regulations (CFR) Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonization (ICH) Guideline 4.8.10. It must be approved by all responsible ethical review bodies before any participants can be consented for the study.

9.1.3 Test of Understanding

Study staff should ensure that participants understand the study before screening them. This process involves reviewing the informed consent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely. A Test of Understanding is used to document the participant's understanding of key concepts in this Zika vaccine trial. The participant must complete the Test of Understanding—with the assistance of staff, if necessary, in reading and understanding the questions and responses—before screening. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

9.2 Clinical and Laboratory Evaluations – Participants

Please see Section 18.3 for a concise outline of study procedures. Screening should occur within 56 days of enrollment. For visit windows, refer to the Schedule of Procedures (Section 18.3).

9.3 Unscheduled Visits

Unscheduled Visits/Contacts are visits/contact that are not described in the Schedule of Procedures (Section 18.3). Unscheduled visits may occur any time during the study:

- For administrative reasons, e.g., the participant or household contact may have questions for study staff or may need to re-schedule a follow-up visit.
- To obtain laboratory test results from a previous visit.
- For other reasons as requested by the participant or site investigator.

All unscheduled visits will be documented in the participant's study records on applicable source documents and entered into the Case Report Form (CRF).

9.4 Unblinding Visit

After all participants (from all 4 groups) have completed the active follow-up part of the study, and the database is locked, unblinding will occur. Participants will be informed as to whether or not they received study product or placebo. The unblinding visit may be performed in person or by phone.

9.5 HIV Counseling and Testing

HIV testing will be performed as part of screening evaluations. Therefore, HIV counseling will also be performed in compliance with the CDC's guidelines and local guidelines for HIV counseling, testing, and referral. All participants who become HIV-infected during the study will be terminated from the study. Potential and enrolled participants identified as HIV-infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.6 Prior and Concomitant Treatment

All concomitant medications will be recorded on CRFs from the signing of the ICF until the last study visit. Study participants can receive medications such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or antihistamines as needed although they must be documented. Use of these medications as routine prophylaxis prior to study vaccination is discouraged.

9.7 Study discontinuation

A participant will be taken out of the study in the event of:

- Repeated failure to comply with protocol requirements
- Decision by the study sponsor of PI to stop or cancel the study
- Decision by local regulatory authorities or IRB to stop or cancel the study
- Participant's request

9.7.1 Early discontinuation or withdrawal of participants

Each participant has the right to withdraw from the study at any time for any reason without affecting the right to treatment by the investigator/designee. The investigator should make an attempt to contact participants who did not return for scheduled visits or follow-up. Although the participant is not obliged to give reason(s) for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights.

The investigator also has the right to withdraw a participant, e.g. because of worsening health status, intercurrent illness, AEs, or pregnancy (for pregnancy follow-up see Section 10.4). The sponsor reserves the right to request the withdrawal of a participant due to safety issues, protocol violations, or administrative or other reasons.

Any unnecessary withdrawal should be avoided. Should a participant be withdrawn, all efforts should be made to complete and report the observations as thoroughly as possible. Whenever a participant is withdrawn from the study, independent of the reason, a final evaluation must be completed for that participant and the primary reason for which the participant was withdrawn must be stated. All documentation concerning the participant must be as complete as possible.

For those participants who are unable to continue participation in the study, but who do not withdraw consent, an early termination visit will be conducted. Any participant who withdraws consent will not have any further data collected after consent has been withdrawn.

9.7.2 Premature termination of the study

The sponsor reserves the right to discontinue the study for safety, ethical, or administrative reasons. Should the study be discontinued, no further vaccinations will be administered but participants who are still actively participating at the time of discontinuation will be followed through the remainder of their follow up visits.

9.7.3 Study pausing rules

If the trial is placed on safety pause, all enrollment and vaccinations will be suspended pending review. The AEs that may lead to a safety pause or prompt PSRT AE review are summarized below in Table 2. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the PI or PSRT, or participant may be threatened.

Table 2: AE notification and safety pause/AE review rules

Event and relationship to study product	Severity	Occurrence	Site PI action	PSRT or SMC action
SAE, regardless of relationship[1]	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AESI[2]	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
Anaphylaxis, laryngospasm, bronchospasm, or generalized urticaria within 1 day of vaccination	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AE[3], related	Grade 3[4]	Second in SOC[5]	Phone, email or fax notification to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AE[3], related	Grade 3[4]	First	Phone, email or fax notification to PSRT chair within 24 hours	PSRT review within 2 business days to consider pause

[1] Deaths due to accident or trauma do not trigger a study pause and do not need to be referred to the SMC for review.

[2] AESI refers to Adverse Event of Special Interest, defined in section 10.1.1

[3] Within 30 days of vaccination, does not resolve within 2 days, and includes laboratory abnormalities; does not include the following reactogenicity symptoms (fever, fatigue, malaise, myalgia, arthralgia, chills, headache, nausea, vomiting, diarrhea, abdominal pain, rash).

[4] If no evidence of disease is present other than an abnormal laboratory value, the test must be repeated (entailing blood re-draw) at least one time. The verification period will be a maximum of 72 hours after initial awareness of the abnormal laboratory value. When signs and symptoms are present, repeat test will not be needed.

[5] PSRT will determine whether the reported related AE (Grade 3) is a second occurrence of a previously reported AE (Grade 3) in the same organ class (SOC).

For events in the table above, the Site Principal Investigator (PI) notifies the PSRT (within 24 hours). The PSRT will convene within two business days to review these AEs. The PSRT will review and determine disposition (including whether the SMC needs to review the event).

If a study pause is triggered, all enrollment and vaccinations will be held until review by the SMC. Resumption of enrollment and study treatment may be determined by the SMC (in consultation with the FDA, if required) following a cumulative review of the available safety data. If a decision to resume study enrollment and study treatment administration is made, the SMC will record its judgment in a memorandum to the study file and notify the sponsor, who will then forward the memorandum to the principal investigators. The clinical site will be allowed to resume activities upon receipt of written notification from the sponsor or its designee. As needed, the appropriate regulatory authorities will be informed in writing of the decision by the SMC to resume or discontinue study activities. The site is responsible for notifying the IRB according to local standards and regulations. The sponsor is responsible for notifying the FDA.

9.8 Laboratory evaluations

The total approximate volume of blood that will be collected from each participant is presented in Section 18.3. Total blood volume drawn from each participant will not exceed the American

Association of Blood Banks (AABB), and US Food and Drug Administration (FDA) guidelines of 550 mL in any eight-week period.

All biological samples must be collected in the appropriate manner. The investigator will ensure that the personnel and laboratory under his/her supervision comply with these requirements. Further details on shipment, handling, and storage of the samples are provided in the Specimen Handling Guidelines.

Any residual samples will be stored indefinitely following completion of the study at the sponsor-designated laboratories. Any future analyses conducted on the samples will maintain participant anonymity. Participants will have to provide their approval for long-term storage of their biological specimens. Participants will have the right to opt out of having their biological specimens stored once all analyses in the Informed Consent Form are completed. Opting out of this procedure does not impact the participant's ability to participate in the study.

9.9 Potential risks and benefits

9.9.1 Risks related to vaccines

Participants may exhibit general signs and symptoms associated with the intramuscular administration of a vaccine or placebo. Side effects, if observed, are expected to be short term, mild, and not requiring treatment.

Participants may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, hives or even difficulty breathing. Severe reactions are rare. Medications are available at the clinical site to treat serious allergic reactions.

The effect of this vaccine on a fetus or nursing baby is unknown, as well as the effect on semen. Therefore, sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until 12 weeks after final immunization (Week 16, 14, 13, and 12 for Groups 1-4, respectively). Female participants of child-bearing potential will be required to agree to use birth control for sexual intercourse beginning prior to the vaccination and continuing through Week 12 weeks after final immunization (Week 16, 14, 13, and 12 for Groups 1-4, respectively). Women who are pregnant or nursing will be excluded from the study.

9.9.2 Blood draws

Blood drawing may cause pain, bruising, and, rarely, infection at the site where the blood is taken.

9.9.3 Unknown risks

There may be other serious risks that are not known. Participants may believe that this vaccine provides protection against acquiring Zika infection, and therefore not use appropriate precautions to avoid Zika infection. They will receive extensive counseling throughout the study to address this potential problem. It is not known if the study vaccine increases, decreases, or has no effect on the chance of becoming Zika infected when exposed, or if upon becoming Zika infected, the person's disease course will be more or less severe.

9.9.4 Potential benefits

There is no direct benefit to the participant for participation in this clinical trial. Although study participants may benefit from clinical testing and physical examination, they may receive no direct benefit from participation. Society may benefit from knowledge gained in this study that may aid in the development of a Zika vaccine.

10 SAFETY ASSESSMENTS

10.1 Adverse events

10.1.1 Definitions

Adverse events

An AE is any untoward medical occurrence in a patient or participant, and does not necessarily have a causal relationship with a medical treatment. This includes any noxious, pathological, or unintended change in anatomical, physiological, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory-detected changes. These might occur in any phase of the clinical study whether associated with the study vaccine or not. This includes exacerbations of pre-existing conditions or events, intercurrent illness, or vaccine or drug interactions. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation are not considered AEs. Discrete episodes of chronic conditions occurring during a study period should be reported as AEs in order to assess changes in frequency or severity.

Serious adverse events

Seriousness refers to the outcome of an AE. Seriousness is determined by both the investigator and medical monitor, and can also be determined by the sponsor (*FDA 2010 Guidance for Industry and Investigators; Safety Reporting Requirements for INDs and BA/BE Studies*). An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in **death.**
- Is **life-threatening**: i.e. the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Results in persistent or significant **disability/incapacity**: i.e. results in a substantial disruption of the participant's ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as headache, nausea, vomiting, diarrhea, influenza, infusion site reactions, or accidental trauma (e.g. sprained ankle).
- Requires in-patient **hospitalization** or prolongation of existing hospitalization: i.e. the participant is detained (usually at least an overnight stay) at the hospital or emergency ward for treatment that would not have been appropriate in the physician's office or in an out-patient setting. Hospitalization for either elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures[1] (including hospitalization for 'social' reasons) that are not the result of an AE, are **not** considered as SAEs.
- Is a **congenital anomaly or birth defect** in the offspring of a study participant
- Is an **important medical event** that may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above: e.g. interventions such as intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization, or development of drug

[1]A procedure that may take place during the study period and should not interfere with the study drug administration or any of the ongoing protocol-specific procedures.

dependency or drug abuse. Based on medical and scientific judgment, these events should usually be considered serious.

A suspected transmission of any infectious agent via a medicinal product is always considered as an important medical event, i.e. an SAE.

Although **not** considered as an SAE, cancer should be reported in the same way as SAEs.

Note: If anything untoward is reported during an elective procedure, that occurrence must be reported as an AE, either serious or non-serious according to the criteria defined above.

When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious.

Adverse Events of Special Interest

For this study, Neurologic and Neuroinflammatory* Disorders will be considered as Adverse Events of Special Interest (AESI). AESI will be collected for this study through the end of study follow-up period and will be captured and recorded in a CRF with the same timeline as the SAEs (within 24 hours of site awareness); the PSRT will be notified by the data center when such an event is reported. Any AESI that meets a SAE criterion will be reported as SAE as well.

***Neurologic and Neuroinflammatory Disorders:** ADEM, including site specific variants, Cranial Nerve Disorders (including paralyses/paresis), GBS (including Miller Fisher Syndrome and other variants), Immune-mediated Peripheral Neuropathies and Plexopathies, Optic Neuritis, Multiple Sclerosis, Narcolepsy, Transverse Myelitis, meningitis, or meningoencephalitis.

Adverse reactions

An adverse reaction is an AE judged to be related to study vaccine.

Related AEs (adverse reactions) are defined as those judged by the investigator, sponsor or independent safety monitor to be possibly, probably, or definitely related to study vaccine.

When an AE is judged to be related to study vaccine and also is judged to be serious and unexpected, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting. All SAEs in this study will be considered unexpected as there are no expected AEs associated with this vaccine product.

Adverse reactions will be reported in accordance with FDA 2009 Guidance for Clinical Investigators, Sponsors and IRBs; Adverse Events Reporting to IRBs – Improving Human Participant Protection.

10.1.2 Surveillance, reporting, and documentation of adverse events

The recording of AEs is an essential part of study documentation. The investigator is responsible for documenting all AEs as set out in the following sections.

10.1.2.1 Surveillance of adverse events

At each visit through 28 days following last vaccination, all AEs, either observed by the investigator or one of his/her clinical collaborators, or reported by the participants spontaneously or in response to a direct question, will be evaluated by the investigator or designee. All SAEs will be reported through the final visit (Study Day 366).

Participants will be instructed to contact the investigator immediately should he/she experience any signs or symptoms he/she perceives as severe from the time of vaccination through a period of 12 months.

10.1.2.2 Documentation of adverse events

All AEs will be collected on case report forms (CRFs) from the signing of the ICF until 28 days following last vaccination. SAEs will be collected through Study Day 366.

AEs and SAEs should be documented in terms of signs and symptoms observed by the investigator or reported by the participants. Whenever possible, a medical diagnosis should be made. The nature of each event, date and (where appropriate) time of onset, outcome, severity, and causal relationship should be established. Details of any symptomatic/corrective treatment should be recorded on the appropriate page of the CRF.

Hospitalization for routine clinical procedures (or other reason) that is not the result of an AE, are not considered AEs but will be recorded on the AE page of the CRF ('Hospitalization, Not an AE'). The same applies for hospitalization for elective procedures related to a pre-existing condition that did not increase in severity or frequency during the study. If hospitalization was planned before administration of the study vaccine, it will be documented in the Medical History page of the CRF (see below).

The following events will be documented in the Medical History page of the CRF:

- Pre-existing conditions or signs and/or symptoms present in a participant before study start. This includes conditions that were not recognized at study entry but later during the study period.
- Hospitalization arising from a pre-existing condition and planned before the administration of the vaccine.

10.1.2.3 Post-vaccination reactions occurring immediately after each vaccine administration

Participants will be observed for minimum of 30 minutes following vaccine administration. Vital signs (temperature, blood pressure, pulse and respiratory rate) will be taken and qualified study personnel will evaluate for any signs or symptoms of reactions.

Possible reactions may include fever (≥37.7 ºC), fatigue/malaise, headache, myalgia, arthralgia, chills, nausea, vomiting, and arm pain. For life-threatening allergic reactions that occur immediately post-vaccination, the site has specific procedures developed for handling such emergencies.

10.1.2.4 Reactions occurring within seven days following each vaccine administration

Participants will be instructed to notify specified study personnel immediately if any unusual or severe sign or symptom appears after vaccine administration. Participants will maintain a study diary during this period to record potential reactions.

10.1.3 SAE Reporting

The sponsor has a regulatory obligation to report SAEs to the FDA according to established timetables for reporting based on specific criteria. The investigator will report SAEs to the PSRT and IRB within 24 hours from the time they first learn of the event. The SAE form will be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the PSRT and IRB. The investigator will provide his/her assessment of causality to study treatment at the time of the initial SAE report. The investigator will not delay in the reporting of any SAE in order to obtain additional information. Any additional information, if collected, will be reported to the PSRT and IRB as a follow-up to the initial report.

All SAEs must be reported immediately (within 24 hours of discovery) by email or fax to the IRB at the following contact information:

Email: Jessica Ripton, jripton@bidmc.harvard.edu
Fax: (617) 975-8501

The PSRT will perform a clinical review of the information provided to identify any missing data. The PSRT will also contact the study site to clarify any discrepant or missing information, to answer questions and to provide guidance to the site, if needed. The investigator will report the SAE as an acceptable medical diagnosis. If a preliminary diagnosis has not yet been made, then each symptom will be listed separately. A follow-up report will be issued when a diagnosis is made.

The investigator must report SAEs to the appropriate IRB as requested by the board according to local legal requirements.

10.2 Reporting requirements to the local IRB

The PI/designee will be responsible for providing all safety reports and reporting all SAEs, study pauses, social harms, and major deviations to the local regulatory authorities, such as a local IRB, and any regulatory agencies, in a timely manner according to the institution's guidelines and to local regulations.

10.3 Causality assessment of study vaccine to adverse events

Every effort should be made by the investigator to explain any AE and assess its potential causal relationship, i.e. to administration of the study treatment or to alternative causes (e.g. natural history of the underlying diseases, concomitant therapy). This applies to all AEs, whether serious or non-serious.

The investigator will use the following guidelines to assess the causal relationship of an AE to study injection:

Not related:	Adverse experiences felt to be due to extraneous causes that neither follows a known pattern of response nor a reasonable temporal relationship to study vaccine.
Remote (probably not related):	Adverse experiences that are unlikely to be related to study vaccine but which follow a reasonable temporal relationship, such that this cannot be completely excluded or events that could be associated with study vaccine but which are unrelated in time.
Possibly related:	Adverse experiences that may be due to extraneous causes but which follow a known pattern of response and/or a reasonable temporal relationship to study vaccine.
Probably related:	Adverse experiences that cannot be explained by extraneous causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.
Definitely related:	Adverse experiences that have a definite relationship to the study vaccine (e.g. anaphylactic reaction after vaccination) without any other explained causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.

In the final analyses, events categorized as “not related” and “remote” will be considered as not related to study vaccine; events categorized as “possibly related”, “probably related” and “definitely related” will be considered as related to study vaccine.

10.3.1 Severity of adverse events

All AE and lab data will be coded for severity using the adapted Division of Microbiology and Infectious Disease Revised Toxicity Table included in Section 18.1. This Table has been adapted to comply with Clinical Data Interchange Standards Consortium guidelines.

The grading scale contains only 3 severity grades: Mild (Grade 1), Moderate (Grade 2), and Severe (Grade 3). Events that are life-threatening or result in death will be graded as Severe (Grade 3) and indicated to be SAEs with the appropriate outcome.

For AEs not identified in the grading table, the following guidelines will be applied:

Mild	Grade 1	Symptoms causing no or minimal interference with usual social and functional activities
Moderate	Grade 2	Symptoms causing greater than minimal interference with usual social and functional activities
Severe	Grade 3	Symptoms causing inability to perform usual social and functional activities; Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability (also reported as SAE); and Death (also reported as SAE)

The clinical research site team will ascertain accurate recording of all AEs during the study. AE CRFs will be completed by the clinical research site staff on a daily basis as the data become available from the isolation unit, clinic or laboratory.

The investigators will monitor and analyze study data including all AE and lab data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study vaccine. AEs will be deemed either related to study vaccine or not related to study vaccine. To ensure that all AEs are captured in a timely manner, CRFs will be entered in real-time entry, and subjected to analysis to identify AEs that may invoke study pausing rules.

The PI or designee must review AE CRFs to insure prompt and complete identification of all events that require expedited reporting as SAEs, study pausing rules or other serious and unexpected events.

Study vaccine related AEs will be followed by the clinical research site team through resolution or until study completion. Non-study vaccine related AEs will be followed to resolution or study completion, whichever occurs first.

10.3.2 Follow-up of ongoing adverse events and assessment of outcome

10.3.2.1 Follow-up of non-serious adverse events

Non-serious AEs already documented in the CRF at a previous assessment and designated as ‘ongoing’ should be reviewed at subsequent visits. If the event has resolved, the documentation in the CRF should be completed. If the frequency or severity of a non-serious AE changes significantly, a new record of the AE has to be started. If the AE becomes serious, the procedures for reporting of SAEs have to be followed (see Section 10.1.3).

New non-serious AEs will be recorded through 28 days post-vaccination. Ongoing non-serious AEs will be monitored until the Day 366 visit (or until the last study visit for participants who withdraw early (see Section 18.3)).

Outcomes will be assessed as:

1. Resolved
2. Resolved with sequelae
3. Resolving
4. Not resolved
5. Fatal
6. Unknown

Clinically significant abnormal laboratory values will be followed up until they have returned to normal, stabilized, or a satisfactory explanation has been provided.

10.3.2.2 Follow-up of serious adverse events and AESI

All SAEs and AESIs must be followed-up until the event has either resolved, subsided, stabilized, disappeared, or is otherwise explained, or the study participant is lost to follow-up,

but no longer than 12 months after the administration of the study vaccine. Outcomes are assessed as above.

All follow-up activities must be reported in a timely manner to the PSRT (if necessary on one or several consecutive SAE/AESI report forms). All form fields with additional or changed information must be completed and the SAE/AESI Report Form should be forwarded to the PSRT as soon as possible but at the latest within 7 calendar days after receipt of the new information.

Clinically significant laboratory abnormalities reported as SAEs will be followed-up until they have returned to normal, or a satisfactory explanation has been provided.

Reports related to the subsequent course of any SAE/AESI reported for any participant must be submitted to the sponsor.

10.3.3 Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to currently available best treatment. The applied measures should be recorded in the CRF.

10.4 Handling of pregnancy cases

Pregnancy events will be reported through 3 months following final vaccination. All initial reports of pregnancy must be reported to the PSRT by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, stillbirth, and congenital anomaly) are considered SAEs and must be reported using the Serious Adverse Event Form.

Because the effect of the study vaccine on sperm is unknown, pregnancies in partners of male participants included in the study will be reported by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

10.5 Physical examination

Physical examinations will be performed by the investigator or designated medically-trained clinician. The time points of these examinations are specified in Section 18.3. Any abnormalities or changes should be documented in the source document and recorded on the CRF.

A new, clinically significant finding (in the opinion of the investigator) not noted at screening must be captured as an AE. In addition, resolution of any abnormal findings during the study will be noted in the source document and in the CRF.

10.6 Routine safety laboratory

Samples for routine safety laboratory parameters will be collected at the time points specified in Section 18.3.

The following routine safety laboratory parameters will be determined:

At screening:

- **Serum chemistry:** ALT, Alkaline Phosphatase, AST, Calcium, Carbon Dioxide, Chloride, Glucose, Potassium, Sodium, Total Bilirubin, Total Protein, Albumin, Globulin (calculated), Blood urea nitrogen, Creatinine, Estimated GFR, Albumin/Globulin ratio (calculated), BUN/Creatinine ratio (calculated)

- **Complete blood count:** WBC (neutrophils, lymphocytes, monocytes, eosinophils, basophils), RBC, Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Platelets Count

At follow up:

- **Serum chemistry:** ALT, AST, Total Bilirubin, Blood urea nitrogen, Creatinine, BUN/Creatinine ratio (calculated)

- **Complete blood count:** Hemoglobin, Platelets Count, WBC

Laboratory values will be graded according to the DMID toxicity scale (Section 18.1) and, if clinically significant, reported as AEs.

10.7 Vital signs

Vital sign measurements will be performed at time points specified in Section 18.3.

The following measurements will be performed:

- Heart rate (bpm)
- Systolic and diastolic blood pressure (mmHg)
- Respiratory rate
- Body temperature (oral)

A confirmatory vital signs measurement can be performed if inconsistent with a prior measurement. If any clinically significant changes in vital signs are noted, they will be reported as AEs and followed to resolution, or until reaching a clinically stable endpoint.

10.9 Protocol Safety Review Team and Safety Monitoring Committee

The Protocol Safety Review Team (PSRT) will include the Protocol Chair, Protocol Co-Chairs, Site Principal Investigator, co-investigators and partners at WRAIR. Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs not requiring a study pause should also be reviewed by the SMC.

The Safety Monitoring Committee (SMC) will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC is an independent multidisciplinary

group consisting of experts in virus infection (e.g., dengue, West Nile Virus, HIV etc.) that, collectively, has experience in the management, conduct, and monitoring of vaccine trials. Members of the SMC are not directly affiliated with this protocol and are not situated at the study site. The SMC also will review individual Expedited Adverse Event (EAE) reports. The SMC will conduct additional special reviews at the request of the PSRT. Additional detail is provided in Section 6.3.

11 IMMUNOGENICITY ASSAYS

11.1 Zika-associated immunogenicity

Blood samples for the determination of cellular and humoral responses will be collected as specified in Section 18.3.

Humoral response assays to Zika will likely include, but are not limited to: microneutralization assays, ELISA, and plaque reduction neutralization assays. The **microneutralization assay** is a flavivirus high-throughput ELISA based microneutralization test in vero cells. Neutralization titer, expressed as MN50 titer, is regarded as the reciprocal of the serum dilution capable of neutralizing 50 percent of the virus dose in the ELISA part of the test. Seropositivity in the assay is a MN50 titer greater then or equal MN50 of 10. **ZIKV ELISA** assays will be performed using ZIKV Env protein; endpoint titers will be defined as the highest reciprocal serum dilution that yielded an absorbance >2-fold over background values and log10 endpoint titers will be reported. The **plaque reduction neutralization test** is used to quantify the titer of neutralizing antibody for ZIKV virus. The concentration of plaque forming units can be estimated by the number of plaques (regions of infected cells) formed after a few days. The concentration of serum to reduce the number of plaques compared to the serum free virus gives the measure of how much antibody is present or how effective it is.

Table 3 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 3: Humoral immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Principle Timepoint(s)
Humoral responses	Secondary	Serum	ZIKV microneutralization (MN) assay	Log10 MN50 titers	Group 1: D1, D57*, D181 Group 2: D1, D43*, D181 Group 3: D1, D36*, D181 Group 4: D1, D29*, D181 * Peak
	Secondary	Serum	ZIKV Env ELISA	Log10 endpoint titers	Group 1: D1, D57*, D181 Group 2: D1, D43*, D181 Group 3: D1, D36*, D181 Group 4: D1, D29*, D181 * Peak
	Exploratory	Serum	ZIKV plaque reduction assays	Log10 titer	Group 1: D1, D57*, D181 Group 2: D1, D43*, D181 Group 3: D1, D36*, D181 Group 4: D1, D29*, D181 * Peak

Evaluations of cellular immune responses to Zika will likely include, but are not limited to, interferon gamma producing cells (ELISPOT) assays. ZIKV-specific cellular immune responses will be assessed by interferon-γ (IFN-γ) ELISPOT assays using pools of overlapping 15-amino-acid peptides covering the prM, Env, Cap, and NS1 proteins. Table 4 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 4: Cellular immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Primary Timepoint(s)
T-cell responses	Exploratory	PBMC	Interferon gamma producing cells (ELISPOT)	Number of spot forming cells per 10^6 PBMC	Group 1: D1, D57*, D181 Group 2: D1, D43*, D181 Group 3: D1, D36*, D181 Group 4: D1, D29*, D181 * Peak

Immunologic assays may be performed and analyzed in a blinded fashion after all participants of a given group reach the peak immunogenicity time-point for their group (D57, D43, D36, and D29 for Groups 1-4, respectively). This will allow more rapid accrual of data to facilitate product development strategies. The optimal choice of immunogenicity assays will take into consideration the latest technological advances.

11.2 DNA/RNA Assays

Blood samples collected for cellular immunogenicity may also be used for exploratory DNA and RNA micro-array and deep-sequencing assays.

11.4 Additional Immunogenicity Assays

Additional immunogenicity assessments of systemic and mucosal responses may potentially include the following: flavivirus serology, epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, and Zika epitope-specific T cell responses.

12 DATA EVALUATION AND STATISTICS

All data entry will be performed by qualified and trained study staff. When all data have been entered and validated, the final database will be locked. An interim blinded analysis will be performed 28 days following the final immunization (group assignments will be known). A second interim blinded analysis will be performed at the 6 month time-point. Final analysis will be performed when D366 data has been collected.

12.1 Analysis populations and data sets

12.1.1 Safety population

All participants who received ZPIV or placebo, and for whom any post-dose data is available, will be included in the safety population.

12.1.2 Immunogenicity population

The immunogenicity population will consist of all participants who received ZPIV or placebo, and who have at least one measured post-dose blood sample collected.

12.2 Endpoints

Safety and tolerability:

- Incidence, intensity, and relationship to vaccination of solicited local and systemic adverse events (AEs) during the 7-day follow-up period (Days 1-7) after each ZPIV dose.
- Incidence, intensity, and relationship to vaccination of unsolicited AEs during the 28-day follow-up period (Days 1-29 after each ZPIV dose)
- Grade 2 and Grade 3 laboratory abnormalities at Day 8 after each ZPIV dose.
- Incidence of serious adverse events (SAEs) and related AEs from Day 1 through Day 366.

Immunogenicity:

- Principle Endpoints: Proportion (95% CI) of participants per dose group with positive responses and mean response (e.g. GMT) per group with 95% CI for the following 2 parameters:
    - ZIKV microneutralization Log10 MN50 titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D1.
    - Zika Env-specific Log10 endpoint ELISA titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D1.

- Additional Endpoints:
    - Plaque reduction neutralization test titer at 14 and 28 days following each vaccination, and at 3, 6, 9, and 12 months from D1.

- Durability and kinetics of humoral immune responses: Log10 MN50 and Log10 endpoint ELISA titers at 14 and 28 days following *first* (and second) vaccination, and at 3, 9 and 12 months.
- Cellular immunogenicity: IFN-γ ELISPOT responses to prM, Env, Cap, and NS1 peptides at 28 days following final vaccination and at 6 months from D1.

12.3 Sample size consideration

The number of participants chosen for this study will provide a preliminary safety and immunogenicity assessment. The sample size is not based on formal hypothesis testing considerations, but within the range of participants (i.e., 20-80) recommended in the Code of Federal Regulations (CFR 312.21) for first-in-human product Phase 1 evaluations. Placebo recipients are included for blinding and safety purposes and will provide additional control specimens for immunogenicity assays.

13 ETHICAL AND LEGAL REQUIREMENTS

13.1 General requirements

The study will be performed according to this Study Protocol and in compliance with the Declaration of Helsinki, the guidelines of the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) and the respective local legal requirements including the following: US Code of Federal Regulations 45CFR Pt 46; 21CFR Pt 50, 21CFR Pt 56 and 21CFR Pt 312.

13.2 Institutional Review Board/Ethics Committee

Before the start of the study, the investigator will submit the Study Protocol, Informed Consent Form, and other study-related documents as required by applicable laws and regulations to the responsible IRB for written approval.

The investigator will inform the IRB according to applicable laws and regulations about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

Unless otherwise instructed by the IRB or local regulation the investigator must submit to the IRB:

- All subsequent Amendments to the Study Protocol, changes to the Informed Consent Form or revisions of other documents originally submitted for review
- New or revised participant recruiting materials approved by the sponsor, if applicable
- All subsequent changes of logistical or administrative aspects (for information)
- Serious and/or unexpected AEs occurring during the study, where required
- New information that may affect adversely the safety of the participants or the conduct of the study
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Annual update and/or request for re-approval, where required
- Date of study completion, where required

13.3 Regulatory authorities

Before initiating the study, the sponsor will submit any required application to the regulatory authorities and obtain approval according to applicable laws and regulations. The sponsor will also inform the regulatory authorities about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

13.4 Participant information and informed consent

The study informed consent describes the investigational products to be used and all aspects involved in protocol participation. A properly executed written informed consent, in compliance with the Declaration of Helsinki, guidelines of the Council of International Organization of Medical Sciences (CIOMS), the Belmont Report, the US Code of Federal Regulations 21 CFR 50, must be obtained from each participant prior to entry into the trial or prior to performing any unusual or non-routine procedure that involves risk to the participant. The investigator must

provide a copy of the approved informed consent to the participant and a signed copy must be maintained in the participant's record file. Before a participant's participation in the study, it is the investigator's responsibility to obtain this written informed consent from the participant, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or study medications are administered.

All items must be explained by the investigator or designee in a language that is easy to understand. Participants will also be informed that the participation is voluntary and that they have the right to withdraw at any time without giving reasons and without any disadvantages for their subsequent care. Participants will confirm their consent in writing before study start and any study-specific procedure.

Participants must be given enough time to consider participation in the study.

13.5 Indemnity

The Sponsor and Institution are responsible to have appropriate liability insurance. For research-related injuries and/or medical problems determined to result from receiving the Investigational Product, treatment including necessary emergency treatment and proper follow-up care will be made available to the participant free of charge at the expense of the Sponsor.

13.6 Data access and protection

Confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

The collection and processing of personal data from participants enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of participants confidential.

The informed consent includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to the participant's source data/documents for study-related monitoring, audit, IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The participant has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

13.7 Future use and storage and blood samples

Each study participant will be asked to consent voluntarily for their blood samples to be stored for other research studies that may be done after this study is completed. Future testing may involve DNA/RNA tests. For participants unwilling to have their blood samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed after all the tests specified for this study have been concluded.

All samples for which consent has been obtained, and for which additional material is available after study-specified testing is complete, will be stored for future testing. All applicable approvals will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB.

13.8 Reimbursement

Participants and household contacts will be reimbursed for time and inconvenience in accordance with the standards and legal obligations for compensation required by each site. Any applicable guidelines by IRBs/ECs for compensation of research participants and household contacts will be sought and followed.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

Monitoring of clinic activities will be performed regularly to ensure that the study is carried out according to this Study Protocol and in compliance with GCP and applicable legal requirements.

Source documents will be reviewed for verification of consistency with CRF data. The investigator guarantees direct access to source documents for monitoring purposes. Source data verification will be performed in accordance with data protection regulations and guidelines. All information reviewed will be handled according to these rules and regulations.

The monitor will review each participant's data as outlined in the study specific Clinical Monitoring Plan.

14.2 Audit and inspections

Audits and inspections may be carried out by qualified delegates authorized by the sponsor or by authorities. The investigator consents to cooperate and to allow direct access to all source documents and other study-related data during an audit or inspection. All information disclosed will be handled in accordance with applicable data protection rules and regulations.

14.3 Data quality assurance

All CRF data will be entered into a validated, 21CFR Part 11 compliant, computerized clinical data management system.

The site is required to have a plan in place for assuring the quality of the research being conducted.

15 DOCUMENTATION, ARCHIVING, AND PUBLICATIONS

15.1 Documentation

Source documents are original documents, data, and records from which the participant's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, radiographs, and correspondence.

The Investigator and staff are responsible for ensuring maintenance of a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the sponsor, FDA, and/or applicable regulatory authorities.

All CRF entries have to be verifiable by the source data in the participant file. This does not apply to CRF entries that are defined as source data.

15.2 Archiving

The Investigator is responsible for the archiving of the Investigator's file, the participant's file, and the source data according to national and international legal requirements.

Any records related to the conduct of the study may not be destroyed without written authorization by the sponsor. According to GCP requirements, study records should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

15.3 Clinical Study Reports and Publications

The results of the clinical study will be documented in the Clinical Study Report.
The study results may be published and/or presented at scientific meetings. Terms of publication will be addressed in an agreement between the sponsor and participating partners.

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17 PROTOCOL SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the appendices and provide the necessary assurances that this study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirement(s).

Protocol Chair:

Signed: _____ Date: _____

Kathryn E. Stephenson, MD, MPH
Beth Israel Deaconess Medical Center

Sponsor-Investigator:

Signed: _____ Date: _____

Kathryn E. Stephenson, MD, MPH
Beth Israel Deaconess Medical Center

18 APPENDICIES

18.1 Toxicity Tables

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE (ADAPTED)

Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal R_X = Therapy
Req = Required	Mod = Moderate
IV = Intravenous	ADL = Activities of Daily Living
Dec = Decreased	

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1	**Mild** Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	**Moderate** Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	**Severe** Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible; or **Life-threatening*** Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable (***also reported as SAE)**

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be graded as Severe (Grade 3) and reported as an SAE. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.
- For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.
- These tables have been modified to comply with Clinical Data Interchange Standards Consortium guidance.

HEMATOLOGY			
	Grade 1	Grade 2	Grade 3
Hemoglobin	9.5 - 10.5 gm/dL	8.0 - 9.4gm/dL	< 7.9 gm/dL
Absolute Neutrophil Count	1000-1500/ mm^3	750-999/ mm^3	< 749/ mm^3
Platelets	75,000-99,999/ mm^3	50,000-74,999/ mm^3	< 49,999/ mm^3
WBCs	11,000-13,000/ mm^3	13,000-15,000 / mm^3	<1,000 / mm^3 or > 15,000/
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: <100 mg/dL High: >600 mg/dL	Low: < 50 mg/dL, OR Fibrinogen associated with gross bleeding or with disseminated coagulation ----------
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	> 51 mcg/ml
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	> 1.51 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	> 2.34 x ULN
Methemoglobin	5.0 - 9.9 %	10.0 - 14.9 %	> 15.0 %

CHEMISTRIES			
	Grade 1	Grade 2	Grade 3
Hyponatremia	130-135 mEq/L	123-129 mEq/L	< 122 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	> 158 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	< 2.4 mEq/L or intensive replacement therapy or hospitalization required, abnormal potassium *with* paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	> 6.6 mEq/l
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	< 39 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	> 251 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	< 6.9 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany

CHEMISTRIES (continued)			
	Grade 1	Grade 2	Grade 3
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	> 12.6 mg/dL or abnormal calcium *with* life threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	< 0.8 mEq/L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	< 1.4 mg/dL intensive therapy or hospitalization required, or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	> 1.5 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	> 2.0 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	> 5.1 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/dL	> 12.1 mg/dL
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	> 3.1 x ULN or dialysis required

ENZYMES			
	Grade 1	Grade 2	Grade 3
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	> 2.1 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	> 2.1 x ULN

URINALYSIS			
	Grade 1	Grade 2	Grade 3
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or > 2 gm loss/day, OR nephrotic syndrome
Hematuria	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts OR obstructive or required transfusion

CARDIOVASCULAR			
	Grade 1	Grade 2	Grade 3
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required, OR unstable dysrythmia; hospitalization
Hypertension	transient increase > 20 mm/Hg; no treatment	recurrent, chronic increase > 20mm/Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible, OR end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required, OR mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment
Pericarditis	minimal effusion	mild/ moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes, OR tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused, OR massive blood loss; > 3 units transfused

RESPIRATORY			
	Grade 1	Grade 2	Grade 3
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present OR cyanosis: $FEV_1 < 25\%$ of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest OR dyspnea requiring Oxygen therapy

GASTROINTESTINAL			
	Grade 1	Grade 2	Grade 3
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids OR hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids OR physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	Obstipation requiring manual evacuation or enema OR obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/day or mild diarrhea last < 1 week	moderate or persistent; 5-7 loose stools/day or diarrhea lasting >1 week	>7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required OR hypotensive shock or physiologic consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods, OR unable to drink fluids; requires IV fluids

NEUROLOGICAL			
	Grade 1	Grade 2	Grade 3
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia, OR incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation, OR acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited, OR paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement, OR incapacitating; or not
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in grea toes) in focal area or symmetrical distribution; or change ir taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities), OR sensory loss involves limbs and trunk; paralysis; or seizures

MUSCULOSKELETAL			
	Grade 1	Grade 2	Grade 3
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living, OR disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living, OR permanent and/or disabling joint distruction
Myalgia	myalgia with no limitation of activity	Muscle tenderness (at other than injection site) or with moderate impairment of activity	severe muscle tenderness with marked impairment of activity, OR frank myonecrosis

SKIN			
	Grade 1	Grade 2	Grade 3
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration, OR exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15mm	15-30 mm	>30mm
Erythema	< 15mm	15-30 mm	>30mm
Edema	< 15mm	15-30 mm	>30mm
Rash at Injection Site	< 15mm	15-30 mm	>30mm
Pruritus	slight itching at injection site	Moderate itching at injection extremity	itching over entire body

SYSTEMIC			
	Grade 1	Grade 2	Grade 3
Allergic Reaction	pruritus without rash	Localized urticaria	Generalized urticaria; angioedema, OR anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy, OR intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	> 39.6 C or > 103 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25-50% > 48 hours	normal activity decreased > 50% can't work, OR unable to care for self

18.2 Key Study Roles

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18.3 Schedule Of Procedures

Table 2a

Group 1 Schedule:

*Visits 2 and 6 are phone calls

Tests and Observations (Days)	D-1	D1 (1)	D4 (4)	D8 (8)	D15 (15)	D29 (29) (1)	D32 (4)	D36 (8)	D43 (15)	D57 (29)	D91	D181	D271	D366
Visit	**Screen 00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**
Clinical Assessments														
Consent, Demographics	X													
Medical History Review	X	X				X								X
Inclusion and Exclusion	X	X												
Medications[a]	X	X		X	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X		X	X	X	X	X	X	X
Laboratory Tests														
Screening Labs (40 mL)[d]	X													
Other Flavivirus Serologies[e] (10 mL)		X												
CBC w/diff and Chemistry Panel (15 mL)[f]		X		X	X	X		X	X	X	X	X	X	X
Pregnancy[g]	X	X				X								
Urinalysis[h]	X	X				X								X
Humoral (40 mL at D0, 30 mL at >D0)		X			X	X			X	X	X	X	X	X
CMI (40 ml)		X			X	X			X	X	X	X	X	X
Study Related Procedures														
Vaccination		**X**				**X**								
Distribute Diary		X				X								
Review Diary[i]				X				X						
Phone AEs[j]			X				X							
Blood Volume (mL)	40	105	0	15	85	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 1 through study discharge)
[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.
[c] Vital signs will be performed pre and post vaccination at 1 and 29 day visits
[d] FSH (if indicated), HIV-1 serology, Hepatitis B surface antigen, Hepatitis C antibody, WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, Plt, ALT, ALP, AST, Ca, Bicarb, Cl, Gluc, K, Na, Tot Bili, Tot Pr, Alb, BUN, Cr
[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.
[f] Hb, Plt, WBC, ALT, AST, Tot Bili, BUN, Cr
[g] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day
[h] Dipstick with reflex to microscopic urinalysis if blood, protein, or glucose is abnormal
[i] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination
[j] Detail AEs including injection site reactions via telephone on these days

Table 2b

Group 2 Schedule:

*Visits 2 and 5 are phone calls

Tests and Observations (Days)	D-1	D1 (1)	D4 (4)	D8 (8)	D15 (15) (1)	D18 (4)	D22 (8)	D29 (29) (15)	D43 (29)	D91	D181	D271	D366
Visit	**Screen 00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Clinical Assessments													
Consent, Demographics	X												
Medical History Review	X	X			X								X
Inclusion and Exclusion	X	X											
Medications[a]	X	X		X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X		X	X	X	X	X	X	X
Laboratory Assessments													
Screening Labs (40 mL)[d]	X												
Other Flavivirus Serologies[e] (10 mL)		X											
CBC w/diff and Chemistry Panel (15 mL)[f]		X		X	X		X	X	X	X	X	X	X
Pregnancy[g]	X	X			X								
Urinalysis[h]	X	X			X								X
Humoral (40 mL at D0, 30 mL at >D0)		X			X			X	X	X	X	X	X
CMI (40 ml)		X			X			X	X	X	X	X	X
Study Related Procedures													
Vaccination		**X**			**X**								
Distribute Diary		X			X								
Review Diary[i]				X			X						
Phone AEs[j]			X			X							
Blood Volume (mL)	40	105	0	15	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 1 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[c] Vital signs will be performed pre and post vaccination at 1 and 15 day visits

[d] FSH (if indicated), HIV-1 serology, Hepatitis B surface antigen, Hepatitis C antibody, WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, Plt, ALT, ALP, AST, Ca, Bicarb, Cl, Gluc, K, Na, Tot Bili, Tot Pr, Alb, BUN, Cr

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Hb, Plt, WBC, ALT, AST, Tot Bili, BUN, Cr

[g] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[h] Dipstick with reflex to microscopic urinalysis if blood, protein, or glucose is abnormal

[i] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[j] Detail AEs including injection site reactions via telephone on these days

Table 2c

Group 3 Schedule:

*Visits 2 and 4 are phone calls

Tests and Observations (Days)	D-1	D1 (1)	D4 (4)	D8 (8) (1)	D11 (11) (4)	D15 (15) (8)	D22 (22) (15)	D29 (29) (22)	D36 (29)	D91	D181	D271	D366
Visit	**Screen 00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Clinical Assessments													
Consent, Demographics	X												
Medical History Review	X	X		X									X
Inclusion and Exclusion	X	X											
Medications[a]	X	X		X		X	X	X	X	X	X	X	X
Physical Exam[b]	X	X		X		X	X	X	X	X	X	X	X
Vital signs[c]	X	X		X		X	X	X	X	X	X	X	X
Laboratory Assessments													
Screening Labs (40 mL)[d]	X												
Other Flavivirus Serologies[e] (10 mL)		X											
CBC w/diff and Chemistry Panel (15 mL)f		X		X		X	X	X	X	X	X	X	X
Pregnancy[g]	X	X		X									
Urinalysis[h]	X	X		X									X
Humoral (40 mL at D0, 30 mL for >D0)		X		X		X	X	X	X	X	X	X	X
CMI (40 ml)		X		X		X	X	X	X	X	X	X	X
Study Related Procedures													
Vaccination		**X**		**X**									
Distribute Diary		X		X									
Review Diary[i]				X		X							
Phone AEs [j]			X		X								
Blood Volume (mL)	40	105	0	85	0	85	85	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 1 through study discharge)
[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.
[c] Vital signs will be performed pre and post vaccination at 1 and 8 day visits
[d] FSH (if indicated), HIV-1 serology, Hepatitis B surface antigen, Hepatitis C antibody, WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, Plt, ALT, ALP, AST, Ca, Bicarb, Cl, Gluc, K, Na, Tot Bili, Tot Pr, Alb, BUN, Cr
[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.
[f] Hb, Plt, WBC, ALT, AST, Tot Bili, BUN, Cr
[g] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day
[h] Dipstick with reflex to microscopic urinalysis if blood, protein, or glucose is abnormal
[i] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination
[j] Detail AEs including injection site reactions via telephone on these days

Table 2d

Group 4 Schedule:

*Visit 2 is a phone call

Tests and Observations (Days)	D-1	D1 (1)	D4 (4)	D8 (8)	D15 (15)	D29 (29)	D91	D181	D271	D366
Visit	**Screen 00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**
Clinical Assessments										
Consent, Demographics	X									
Medical History Review	X	X								X
Inclusion and Exclusion	X	X								
Medications[a]	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X	X	X	X	X
Laboratory Assessments										
Screening Labs (40 mL)[d]	X									
Other Flavivirus Serologies[e] (10 mL)		X								
CBC w/diff and Chemistry Panel (15 mL)[f]		X		X	X	X	X	X	X	X
Pregnancy[g]	X	X								
Urinalysis[h]	X	X								X
Humoral (40 mL at D0, 30 mL at >D0)		X			X	X	X	X	X	X
CMI (40 ml)		X			X	X	X	X	X	X
Study Related Procedures										
Vaccination		**X**								
Distribute Diary		X								
Review Diary[i]				X						
Phone AEs[j]			X							
Blood Volume (mL)	40	105	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 1 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[c] Vital signs will be performed pre and post vaccination at day 1

[d] FSH (if indicated), HIV-1 serology, Hepatitis B surface antigen, Hepatitis C antibody,WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, Plt, ALT, ALP, AST, Ca, Bicarb, Cl, Gluc, K, Na, Tot Bili, Tot Pr, Alb, BUN, Cr

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Hb, Plt, WBC, ALT, AST, Tot Bili, BUN, Cr

[g] Serum pregnancy test at screening and urine pregnancy test at vaccination day

[h] Dipstick with reflex to microscopic urinalysis if blood, protein, or glucose is abnormal

[i] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination

[j] Detail AEs including injection site reactions via telephone on these days

Protocol Title:	A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
Short Title:	Zika Virus Purified Inactivated Vaccine (ZPIV) Accelerated Vaccination Schedule Study
Protocol Number:	Z001
Phase:	Phase 1
Sponsor:	Kathryn E. Stephenson Boston, MA
Sponsor Status:	Sponsor-Investigator
Study Location:	Center for Virology and Vaccine Research Clinical Trials Unit, Beth Israel Deaconess Medical Center
Study Period:	October 2016 – November 2017
Collaborating Institutions:	Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, USA, Vaccine Research Center/National Institute of Allergy and Infectious Diseases (VRC/NIAID), Bethesda, MD, USA
Date of Protocol Version:	February 27, 2017 4.0

TABLE OF CONTENTS

List of Abbreviations

AE	Adverse event
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCVI	Antibody-dependent cell-mediated virus inhibition
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotranferase
AST	Aspartate aminotransferase
BIDMC	Beth Israel Deaconess Medical Center
CBC	Complete blood count
$CD4^+$	A functional subclass of T cells, helper T lymphocytes (Th), that are necessary for augmentation and coordination of innate and adaptive effector responses, humoral and cellular
$CD8^+$	Cytotoxic T-cells that destroy host cells that have become infected by viruses or other intracellular pathogens
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CRF	Case report form
CTL	Cytotoxic T lymphocyte
CVVR	Center for Virology and Vaccine Research
DNA	Deoxyribonucleic acid
DMC	Data Management Center
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FDA	US Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMS	Global Medical Safety
HIV-1	Human immunodeficiency virus, type 1
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICF	Informed Consent Form
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
mL	Mililiters
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PI	Principal investigator
PO	Per Oral
PSRT	Protocol Safety Review Team
RBC	Red blood cell count
rtPCR	Real-time PCR
SAE	Serious adverse event
SMC	Safety Monitoring Committee
SST	Serum separating tubes
SUSAR	Suspected unexpected serious adverse reaction
$TCID_{50}$	50% Tissue Culture Infective Dose
VP	Viral particles

WBC	White blood cell count
ZPIV	Zika Virus Purified Inactivated Vaccine

1 OVERVIEW

Title

A Phase 1, Randomized, Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine in Healthy Adults

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 112, 196, 280, and 364 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

Study Products and Routes of Administration

- **Zika Virus Purified Inactivated Vaccine (ZPIV) with Alum Adjuvant:** Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at the manufacturing facility prior to shipment to clinic.
- **Placebo**: The placebo product is saline.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		36					

Participants

Healthy participants aged 18-50 years:

- 30 vaccinees
- 6 placebo recipients
- 36 total participants

Design

Single center, randomized, controlled, double-blind phase 1 trial

Duration per participant

12 months active follow-up per participant

Estimated total study duration

18 months (includes screening and active follow-up)

Investigational New Drug (IND) Sponsor-Investigator

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Study Site

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Safety Monitoring

Protocol Safety Review Team (PSRT)
Safety Monitoring Committee (SMC)

2 BACKGROUND AND RATIONALE

2.1 Introduction

Zika virus (ZIKV) is an emerging vector-borne RNA virus that, since early 2015, has caused an increased incidence of systemic disease and neurologic complications in an expanded region of the Western Hemisphere. ZIKV, of the family *Flaviviridae,* is related to other pathogens of global importance to humans, including dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV) and tick-borne encephalitic virus (TBEV). ZIKV was first isolated in Uganda in 1947 from a sentinel rhesus macaque and has since diverged along two lineages: African and Asian. The virus is almost exclusively transmitted by the *Aedes aegypti* mosquito but has been isolated from several other species of the genus *Aedes,* including *A. albopictus, A. africanus* and *A. luteocephalus*. The majority of human ZIKV infections result in an asymptomatic infection or a benign, self-limited acute febrile illness. ZIKV disease typically manifests as a rash, fever, conjunctivitis and arthralgia. Until 2007 when several outbreak clusters erupted in the South Pacific, ZIKV disease was relatively rare and geographically limited. Between 2007 and 2016, however, Zika has been documented in 60 countries and territories, 46 of which recorded their first case in either 2015 or 2016.

A clinical pattern has emerged from the most recent outbreaks that was never observed before. Nine months after local ZIKV transmission was first documented in Brazil, public health officials detected an increase in neonates born with microcephaly in the northeastern part of the country (WHO 2016). Epidemiologic studies, together with *in vitro* and *in vivo* experiments, have confirmed a causal association between ZIKV infections during pregnancy and the consequent occurrence of serious birth defects including microcephaly, brain malformations and ocular defects (Rasmussen SA, 2016). An increase in Guillain-Barré syndrome (GBS), meningitis and encephalitis have also been found in people with primary ZIKV infections (Cao-Lormeau, 2016) (Carteaux G, 2016), (Broutet N, 2016). The neuro-invasiveness of Zika virus infection and its complications ultimately prompted the World Health Organization (WHO) to declare, in February 2016, the emerging ZIKV epidemic as Public Health Emergency of International Concern.

2.2 Rationale for developing and testing a Zika Virus Purified Inactivated Vaccine (ZPIV)

There is no specific treatment or prophylactic currently available for ZIKV disease, other than supportive care and mosquito control. Given the expanding distribution and significant morbidity associated with ZIKV disease, the development of a safe and effective vaccine against ZIKV is a high global public health priority. The ZIKV vaccine to be used in this trial is a formalin-inactivated whole virus vaccine that was produced by the Walter Reed Army Institute of Research (WRAIR). There has been extensive experience with formalin-inactivated vaccines for other viruses, including other flaviviruses, such as the safe and immunogenic experimental dengue purified inactivated vaccine (Martinez 2015) and the licensed IXIARO® Japanese encephalitis vaccine. Experience with these other flavivirus vaccines has informed the design and development of the current ZIKV vaccine. However, the safety, tolerability and immunogenicity of a ZIKV vaccine, including the inactivated vaccine, is still to be determined. Because the safety and immunogenicity profile of this vaccine is still unknown, this Phase I trial, in concert with three others, will address a critical gap necessary to complete an early and rapid assessment of the viability of this ZIKV vaccine candidate. The first two clinical trials will explore the safety and immune response to the vaccine in flavivirus naïve individuals (National

Institutes of Health (NIH) and in participants who have been previously vaccinated with JEV or YFV vaccines (WRAIR). The third trial (NIH) will be conducted in Puerto Rico, whose population has background exposure to dengue virus and potentially yellow fever and West Nile virus as well. The dose and regimen that will be utilized in these initial trials was selected based on past experience with other inactivated flavivirus vaccines. Potential risks related to ZIKV vaccine administration may be similar to those of other inactivated flavivirus vaccines. Two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine developed by WRAIR and a JEV vaccine (IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated.

In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting and fever with the last three AEs occurring after the second administration of the 5 mcg dose. Unsolicited AEs detected among subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

2.3 Rationale for studying an accelerated vaccine schedule

The Phase 1 testing of a one- or two-dose ZPIV vaccine for Zika virus along a compressed schedule will complement the three other Phase 1 trials that are testing a standard prime-boost schedule of 0 and 4 weeks. This trial will test the WRAIR-developed vaccine in 36 healthy adults according to either a one-dose vaccination regimen or two-dose schedules of 0/2 weeks and 0/4 weeks. Investigation into the immunogenicity of a single dose or accelerated schedule of vaccination is salient to vaccine deployment in the setting of an emergent outbreak. The more rapidly a vaccine schedule confers immunity, the better chance an individual has of being protected from disease during the outbreak and the higher likelihood that transmission can be interrupted within populations. An accelerated schedule could not only benefit general populations living in Zika-endemic areas but first responders who need to be rapidly deployed to those areas. Emergency health responders and military personnel would potentially benefit from a shorter vaccination schedule that confers protection equivalent to that afforded by a standard, but more prolonged 1-month regimen. This study will evaluate those abbreviated schedules.

3 PRECLINICAL EXPERIENCE

3.1. *In vitro* characterization of ZPIV

The ZIKV vaccine is formalin-inactivated whole virus vaccine using a 2015 isolate from Puerto Rico (PRVABC59 V3 strain). The vaccine was produced in VERO cells and manufactured via a process that has been used successfully for other flavivirus vaccines (*e.g.* JEV vaccine). The clinical material used to develop the ZPIV was manufactured in Vero cells cultured in medium containing heat inactivated fetal bovine serum, Neomycin, and Streptomycin. Following infection with ZIKV (Puerto Rico PRVABC59 strain), culture supernatants were collected, and clarified by centrifugation and filtration (0.45 µm followed by 0.22 µm). The clarified viral fluids were treated with Benzonase to remove cellular DNA, and then concentrated by ultrafiltration followed filtration (0.45 µm). The concentrated virus was purified by column chromatography using a Capto™Core 700 column. The virus-containing fractions were pooled. The purified pool was filtered (0.22 µm). Sucrose was added and the virus was inactivated for 7 days at 22 °C with 0.05% formalin (1:2000 dilution of 37% formaldehyde). On day 2 of the inactivation, the virus was filtered (0.22 µm). Following inactivation, the formalin-treated virus pool was filtered (0.22 µm), concentrated to 50 µg/mL, and diafiltered to remove residual formaldehyde. Sucrose in PBS was added to a final concentration of 3% sucrose. The bulk was sterile filtered with a 0.22 sterile in-line filter. Inactivation was confirmed by inoculation of Vero cells. The purified inactivated virus (PIV) was then adsorbed to aluminum hydroxide (Alhydrogel®), which will be used to further dilute the adjuvanted vaccine to attain the moderate and low doses (i.e., 2.5 mcg and 1.25 mcg, respectively). Use of alum as an adjuvant is well established and generally well tolerated.

3.2 *In vivo* characterization of ZPIV

ZPIV has been tested in both mouse and rhesus monkey models. A single dose of purified inactivated virus vaccine induced high titers of ZIKV neutralizing antibodies and conferred complete protection, as measured by viremia, in both susceptible mice and nonhuman primates against challenge with a ZIKV strains from Brazil and Puerto Rico. Purified IgG from vaccinated animals also conferred passive protection in adoptive transfer studies in mice. CD4 and CD8 T lymphocyte depletion in vaccinated mice did not abrogate protective efficacy. These data demonstrate that protection against ZIKV challenge can be achieved by single-dose ZPIV and that humoral immunity alone is sufficient for that protection. These studies were carried out with non-GLP vaccine material. Overall, no clinical adverse events or clear changes in hematologies or chemistries were observed in rhesus monkeys following ZIKV PIV vaccination at weeks 0 and 4 (Larocca 2016) (Abbink 2016).

4 CLINICAL EXPERIENCE

4.1 Clinical experience with ZPIV in humans

While there is no human clinical experience with ZPIV, two other Vero cell-derived, formalin-inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent DENV (serotype 1) vaccine (WRAIR) and a JEV vaccine (manufactured as IXIARO®). Studies with both vaccines indicate that formalin inactivated flavivirus vaccines are reasonably well tolerated. In a Phase 1 clinical trial of two doses (2.5 mcg and 5.0 mcg per 0.5 mL) of an inactivated dengue vaccine administered IM in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez LJ, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting, and fever with the last three AEs occurring after the 2nd administration of the 5 mcg dose. Unsolicited AEs detected in subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia.

A formaldehyde-inactivated JEV vaccine (IXIARO®) has been licensed for use in the US since 2009. The package insert indicates that the most common adverse reactions reported were headache, myalgia, influenza-like illness, and fatigue (Valnera Austria GmBH, 2015). No effect was seen on the safety profile of IXIARO® compared to placebo (aluminum hydroxide) when examined by age, sex, or ethnic origin. In a Phase 3 clinical trial involving 2675 subjects who received either two doses of IXIARO® (6 mcg) or placebo (phosphate buffered saline [PBS] plus aluminum hydroxide), there was little difference between the active and placebo groups (Valnera Austria GmBH, 2015). The overall percentage of subjects who experienced at least one AE was 59% in the active group versus 57% in the placebo. Injection site reactions were mild to moderate in severity, and consisted of (in descending order of occurrence) pain, tenderness, erythema, induration, edema and pruritus. The most common systemic AEs post first dose and second dose were headache, myalgia, fatigue, influenza-like illness, nausea, nasopharygnitis, and fever. Sixteen SAEs were reported in 10 subjects who received vaccine and 6 subjects who received placebo. The SAEs that occurred among subjects in the IXIARO® group were dermatomyositis, appendicitis, rectal hemorrhage, limb abscess involving contralateral arm, chest pain, ovarian torsion, ruptured corpus luteal cyst, and three orthopedic injuries. No deaths occurred in the trial. IXIARO® is also licensed at one half the dose (3 mcg) for infants and children aged 2 months to <3 years; the full strength is recommended for use in older children. Fever was the most commonly observed AEs up to 12 years of age.

The association of ZIKV infections with the rare autoimmune disease, Guillain-Barre syndrome (GBS) and acute disseminated encephalomyelitis (ADEM) is a concern for ZIKV vaccines in general. In natural ZIKV infections, it is not yet clear whether the increased incidence of GBS is the result of an autoimmune reaction or is the result of neurotropism of the virus. Meningoencephalitis from ZIKV infection has also recently been reported.

In summary, inactivated flavivirus vaccines appear to be reasonably well tolerated. However, typical safety considerations with inactivated flavivirus vaccines include local reactions of pain, tenderness, erythema, induration, edema and pruritus, and mild to moderate systemic reactions including headache, myalgia, fatigue, influenza-like illness, nausea, and fever. The potential for a vaccine to induce GBS and other neuroinflammatory diseases is not known.

5 OBJECTIVES

Primary Objective

- To evaluate the safety and reactogenicity of ZPIV from initial dose through 28 days after the final dose.

Secondary Objectives

- To evaluate the humoral immune response (neutralizing and binding antibody titers) 14 and 28 days following the final administration of ZPIV, as well as at 6 months from the beginning of the study.

Exploratory Objectives

- To evaluate the durability of the humoral immune response (neutralizing and binding antibody titers) 112, 196, 280, and 364 days following the first dose of ZPIV.
- To evaluate cell-mediated immune (CMI) responses to ZPIV following vaccine administration.
- To qualitatively describe humoral immune responses following administration of ZPIV, through novel methods such as systems serology, RNASeq, and antibody epitope mapping via peptide microarrays.

6 STUDY DESIGN

This is a phase 1 trial of one or more administrations of Zika Virus Purified Inactivated Vaccine (ZPIV). The trial will be conducted under a placebo controlled, double-blind, randomized allocation of study product. This design is intended to reduce the likelihood of observer bias, provide control for confounding variables of intercurrent illness, and aid in the interpretation of laboratory data. There are three groups in the study and blinding will be maintained to vaccine or placebo within each group as noted in the table below.

Table 1: Study Schema

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 mcg	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 mcg	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 mcg	ZPIV			
	B	2		Placebo			
Total		36					

It is anticipated that enrollment will be completed within a 6-month period, and that participants will be actively followed for 12 months. Groups 1 through 3 will be enrolled in sequence to accelerate the collection of peak immunogenicity time-points from the groups with longer schedules (peak time-points are D57, D43, and D29 for Groups 1-3, respectively).

Sentinel Participants: Currently there are plans for two additional phase 1 clinical trials to test the ZPIV study product; these studies will begin before Z001 and will thus be responsible for monitoring the First-in-Human administrations of the vaccine. However, in the event that safety data is not available from at least 3 people from other Phase 1 studies with the vaccine at the proposed dose at the time when Z001 is ready to commence dosing, then we will enroll three sentinel subjects and assess safety for 7 days following dosing of the third sentinel prior to determining whether to vaccinate the remainder of the treatment groups. The randomization blocks ensure that at least 2 of the 3 sentinel subjects will receive active product; the third sentinel may receive either active product or placebo. The decision to vaccinate the remainder of the treatment groups will be made by the Protocol Safety Review Team (see Section 6.3)

6.1 Safety Assessment

To assess the safety of the administered vaccine, participants will document local and systemic reactogenicity on the first day of each vaccine administration period (days 1, 15, and/or 29 depending on the group assignment) and for the subsequent 6 days following each vaccine administration. The investigator will interview each participant about AEs at each visit throughout the study. Two to 3 days after each vaccine administration, a member of the site staff will have a remote safety follow-up communication with the participant by telephone. The

participant will be questioned about occurrence of reactogenicity. The participant will be brought in for a clinic visit based on this assessment, if deemed necessary by the investigator/sub-investigator or upon request of the participant. All participants will also be seen in clinic at the end of each reactogenicity period (days 8, 15, and/or 36 depending on the group assignment). All reactogenic events and AEs will be recorded on case report forms (CRFs) from the signing of the informed consent form (ICF) until the last study visit. To assess the participants' cellular and humoral immune response, blood samples will be taken at the clinic visits. Safety and immunogenicity visits will be conducted as outlined in the Schedule of Procedures (SOP).

6.2 Immunogenicity Assessment

Blood samples will be obtained at various time points after immunization (Section 18.3). These will be assayed for the magnitude of neutralizing and binding antibody titers to Zika virus at 14 and 28 days following each administration of ZPIV, as well as at 112, 196, 280 and 364 days following the first dose of ZPIV. The primary readout for neutralizing antibody assays will be the results of pseudovirus reporter and microneutralization assays. The secondary readout will be the result of plaque reduction neutralization assays. The third readout will be binding antibody titers by ELISA. Exploratory assessments may include epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, Zika-specific T cell responses, and the analysis of Zika-specific immune responses in animal models.

6.3 Monitoring

The Protocol Safety Review Team (PSRT) will review all adverse events (AEs) on a regular and expedited basis as needed. In addition, the PSRT will review safety data reports on a weekly basis until 12 participants have been enrolled after which reports will be reviewed biweekly. If sentinel participants are required (see above), the PSRT will also review safety data from the 3 sentinel participants after the 3rd has been followed for 7 days to determine whether to vaccinate the remainder of the treatment groups. The PSRT will include the Study Chair(s), co-investigators, and representatives from WRAIR and VRC/NIH, including a Department of Defense (DoD) Research Monitor (see below). Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs should also be reviewed by the Safety Monitoring Committee (SMC). The SMC will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC will be completely independent from the protocol team and sponsor. The SMC will evaluate safety and tolerability data on a regular basis. The SMC may review an individual SAE or it may choose to review AEs, SAEs, and laboratory and vital sign data. The SMC may unblind any amount of safety information needed to conduct their assessment. The conclusions of the SMC will be communicated to the investigators and the IRB/Ethics Committees and the national regulatory authorities as appropriate. The sponsor agrees to abide by the decision of the SMC and any directives issued by the national regulatory authorities, the Institutional Review Boards or Ethics Committees.

Department of Defense (DoD) Research Monitor: This study is supported by the Department of Defense (DoD), and therefore an independent research monitor is required. As outlined in the Department of Defense Instruction Number 3216.02, Section 8, the research monitor may perform oversight functions (e.g., observe recruitment, enrollment procedures, etc.) and report their observations and findings to the CCI / IRB or a designated official; the monitor may discuss

the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research; the monitor shall have the authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the CCI / IRB can assess the monitor's report. Research monitors shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the US Army Medical Research and Materiel Command Human Research Protection Office. The monitor for the study will have access to all safety data as a sitting member of the PSRT. The DoD research monitor has been appointed and is the following expert on vaccine development:

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6.4 Randomization Procedures and Enrollment

Participants will be enrolled in the study after ascertainment by one of the study investigators that all of the inclusion and none of the exclusion criteria have been met. Once confirmation of eligibility for the trial has been performed, the participant will be randomized in the electronic database using randomized treatment assignments generated by the Data Management Center (DMC). After successful randomization, a blinded allocation number is provided. The DMC will create the randomization table and the sponsor will monitor the implementation of this process. The blinded allocation number is referenced against a confidential list provided to an unblinded on-site pharmacist to determine the assignment/treatment allocation.

The 12 participants in each group will be randomized in a ratio of 5 vaccinees to 1 placebo recipient.

6.7 Method of Blinding and Unblinding

The participants, clinical staff, investigator, and sponsor personnel will be blinded to treatment allocation throughout the study. The pharmacist with primary responsibility for vaccine dispensing will not be blinded to the treatment and will maintain the randomization code and complete assignments of participants according to the randomization allocation. The pharmacist will also apply masking tape to the syringes to preserve the blind between vaccine and placebo. Routine unblinding of treatment allocations may occur only after all participants have had their last study visit and the database is locked.

7 STUDY POPULATION

The study population will include healthy men or women aged 18-50 years old who are able and willing to provide written informed consent. Participants will be enrolled in the study once eligibility criteria are met.

7.1 Participant Inclusion Criteria

1. Age 18-50 years old.

2. Ability and willingness to provide informed consent.

3. Assessment of understanding: completion of a questionnaire prior to first screening procedure; verbally demonstrate understanding of all questionnaire items answered incorrectly.

4. Available for the duration of the trial.

5. Good general health as shown by medical history, physical exam, and screening laboratory tests.

6. Meets laboratory parameters for hematology, chemistry, and urinalysis*

   **Criteria for hematology, chemistry, and urinalysis defined as follows:*
   - *Hematology*
     - *Hemoglobin ≥10.5 g/dL for women; ≥11 g/dL for men*
     - *Absolute Neutrophil Count (ANC): ≥1000/mm^3*
     - *Platelets: 125,000 to 550,000/mm^3*
   - *Chemistry*
     - *Creatinine: <1.1 x upper limit of normal (ULN)*
     - *AST: <1.25 x ULN*
     - *ALT: <1.25 x ULN*
   - *Normal urinalysis*
     - *Negative urine glucose (if positive urine glucose, then a microscopic urinalysis within institutional range).*
     - *Negative or trace urine protein (if greater than trace protein, then a microscopic urinalysis within institutional range).*
     - *Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis within institutional range).*

7. Female participants must be willing to undergo serum or urine beta human chorionic gonadotropin pregnancy tests at time points indicated in Section 18.3 and must test negative prior to vaccination.

8. All sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until Week 12.

9. If a woman of child-bearing potential, committed to use an effective method of contraception* when sexually active with men from the day of first vaccination until Week 12.

**Effective methods of contraception include the following:*

- *Condoms (male or female) with or without spermicide.*
- *Diaphragm or cervical cap with spermicide.*
- *Intrauterine device.*
- *Hormonal contraception.*
- *Successful vasectomy in the male partner (considered successful if a woman reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity post-vasectomy).*
- *Not be of reproductive potential, such as having undergone hysterectomy, bilateral oophorectomy, or tubal ligation, or being postmenopausal (>45 years of age with amenorrhea for at least 2 years, or any age with amenorrhea for at least 6 months and a serum follicle stimulating hormone [FSH] level >40 IU/L).*

Participant Exclusion Criteria:

1. History of known flavivirus infection or previous receipt of flavivirus vaccine.

2. Positive serology for HIV-1, Hepatitis B surface antigen, or anti-hepatitis C virus antibodies prior to enrollment.

3. Planned travel to areas with active Zika virus transmission during the study period.

4. Recent (within 3 weeks) travel to an area with active Zika virus transmission.

5. Current or planned participation in another clinical trial of an experimental agent during the study period.

6. Pregnant or lactating.

7. Any condition* for which participation would not be in the best interest of the subject (eg, compromise the well-being) or that could prevent, limit, or confound the study endpoints.

   **,At the discretion of the investigator and Includes any clinically significant acute or chronic medical condition.*

8. Use of anticancer, antituberculosis or other medications considered significant by the investigator within the previous 6 months.

9. Receipt of live-attenuated vaccine within the previous 60 days or planned receipt within 60 days after vaccination with Investigational Product*

   **Within 14 days for live attenuated influenza vaccine [LAIV]*

10. Receipt of other vaccine (e.g., influenza, pneumococcal), allergy treatment with antigen injections or tuberculin skin test within the previous 14 days or planned receipt within 14 days after vaccination with Investigational Product

11. Receipt of blood transfusion or blood-derived products within the previous 3 months.

12. Previous severe local or systemic reactions to vaccination.

13. History of splenectomy

14. History of seizure in the last 3 years (participants with a history of seizures who have neither required medications nor had a seizure for 3 years are not excluded)

15. Known autoimmune disease

16. Asthma other than mild, well-controlled asthma*.

**Participants should be excluded that:*
- *a. Use a bronchodilator (beta 2 agonist) daily, or*
- *b. In the past year have (any of the following):*
  - *i. Had > 1 exacerbation of symptoms treated with oral steroids*
  - *ii. Routinely used moderate to high dose inhaled corticosteroids (e.g., more than the equivalent of 250 mcg fluticasone; 400 mcg budesonide; 500 mcg beclomethasone; or 1000 mcg triamcinolone/flunisolide, as a daily dose) or theophylline*
  - *iii. Needed emergency care, urgent care, hospitalization, or intubation for asthma*
- *c. Prophylactic bronchodilator use prior to exercise is not exclusionary*

17. Diabetes mellitus type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

18. Thyroidectomy, or thyroid disease requiring medication during the last 12 months

19. Angioedema within the last 3 years if episodes are considered serious or have required medication within the last 2 years

20. Uncontrolled Hypertension*

**Uncontrolled hypertension is defined as:*
- *a. If a person has been diagnosed with hypertension during screening or previously, exclude for hypertension that is not well controlled. Well- controlled hypertension is defined as blood pressure consistently ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm*
- *b. If a person has NOT been diagnosed with hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 90 mm Hg at enrolment*

21. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

22. Malignancy*

**Not excluded: a participant with a surgical excision and subsequent observation period that in the investigator's estimation has a reasonable assurance of sustained cure or is unlikely to recur during the study period*

23. Psychiatric condition* that compromises safety of the participant or precludes compliance with the protocol

**Specifically excluding persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years*

24. History of neuroinflammatory disorder (e.g., Guillan-Barre Syndrome and Bell's Palsy)

8 STUDY PRODUCTS

8.1 Study Products

Detailed instructions for study products including preparation, storage and documentation are provided under separate cover in the Study Operations Manual. Additional information is also provided in the Investigator's Brochure for the ZPIV product.

8.1.1 Zika Virus Purified Inactivated Vaccine (ZPIV)

Zika Virus Purified Inactivated Vaccine (ZPIV) is from the ZIKV-PR strain (PRVABC59). The dose will be 5 mcg delivered as an intramuscular (left or right deltoid) injection. Alum adjuvant will be co-formulated with ZPIV in vials at manufacturing facility prior to shipment to clinic.

8.1.2 Placebo

The placebo is saline.

8.2 Handling of Study Treatments

8.2.1 Packaging and Labeling

Each single-use 2 mL vial of ZPIV vaccine contains 10 µg/mL ZPIV antigen and 1 mg/mL aluminum hydroxide adjuvant with a fill volume of 0.7 ± 0.07 mL of a sterile, preservative-free, PBS solution, for 5 µg PIV protein and 500 µg aluminum hydroxide adjuvant per 0.5 mL dose and for intramuscular injection only.

8.2.2 Shipment and Storage

ZPIV vials should be stored at 2-8 °C. During storage, a clear liquid with a white precipitate will be observed; this is the alum adjuvant and this appearance is to be expected following refrigerated storage. **Do Not Freeze.**

8.2.3 Dose preparation and administration

The unblinded pharmacist (or other unblinded staff member qualified to handle and dispense medication) will dispense ZPIV or placebo in a pre-filled capped syringe with the participant's study ID and allocation number. The vaccine vial should be shaken well to disperse and fully resuspend the alum-adsorbed vaccine, after which it should appear as a turbid white to slightly yellow suspension with white particulates.

9 CONDUCT OF THE STUDY

9.1 Informed Consent

The informed consent form documents that a participant (1) understands the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in this study. Informed consent encompasses all written or verbal study information staff provide to the participant before and during the trial.

The informed consent process continues throughout the study. At each study encounter, staff should consider reviewing the procedures and requirements for that encounter and for the remaining encounters. Additionally, if any new information is learned that might affect the participant's decision to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign a revised informed consent form.

Participants must sign a screening or protocol-specific consent before any procedures to determine eligibility are performed. All recruitment and prescreening materials will be approved by the Institutional Review Board.

9.1.1 Screening Consent Form

An IRB approved general vaccine screening protocol and consent may be used as part of the initial screening procedure for this trial. Results from this IRB approved general screening or prescreening may be used to determine eligibility in this protocol, provided the tests are conducted within the time period specified in the eligibility criteria.

9.1.2 Protocol-Specific Consent Form

The protocol-specific consent form describes the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. The consent form is developed in accordance with IRB requirements and the principles of informed consent as described in Title 45, Code of Federal Regulations (CFR) Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonization (ICH) Guideline 4.8.10. It must be approved by all responsible ethical review bodies before any participants can be consented for the study.

9.1.3 Test of Understanding

Study staff should ensure that participants understand the study before screening them. This process involves reviewing the informed consent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely. A Test of Understanding is used to document the participant's understanding of key concepts in this Zika vaccine trial. The participant must complete the Test of Understanding—with the assistance of staff, if necessary, in reading and understanding the questions and responses—before screening. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

9.2 Clinical and Laboratory Evaluations – Participants

Please see Section 18.3 for a concise outline of study procedures. Screening should occur within 56 days of enrollment. For visit windows, refer to the Schedule of Procedures (Section 18.3).

9.3 Unscheduled Visits

Unscheduled Visits/Contacts are visits/contact that are not described in the Schedule of Procedures (Section 18.3). Unscheduled visits may occur any time during the study:

- For administrative reasons, e.g., the participant or household contact may have questions for study staff or may need to re-schedule a follow-up visit.
- To obtain laboratory test results from a previous visit.
- For other reasons as requested by the participant or site investigator.

All unscheduled visits will be documented in the participant's study records on applicable source documents and entered into the Case Report Form (CRF).

9.4 Unblinding Visit

After all participants (from all 3 groups) have completed the active follow-up part of the study, and the database is locked, unblinding will occur. Participants will be informed as to whether or not they received study product or placebo. The unblinding visit may be performed in person or by phone.

9.5 HIV Counseling and Testing

HIV testing will be performed as part of screening evaluations. Therefore, HIV counseling will also be performed in compliance with the CDC's guidelines and local guidelines for HIV counseling, testing, and referral. All participants who become HIV-infected during the study will be terminated from the study. Potential and enrolled participants identified as HIV-infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.6 Prior and Concomitant Treatment

All concomitant medications will be recorded on CRFs from the signing of the ICF until the last study visit. Study participants can receive medications such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or antihistamines as needed although they must be documented. Use of these medications as routine prophylaxis prior to study vaccination is discouraged.

9.7 Study discontinuation

A participant will be taken out of the study in the event of:

- Repeated failure to comply with protocol requirements
- Decision by the study sponsor of PI to stop or cancel the study
- Decision by local regulatory authorities or IRB to stop or cancel the study
- Participant's request

9.7.1 Early discontinuation or withdrawal of participants

Each participant has the right to withdraw from the study at any time for any reason without affecting the right to treatment by the investigator/designee. The investigator should make an attempt to contact participants who did not return for scheduled visits or follow-up. Although the participant is not obliged to give reason(s) for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights.

The investigator also has the right to withdraw a participant, e.g. because of worsening health status, intercurrent illness, AEs, or pregnancy (for pregnancy follow-up see Section 10.4). The sponsor reserves the right to request the withdrawal of a participant due to safety issues, protocol violations, or administrative or other reasons.

Any unnecessary withdrawal should be avoided. Should a participant be withdrawn, all efforts should be made to complete and report the observations as thoroughly as possible. Whenever a participant is withdrawn from the study, independent of the reason, a final evaluation must be completed for that participant and the primary reason for which the participant was withdrawn must be stated. All documentation concerning the participant must be as complete as possible.

For those participants who are unable to continue participation in the study, but who do not withdraw consent, an early termination visit will be conducted. Any participant who withdraws consent will not have any further data collected after consent has been withdrawn.

9.7.2 Premature termination of the study

The sponsor reserves the right to discontinue the study for safety, ethical, or administrative reasons. Should the study be discontinued, no further vaccinations will be administered but participants who are still actively participating at the time of discontinuation will be followed through the remainder of their follow up visits.

9.7.3 Study pausing rules

If the trial is placed on safety pause, all enrollment and vaccinations will be suspended pending review. The AEs that may lead to a safety pause or prompt PSRT AE review are summarized below in Table 2. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the PI or PSRT, or participant may be threatened.

Table 2: AE notification and safety pause/AE review rules

Event and relationship to study product	Severity	Occurrence	Site PI action	PSRT or SMC action
SAE, regardless of relationship[1]	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AESI[2]	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
Anaphylaxis, laryngospasm, bronchospasm, or generalized urticaria within 1 day of vaccination	Any	Any	Phone, email or fax forms to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AE[3], related	Grade 3[4]	Second in SOC[5]	Phone, email or fax notification to PSRT chair within 24 hours	Study pause within 24 hours, refer to SMC for review
AE[3], related	Grade 3[4]	First	Phone, email or fax notification to PSRT chair within 24 hours	PSRT review within 2 business days to consider pause

[1] Deaths due to accident or trauma do not trigger a study pause and do not need to be referred to the SMC for review.

[2] AESI refers to Adverse Event of Special Interest, defined in section 10.1.1

[3] Within 30 days of vaccination, does not resolve within 2 days, and includes laboratory abnormalities; does not include the following reactogenicity symptoms (fever, fatigue, malaise, myalgia, arthralgia, chills, headache, nausea, vomiting, diarrhea, abdominal pain, rash).

[4] If no evidence of disease is present other than an abnormal laboratory value, the test must be repeated (entailing blood re-draw) at least one time. The verification period will be a maximum of 72 hours after initial awareness of the abnormal laboratory value. When signs and symptoms are present, repeat test will not be needed.

[5] PSRT will determine whether the reported related AE (Grade 3) is a second occurrence of a previously reported AE (Grade 3) in the same organ class (SOC).

For events in the table above, the Site Principal Investigator (PI) notifies the PSRT (within 24 hours). The PSRT will convene within two business days to review these AEs. The PSRT will review and determine disposition (including whether the SMC needs to review the event).

If a study pause is triggered, all enrollment and vaccinations will be held until review by the SMC. Resumption of enrollment and study treatment may be determined by the SMC (in consultation with the FDA, if required) following a cumulative review of the available safety data. If a decision to resume study enrollment and study treatment administration is made, the SMC will record its judgment in a memorandum to the study file and notify the sponsor, who will then forward the memorandum to the principal investigators. The clinical site will be allowed to resume activities upon receipt of written notification from the sponsor or its designee. As needed, the appropriate regulatory authorities will be informed in writing of the decision by the SMC to resume or discontinue study activities. The site is responsible for notifying the IRB according to local standards and regulations. The sponsor is responsible for notifying the FDA.

9.8 Laboratory evaluations

The total approximate volume of blood that will be collected from each participant is presented in Section 18.3. Total blood volume drawn from each participant will not exceed the American

Association of Blood Banks (AABB), and US Food and Drug Administration (FDA) guidelines of 550 mL in any eight-week period.

All biological samples must be collected in the appropriate manner. The investigator will ensure that the personnel and laboratory under his/her supervision comply with these requirements. Further details on shipment, handling, and storage of the samples are provided in the Specimen Handling Guidelines.

Any residual samples will be stored indefinitely following completion of the study at the sponsor-designated laboratories. Any future analyses conducted on the samples will maintain participant anonymity. Participants will have to provide their approval for long-term storage of their biological specimens. Participants will have the right to opt out of having their biological specimens stored once all analyses in the Informed Consent Form are completed. Opting out of this procedure does not impact the participant's ability to participate in the study.

9.9 Potential risks and benefits

9.9.1 Risks related to vaccines

Participants may exhibit general signs and symptoms associated with the intramuscular administration of a vaccine or placebo. Side effects, if observed, are expected to be short term, mild, and not requiring treatment.

Participants may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, hives or even difficulty breathing. Severe reactions are rare. Medications are available at the clinical site to treat serious allergic reactions.

The effect of this vaccine on a fetus or nursing baby is unknown, as well as the effect on semen. Therefore, sexually active males (unless anatomically sterile) must be willing to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until 12 weeks after final immunization (Week 16, 14, and 12 for Groups 1-3, respectively). Female participants of child-bearing potential will be required to agree to use birth control for sexual intercourse beginning prior to the vaccination and continuing through Week 12 weeks after final immunization (Week 16, 14, and 12 for Groups 1-3, respectively). Women who are pregnant or nursing will be excluded from the study.

9.9.2 Blood draws

Blood drawing may cause pain, bruising, and, rarely, infection at the site where the blood is taken.

9.9.3 Unknown risks

There may be other serious risks that are not known. Participants may believe that this vaccine provides protection against acquiring Zika infection, and therefore not use appropriate precautions to avoid Zika infection. They will receive extensive counseling throughout the study to address this potential problem. It is not known if the study vaccine increases, decreases, or has no effect on the chance of becoming Zika infected when exposed, or if upon becoming Zika infected, the person's disease course will be more or less severe.

9.9.4 Potential benefits

There is no direct benefit to the participant for participation in this clinical trial. Although study participants may benefit from clinical testing and physical examination, they may receive no direct benefit from participation. Society may benefit from knowledge gained in this study that may aid in the development of a Zika vaccine.

10 SAFETY ASSESSMENTS

10.1 Adverse events

10.1.1 Definitions

Adverse events

An AE is any untoward medical occurrence in a patient or participant, and does not necessarily have a causal relationship with a medical treatment. This includes any noxious, pathological, or unintended change in anatomical, physiological, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory-detected changes. These might occur in any phase of the clinical study whether associated with the study vaccine or not. This includes exacerbations of pre-existing conditions or events, intercurrent illness, or vaccine or drug interactions. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation are not considered AEs. Discrete episodes of chronic conditions occurring during a study period should be reported as AEs in order to assess changes in frequency or severity.

Serious adverse events

Seriousness refers to the outcome of an AE. Seriousness is determined by both the investigator and medical monitor, and can also be determined by the sponsor (*FDA 2010 Guidance for Industry and Investigators; Safety Reporting Requirements for INDs and BA/BE Studies*). An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in **death.**
- Is **life-threatening**: i.e. the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Results in persistent or significant **disability/incapacity**: i.e. results in a substantial disruption of the participant's ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance, such as headache, nausea, vomiting, diarrhea, influenza, infusion site reactions, or accidental trauma (e.g. sprained ankle).
- Requires in-patient **hospitalization** or prolongation of existing hospitalization: i.e. the participant is detained (usually at least an overnight stay) at the hospital or emergency ward for treatment that would not have been appropriate in the physician's office or in an out-patient setting. Hospitalization for either elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures[1] (including hospitalization for 'social' reasons) that are not the result of an AE, are **not** considered as SAEs.
- Is a **congenital anomaly or birth defect** in the offspring of a study participant
- Is an **important medical event** that may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above: e.g. interventions such as intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization, or development of drug

[1]A procedure that may take place during the study period and should not interfere with the study drug administration or any of the ongoing protocol-specific procedures.

dependency or drug abuse. Based on medical and scientific judgment, these events should usually be considered serious.

A suspected transmission of any infectious agent via a medicinal product is always considered as an important medical event, i.e. an SAE.

Although **not** considered as an SAE, cancer should be reported in the same way as SAEs.

Note: If anything untoward is reported during an elective procedure, that occurrence must be reported as an AE, either serious or non-serious according to the criteria defined above.

When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious.

Adverse Events of Special Interest

For this study, Neurologic and Neuroinflammatory* Disorders will be considered as Adverse Events of Special Interest (AESI). AESI will be collected for this study through the end of study follow-up period and will be captured and recorded in a CRF with the same timeline as the SAEs (within 24 hours of site awareness); the PSRT will be notified by the data center when such an event is reported. Any AESI that meets a SAE criterion will be reported as SAE as well.

***Neurologic and Neuroinflammatory Disorders:** ADEM, including site specific variants, Cranial Nerve Disorders (including paralyses/paresis), GBS (including Miller Fisher Syndrome and other variants), Immune-mediated Peripheral Neuropathies and Plexopathies, Optic Neuritis, Multiple Sclerosis, Narcolepsy, Transverse Myelitis, meningitis, or meningoencephalitis.

Adverse reactions

An adverse reaction is an AE judged to be related to study vaccine.

Related AEs (adverse reactions) are defined as those judged by the investigator, sponsor or independent safety monitor to be possibly, probably, or definitely related to study vaccine.

When an AE is judged to be related to study vaccine and also is judged to be serious and unexpected, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting. All SAEs in this study will be considered unexpected as there are no expected AEs associated with this vaccine product.

Adverse reactions will be reported in accordance with FDA 2009 Guidance for Clinical Investigators, Sponsors and IRBs; Adverse Events Reporting to IRBs – Improving Human Participant Protection.

10.1.2 Surveillance, reporting, and documentation of adverse events

The recording of AEs is an essential part of study documentation. The investigator is responsible for documenting all AEs as set out in the following sections.

10.1.2.1 Surveillance of adverse events

At each visit through 28 days following last vaccination, all AEs, either observed by the investigator or one of his/her clinical collaborators, or reported by the participants spontaneously or in response to a direct question, will be evaluated by the investigator or designee. All SAEs will be reported through the final visit (Study Day 364).

Participants will be instructed to contact the investigator immediately should he/she experience any signs or symptoms he/she perceives as severe from the time of vaccination through a period of 12 months.

10.1.2.2 Documentation of adverse events

All AEs will be collected on case report forms (CRFs) following first vaccination until 28 days following last vaccination. SAEs will be collected through Study Day 364.

AEs and SAEs should be documented in terms of signs and symptoms observed by the investigator or designee or reported by the participants. Whenever possible, a medical diagnosis should be made. The nature of each event, date and (where appropriate) time of onset, outcome, severity, and causal relationship should be established. Details of any symptomatic/corrective treatment should be recorded on the appropriate page of the CRF.

Hospitalization for routine clinical procedures (or other reason) that is not the result of an AE, are not considered AEs but will be recorded on the AE page of the CRF ('Hospitalization, Not an AE'). The same applies for hospitalization for elective procedures related to a pre-existing condition that did not increase in severity or frequency during the study. If hospitalization was planned before administration of the study vaccine, it will be documented in the Medical History page of the CRF (see below).

The following events will be documented in the Medical History page of the CRF:

- Pre-existing conditions or signs and/or symptoms present in a participant before study start. This includes conditions that were not recognized at study entry but later during the study period.
- Hospitalization arising from a pre-existing condition and planned before the administration of the vaccine.

10.1.2.3 Post-vaccination reactions occurring immediately after each vaccine administration

Participants will be observed for minimum of 30 minutes following vaccine administration. Vital signs (temperature, blood pressure, pulse and respiratory rate) will be taken and qualified study personnel will evaluate for any signs or symptoms of reactions.

Possible reactions may include fever (≥37.7 ºC), fatigue/malaise, headache, myalgia, arthralgia, chills, nausea, vomiting, and arm pain. For life-threatening allergic reactions that occur immediately post-vaccination, the site has specific procedures developed for handling such emergencies.

10.1.2.4 Reactions occurring within seven days following each vaccine administration

Participants will be instructed to notify specified study personnel immediately if any unusual or severe sign or symptom appears after vaccine administration. Participants will maintain a study diary during this period to record potential reactions.

10.1.3 SAE Reporting

The sponsor has a regulatory obligation to report SAEs to the FDA according to established timetables for reporting based on specific criteria. The investigator will report SAEs to the PSRT and IRB within 24 hours from the time they first learn of the event. The SAE form will be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the PSRT and IRB. The investigator will provide his/her assessment of causality to study treatment at the time of the initial SAE report. The investigator will not delay in the reporting of any SAE in order to obtain additional information. Any additional information, if collected, will be reported to the PSRT and IRB as a follow-up to the initial report.

All SAEs must be reported immediately (within 24 hours of discovery) by email or fax to the IRB at the following contact information:

Email: Jessica Ripton, jripton@bidmc.harvard.edu
Fax: (617) 975-8501

The PSRT will perform a clinical review of the information provided to identify any missing data. The PSRT will also contact the study site to clarify any discrepant or missing information, to answer questions and to provide guidance to the site, if needed. The investigator will report the SAE as an acceptable medical diagnosis. If a preliminary diagnosis has not yet been made, then each symptom will be listed separately. A follow-up report will be issued when a diagnosis is made.

The investigator must report SAEs to the appropriate IRB as requested by the board according to local legal requirements.

10.2 Reporting requirements to the local IRB

The PI/designee will be responsible for providing all safety reports and reporting all SAEs, study pauses, social harms, and major deviations to the local regulatory authorities, such as a local IRB, and any regulatory agencies, in a timely manner according to the institution's guidelines and to local regulations.

10.3 Causality assessment of study vaccine to adverse events

Every effort should be made by the investigator to explain any AE and assess its potential causal relationship, i.e. to administration of the study treatment or to alternative causes (e.g. natural history of the underlying diseases, concomitant therapy). This applies to all AEs, whether serious or non-serious.

The investigator will use the following guidelines to assess the causal relationship of an AE to study injection:

Not related:	Adverse experiences felt to be due to extraneous causes that neither follows a known pattern of response nor a reasonable temporal relationship to study vaccine.
Remote (probably not related):	Adverse experiences that are unlikely to be related to study vaccine but which follow a reasonable temporal relationship, such that this cannot be completely excluded or events that could be associated with study vaccine but which are unrelated in time.
Possibly related:	Adverse experiences that may be due to extraneous causes but which follow a known pattern of response and/or a reasonable temporal relationship to study vaccine.
Probably related:	Adverse experiences that cannot be explained by extraneous causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.
Definitely related:	Adverse experiences that have a definite relationship to the study vaccine (e.g. anaphylactic reaction after vaccination) without any other explained causes; which follow a known pattern of response and/or a reasonable temporal relationship; which disappear or decrease on cessation of study vaccine and reappear on re-challenge.

In the final analyses, events categorized as “not related” and “remote” will be considered as not related to study vaccine; events categorized as “possibly related”, “probably related” and “definitely related” will be considered as related to study vaccine.

10.3.1 Severity of adverse events

All AE and lab data will be coded for severity using the adapted Division of Microbiology and Infectious Disease Revised Toxicity Table included in Section 18.1. This Table has been adapted to comply with Clinical Data Interchange Standards Consortium guidelines.

The grading scale contains only 3 severity grades: Mild (Grade 1), Moderate (Grade 2), and Severe (Grade 3). Events that are life-threatening or result in death will be graded as Severe (Grade 3) and indicated to be SAEs with the appropriate outcome.

For AEs not identified in the grading table, the following guidelines will be applied:

Mild	Grade 1	Symptoms causing no or minimal interference with usual social and functional activities
Moderate	Grade 2	Symptoms causing greater than minimal interference with usual social and functional activities
Severe	Grade 3	Symptoms causing inability to perform usual social and functional activities; Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability (also reported as SAE); and Death (also reported as SAE)

The clinical research site team will ascertain accurate recording of all AEs during the study. AE CRFs will be completed by the clinical research site staff on a daily basis as the data become available from the isolation unit, clinic or laboratory.

The investigators will monitor and analyze study data including all AE and lab data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study vaccine. AEs will be deemed either related to study vaccine or not related to study vaccine. To ensure that all AEs are captured in a timely manner, CRFs will be entered in real-time entry, and subjected to analysis to identify AEs that may invoke study pausing rules.

The PI or designee must review AE CRFs to insure prompt and complete identification of all events that require expedited reporting as SAEs, study pausing rules or other serious and unexpected events.

Study vaccine related AEs will be followed by the clinical research site team through resolution or until study completion. Non-study vaccine related AEs will be followed to resolution or study completion, whichever occurs first.

10.3.2 Follow-up of ongoing adverse events and assessment of outcome

10.3.2.1 Follow-up of non-serious adverse events

Non-serious AEs already documented in the CRF at a previous assessment and designated as ‘ongoing’ should be reviewed at subsequent visits. If the event has resolved, the documentation in the CRF should be completed. If the frequency or severity of a non-serious AE changes significantly, a new record of the AE has to be started. If the AE becomes serious, the procedures for reporting of SAEs have to be followed (see Section 10.1.3).

New non-serious AEs will be recorded through 28 days post-vaccination. Ongoing non-serious AEs will be monitored until the Day 366 visit (or until the last study visit for participants who withdraw early (see Section 18.3)).

Outcomes will be assessed as:

1. Resolved
2. Resolved with sequelae
3. Resolving
4. Not resolved
5. Fatal
6. Unknown

Clinically significant abnormal laboratory values will be followed up until they have returned to normal, stabilized, or a satisfactory explanation has been provided.

10.3.2.2 Follow-up of serious adverse events and AESI

All SAEs and AESIs must be followed-up until the event has either resolved, subsided, stabilized, disappeared, or is otherwise explained, or the study participant is lost to follow-up,

but no longer than 12 months after the administration of the study vaccine. Outcomes are assessed as above.

All follow-up activities must be reported in a timely manner to the PSRT (if necessary on one or several consecutive SAE/AESI report forms). All form fields with additional or changed information must be completed and the SAE/AESI Report Form should be forwarded to the PSRT as soon as possible but at the latest within 7 calendar days after receipt of the new information.

Clinically significant laboratory abnormalities reported as SAEs will be followed-up until they have returned to normal, or a satisfactory explanation has been provided.

Reports related to the subsequent course of any SAE/AESI reported for any participant must be submitted to the sponsor.

10.3.3 Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to currently available best treatment. The applied measures should be recorded in the CRF.

10.4 Handling of pregnancy cases

Pregnancy events will be reported through 3 months following final vaccination. All initial reports of pregnancy must be reported to the PSRT by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, stillbirth, and congenital anomaly) are considered SAEs and must be reported using the Serious Adverse Event Form.

Because the effect of the study vaccine on sperm is unknown, pregnancies in partners of male participants included in the study will be reported by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

10.5 Physical examination

Physical examinations will be performed by the investigator or designated medically-trained clinician. The time points of these examinations are specified in Section 18.3. Any abnormalities or changes should be documented in the source document and recorded on the CRF.

A new, clinically significant finding (in the opinion of the investigator) not noted at screening must be captured as an AE. In addition, resolution of any abnormal findings during the study will be noted in the source document and in the CRF.

10.6 Routine safety laboratory

Samples for routine safety laboratory parameters will be collected at the time points specified in Section 18.3.

The following routine safety laboratory parameters will be determined:

At screening:

- **Serum chemistry:** ALT, Alkaline Phosphatase, AST, Calcium, Carbon Dioxide, Chloride, Glucose, Potassium, Sodium, Total Bilirubin, Total Protein, Albumin, Globulin (calculated), Blood urea nitrogen, Creatinine, Estimated GFR, Albumin/Globulin ratio (calculated), BUN/Creatinine ratio (calculated)

- **Complete blood count:** WBC (neutrophils, lymphocytes, monocytes, eosinophils, basophils), RBC, Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Platelets Count

At follow up:

- **Serum chemistry:** ALT, AST, Total Bilirubin, Blood urea nitrogen, Creatinine, BUN/Creatinine ratio (calculated)

- **Complete blood count:** Hemoglobin, Platelets Count, WBC

Laboratory values will be graded according to the DMID toxicity scale (Section 18.1) and, if clinically significant, reported as AEs.

10.7 Vital signs

Vital sign measurements will be performed at time points specified in Section 18.3.

The following measurements will be performed:

- Heart rate (bpm)
- Systolic and diastolic blood pressure (mmHg)
- Respiratory rate
- Body temperature (oral)

A confirmatory vital signs measurement can be performed if inconsistent with a prior measurement. If any clinically significant changes in vital signs are noted, they will be reported as AEs and followed to resolution, or until reaching a clinically stable endpoint.

10.9 Protocol Safety Review Team and Safety Monitoring Committee

The Protocol Safety Review Team (PSRT) will include the Protocol Chair, Protocol Co-Chairs, Site Principal Investigator, co-investigators and partners at WRAIR. Additional members could include associate investigators, senior clinical research nursing staff, and site staff. The PSRT will decide by consensus whether AEs not requiring a study pause should also be reviewed by the SMC.

The Safety Monitoring Committee (SMC) will be appointed by the sponsor before the start of the study and will operate according to its charter. The SMC is an independent multidisciplinary

group consisting of experts in virus infection (e.g., dengue, West Nile Virus, HIV etc.) that, collectively, has experience in the management, conduct, and monitoring of vaccine trials. Members of the SMC are not directly affiliated with this protocol and are not situated at the study site. The SMC also will review individual Expedited Adverse Event (EAE) reports. The SMC will conduct additional special reviews at the request of the PSRT. Additional detail is provided in Section 6.3.

11 IMMUNOGENICITY ASSAYS

11.1 Zika-associated immunogenicity

Blood samples for the determination of cellular and humoral responses will be collected as specified in Section 18.3.

Humoral response assays to Zika will likely include, but are not limited to: microneutralization assays, ELISA, and plaque reduction neutralization assays. The **microneutralization assay** is a flavivirus high-throughput ELISA based microneutralization test in vero cells. Neutralization titer, expressed as MN50 titer, is regarded as the reciprocal of the serum dilution capable of neutralizing 50 percent of the virus dose in the ELISA part of the test. Seropositivity in the assay is a MN50 titer greater then or equal MN50 of 10. **ZIKV ELISA** assays will be performed using ZIKV Env protein; endpoint titers will be defined as the highest reciprocal serum dilution that yielded an absorbance >2-fold over background values and log10 endpoint titers will be reported. The **plaque reduction neutralization test** is used to quantify the titer of neutralizing antibody for ZIKV virus. The concentration of plaque forming units can be estimated by the number of plaques (regions of infected cells) formed after a few days. The concentration of serum to reduce the number of plaques compared to the serum free virus gives the measure of how much antibody is present or how effective it is.

Table 3 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 3: Humoral immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Principle Timepoint(s)
Humoral responses	Secondary	Serum	ZIKV microneutralization (MN) assay	Log10 MN50 titers	Group 1: D1, D57*, D196 Group 2: D1, D43*, D196 Group 3: D1, D29*, D196 * Peak
	Secondary	Serum	ZIKV Env ELISA	Log10 endpoint titers	Group 1: D1, D57*, D196 Group 2: D1, D43*, D196 Group 3: D1, D29*, D196 * Peak
	Exploratory	Serum	ZIKV plaque reduction assays	Log10 titer	Group 1: D1, D57*, D196 Group 2: D1, D43*, D196 Group 3: D1, D29*, D196 * Peak

Evaluations of cellular immune responses to Zika will likely include, but are not limited to, interferon gamma producing cells (ELISPOT) assays. ZIKV-specific cellular immune responses will be assessed by interferon-γ (IFN-γ) ELISPOT assays using pools of overlapping 15-amino-acid peptides covering the prM, Env, Cap, and NS1 proteins. Table 4 describes the principle timepoints that will be analyzed to determine the endpoints of the study.

Table 4: Cellular immune response assays

Rationale	Objective/ endpoint	System	Assay method	Unit	Primary Timepoint(s)
T-cell responses	Exploratory	PBMC	Interferon gamma producing cells (ELISPOT)	Number of spot forming cells per 10^6 PBMC	Group 1: D1, D57*, D196 Group 2: D1, D43*, D196 Group 3: D1, D29*, D196 * Peak

Immunologic assays may be performed and analyzed in a blinded fashion after all participants of a given group reach the peak immunogenicity time-point for their group (D57, D43, and D29 for Groups 1-3, respectively). This will allow more rapid accrual of data to facilitate product development strategies. The optimal choice of immunogenicity assays will take into consideration the latest technological advances.

11.2 DNA/RNA Assays

Blood samples collected for cellular immunogenicity may also be used for exploratory DNA and RNA micro-array and deep-sequencing assays.

11.4 Additional Immunogenicity Assays

Additional immunogenicity assessments of systemic and mucosal responses may potentially include the following: flavivirus serology, epitope specific neutralization patterns, systems serology (including measurements of Zika-specific antibody function, subclass and epitope specificity), the identification and development of vaccine-induced Zika-specific monoclonal antibodies, and Zika epitope-specific T cell responses.

12 DATA EVALUATION AND STATISTICS

All data entry will be performed by qualified and trained study staff. When all data have been entered and validated, the final database will be locked. An interim blinded analysis will be performed 28 days following the final immunization (group assignments will be known). A second interim blinded analysis will be performed at the 6 month time-point. Final analysis will be performed when D364 data has been collected.

12.1 Analysis populations and data sets

12.1.1 Safety population

All participants who received ZPIV or placebo, and for whom any post-dose data is available, will be included in the safety population.

12.1.2 Immunogenicity population

The immunogenicity population will consist of all participants who received ZPIV or placebo, and who have at least one measured post-dose blood sample collected.

12.2 Endpoints

Safety and tolerability:

- Incidence, intensity, and relationship to vaccination of solicited local and systemic adverse events (AEs) during the 7-day follow-up period (Days 1-7) after each ZPIV dose.
- Incidence, intensity, and relationship to vaccination of unsolicited AEs during the 28-day follow-up period (Days 1-29 after each ZPIV dose)
- Grade 2 and Grade 3 laboratory abnormalities at Day 8 after each ZPIV dose.
- Incidence of serious adverse events (SAEs) and related AEs from Day 1 through Day 364.

Immunogenicity:

- Principle Endpoints: Proportion (95% CI) of participants per dose group with positive responses and mean response (e.g. GMT) per group with 95% CI for the following 2 parameters:
    - ZIKV microneutralization Log10 MN50 titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D1.
    - Zika Env-specific Log10 endpoint ELISA titers at peak immunogenicity (e.g., 28 days following final vaccination) and at 6 months from D1.

- Additional Endpoints:
    - Plaque reduction neutralization test titer at 14 and 28 days following each vaccination, and at 6, 9, and 12 months from D1.

- Durability and kinetics of humoral immune responses: Log10 MN50 and Log10 endpoint ELISA titers at 14 and 28 days following *first* (and second) vaccination, and at 6, 9, and 12 months.
- Cellular immunogenicity: IFN-γ ELISPOT responses to prM, Env, Cap, and NS1 peptides at 28 days following final vaccination and at 6 months from D1.

12.3 Sample size consideration

The number of participants chosen for this study will provide a preliminary safety and immunogenicity assessment. The sample size is not based on formal hypothesis testing considerations, but within the range of participants (i.e., 20-80) recommended in the Code of Federal Regulations (CFR 312.21) for first-in-human product Phase 1 evaluations. Placebo recipients are included for blinding and safety purposes and will provide additional control specimens for immunogenicity assays.

13 ETHICAL AND LEGAL REQUIREMENTS

13.1 General requirements

The study will be performed according to this Study Protocol and in compliance with the Declaration of Helsinki, the guidelines of the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) and the respective local legal requirements including the following: US Code of Federal Regulations 45CFR Pt 46; 21CFR Pt 50, 21CFR Pt 56 and 21CFR Pt 312.

13.2 Institutional Review Board/Ethics Committee

Before the start of the study, the investigator will submit the Study Protocol, Informed Consent Form, and other study-related documents as required by applicable laws and regulations to the responsible IRB for written approval.

The investigator will inform the IRB according to applicable laws and regulations about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

Unless otherwise instructed by the IRB or local regulation the investigator must submit to the IRB:

- All subsequent Amendments to the Study Protocol, changes to the Informed Consent Form or revisions of other documents originally submitted for review
- New or revised participant recruiting materials approved by the sponsor, if applicable
- All subsequent changes of logistical or administrative aspects (for information)
- Serious and/or unexpected AEs occurring during the study, where required
- New information that may affect adversely the safety of the participants or the conduct of the study
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Annual update and/or request for re-approval, where required
- Date of study completion, where required

13.3 Regulatory authorities

Before initiating the study, the sponsor will submit any required application to the regulatory authorities and obtain approval according to applicable laws and regulations. The sponsor will also inform the regulatory authorities about Amendments to the Study Protocol including, but not limited to, any new information that requires an ethical reconsideration of the Study Protocol.

13.4 Participant information and informed consent

The study informed consent describes the investigational products to be used and all aspects involved in protocol participation. A properly executed written informed consent, in compliance with the Declaration of Helsinki, guidelines of the Council of International Organization of Medical Sciences (CIOMS), the Belmont Report, the US Code of Federal Regulations 21 CFR 50, must be obtained from each participant prior to entry into the trial or prior to performing any unusual or non-routine procedure that involves risk to the participant. The investigator must

provide a copy of the approved informed consent to the participant and a signed copy must be maintained in the participant's record file. Before a participant's participation in the study, it is the investigator's responsibility to obtain this written informed consent from the participant, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or study medications are administered.

All items must be explained by the investigator or designee in a language that is easy to understand. Participants will also be informed that the participation is voluntary and that they have the right to withdraw at any time without giving reasons and without any disadvantages for their subsequent care. Participants will confirm their consent in writing before study start and any study-specific procedure.

Participants must be given enough time to consider participation in the study.

13.5 Indemnity

The Sponsor and Institution are responsible to have appropriate liability insurance. For research-related injuries and/or medical problems determined to result from receiving the Investigational Product, treatment including necessary emergency treatment and proper follow-up care will be made available to the participant free of charge at the expense of the Sponsor.

13.6 Data access and protection

Confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

The collection and processing of personal data from participants enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of participants confidential.

The informed consent includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to the participant's source data/documents for study-related monitoring, audit, IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The participant has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

13.7 Future use and storage and blood samples

Each study participant will be asked to consent voluntarily for their blood samples to be stored for other research studies that may be done after this study is completed. Future testing may involve DNA/RNA tests. For participants unwilling to have their blood samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed after all the tests specified for this study have been concluded.

All samples for which consent has been obtained, and for which additional material is available after study-specified testing is complete, will be stored for future testing. All applicable approvals will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB.

13.8 Reimbursement

Participants and household contacts will be reimbursed for time and inconvenience in accordance with the standards and legal obligations for compensation required by each site. Any applicable guidelines by IRBs/ECs for compensation of research participants and household contacts will be sought and followed.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

Monitoring of clinic activities will be performed regularly to ensure that the study is carried out according to this Study Protocol and in compliance with GCP and applicable legal requirements.

Source documents will be reviewed for verification of consistency with CRF data. The investigator guarantees direct access to source documents for monitoring purposes. Source data verification will be performed in accordance with data protection regulations and guidelines. All information reviewed will be handled according to these rules and regulations.

Monitor(s) will review each participant's data as outlined in the study specific Data, Investigational Product, and Safety Monitoring Plans.

14.2 Audit and inspections

Audits and inspections may be carried out by qualified delegates authorized by the sponsor or by authorities. The investigator consents to cooperate and to allow direct access to all source documents and other study-related data during an audit or inspection. All information disclosed will be handled in accordance with applicable data protection rules and regulations.

14.3 Data quality assurance

All CRF data will be entered into a validated, 21CFR Part 11 compliant, computerized clinical data management system.

The site is required to have a plan in place for assuring the quality of the research being conducted.

15 DOCUMENTATION, ARCHIVING, AND PUBLICATIONS

15.1 Documentation

Source documents are original documents, data, and records from which the participant's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, radiographs, and correspondence.

The Investigator and staff are responsible for ensuring maintenance of a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the sponsor, FDA, and/or applicable regulatory authorities.

All CRF entries have to be verifiable by the source data in the participant file. This does not apply to CRF entries that are defined as source data.

15.2 Archiving

The Investigator is responsible for the archiving of the Investigator's file, the participant's file, and the source data according to national and international legal requirements.

Any records related to the conduct of the study may not be destroyed without written authorization by the sponsor. According to GCP requirements, study records should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

15.3 Clinical Study Reports and Publications

The results of the clinical study will be documented in the Clinical Study Report.
The study results may be published and/or presented at scientific meetings. Terms of publication will be addressed in an agreement between the sponsor and participating partners.

16 REFERENCES

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17 PROTOCOL SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the appendices and provide the necessary assurances that this study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) and the applicable regulatory requirement(s).

Protocol Chair:

Signed: _____ Date: _____

Kathryn E. Stephenson, MD, MPH
Beth Israel Deaconess Medical Center

Sponsor-Investigator:

Signed: _____ Date: _____

Kathryn E. Stephenson, MD, MPH
Beth Israel Deaconess Medical Center

18 APPENDICIES

18.1 Toxicity Tables

DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASES (DMID) ADULT TOXICITY TABLE (ADAPTED)

Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal R_x = Therapy
Req = Required	Mod = Moderate
IV = Intravenous	ADL = Activities of Daily Living
Dec = Decreased	

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1	**Mild** Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	**Moderate** Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	**Severe** Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible; or **Life-threatening*** Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable (***also reported as SAE)**

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be graded as Severe (Grade 3) and reported as an SAE. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.
- For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.
- These tables have been modified to comply with Clinical Data Interchange Standards Consortium guidance.

HEMATOLOGY			
	Grade 1	Grade 2	Grade 3
Hemoglobin	9.5 - 10.5 gm/dL	8.0 - 9.4gm/dL	< 7.9 gm/dL
Absolute Neutrophil Count	1000-1500/ mm^3	750-999/ mm^3	< 749/ mm^3
Platelets	75,000-99,999/ mm^3	50,000-74,999/ mm^3	< 49,999/ mm^3
WBCs	11,000-13,000/ mm^3	13,000-15,000 / mm^3	<1,000 / mm^3 or > 15,000/
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: <100 mg/dL High: >600 mg/dL	Low: < 50 mg/dL, OR Fibrinogen associated with gross bleeding or with disseminated coagulation ----------
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	> 51 mcg/ml
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	> 1.51 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	> 2.34 x ULN
Methemoglobin	5.0 - 9.9 %	10.0 - 14.9 %	> 15.0 %

CHEMISTRIES			
	Grade 1	Grade 2	Grade 3
Hyponatremia	130-135 mEq/L	123-129 mEq/L	< 122 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	> 158 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	< 2.4 mEq/L or intensive replacement therapy or hospitalization required, abnormal potassium *with* paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	> 6.6 mEq/l
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	< 39 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	> 251 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	< 6.9 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany

CHEMISTRIES (continued)			
	Grade 1	Grade 2	Grade 3
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	> 12.6 mg/dL or abnormal calcium *with* life threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	< 0.8 mEq/L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	< 1.4 mg/dL intensive therapy or hospitalization required, or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	> 1.5 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	> 2.0 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	> 5.1 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/dL	> 12.1 mg/dL
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	> 3.1 x ULN or dialysis required

ENZYMES			
	Grade 1	Grade 2	Grade 3
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	> 3.0 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	> 2.1 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	> 2.1 x ULN

URINALYSIS			
	Grade 1	Grade 2	Grade 3
Glycosuria (random collection tested by dipstick)	Trace to 1+ or ≤ 250 mg	2+ or > 250 to < 500 mg	> 2+ or > 500 mg
Proteinuria (random collection tested by dipstick)	1+	2+	3+ or higher
Hematuria (not to be reported based on dipstick findings or on blood believed to be of menstrual origin)	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts OR obstructive or required transfusion

CARDIOVASCULAR			
	Grade 1	Grade 2	Grade 3
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required, OR unstable dysrythmia; hospitalization
Hypertension	transient increase > 20 mm/Hg; no treatment	recurrent, chronic increase > 20mm/Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible, OR end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required, OR mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment
Pericarditis	minimal effusion	mild/ moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes, OR tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused, OR massive blood loss; > 3 units transfused

RESPIRATORY			
	Grade 1	Grade 2	Grade 3
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present OR cyanosis: $FEV_1 < 25\%$ of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest OR dyspnea requiring Oxygen therapy

GASTROINTESTINAL			
	Grade 1	Grade 2	Grade 3
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids OR hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids OR physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	Obstipation requiring manual evacuation or enema OR obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/day or mild diarrhea last < 1 week	moderate or persistent; 5-7 loose stools/day or diarrhea lasting >1 week	>7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required OR hypotensive shock or physiologic consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods, OR unable to drink fluids; requires IV fluids

NEUROLOGICAL			
	Grade 1	Grade 2	Grade 3
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia, OR incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation, OR acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited, OR paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement, OR incapacitating; or not
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in grea toes) in focal area or symmetrical distribution; or change i taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities), OR sensory loss involves limbs and trunk; paralysis; or seizures

MUSCULOSKELETAL			
	Grade 1	Grade 2	Grade 3
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living, OR disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living, OR permanent and/or disabling joint distruction
Myalgia	myalgia with no limitation of activity	Muscle tenderness (at other than injection site) or with moderate impairment of activity	severe muscle tenderness with marked impairment of activity, OR frank myonecrosis

SKIN			
	Grade 1	Grade 2	Grade 3
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration, OR exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15mm	15-30 mm	>30mm
Erythema	< 15mm	15-30 mm	>30mm
Edema	< 15mm	15-30 mm	>30mm
Rash at Injection Site	< 15mm	15-30 mm	>30mm
Pruritus	slight itching at injection site	Moderate itching at injection extremity	itching over entire body

SYSTEMIC			
	Grade 1	Grade 2	Grade 3
Allergic Reaction	pruritus without rash	Localized urticaria	Generalized urticaria; angioedema, OR anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy, OR intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	> 39.6 C or > 103 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25-50% > 48 hours	normal activity decreased > 50% can't work, OR unable to care for self

18.2 Key Study Roles

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18.3 Schedule Of Procedures

Table 2a

Group 1 Schedule:

*Visits 2 and 6 are phone calls

Tests and Observations (Days)	D-1	D1 (1)	D4 (4)	D8 (8)	D15 (15)	D29 (29) (1)	D32 (4)	D36 (8)	D43 (15)	D57 (29)	D112	D196	D280	D364
Window	D-56 to -D1		24-72 hrs from visit 1	+/- 1 day	+/- 1 day	+/- 1 day	24-72 hrs from visit 5	+/- 1 day	+/- 1 day	+/- 5 days	+/- 10 days	+/- 10 days	+/- 10 days	+/- 10 days
Visit	Screen 00	01	02	03	04	05	06	07	08	09	10	11	12	13
Clinical Assessments														
Consent, Demographics	X													
Medical History Review	X	X				X								X
Inclusion and Exclusion	X	X												
Medications[a]	X	X		X	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X		X	X	X	X	X	X	X
Laboratory Tests														
Screening Labs (40 mL)[d]	X													
Other Flavivirus Serologies[e] (10 mL)		X												
CBC w/diff and Chemistry Panel (15 mL)[f]		X		X	X	X		X	X	X	X	X	X	X
Pregnancy[g]	X	X				X								
Urinalysis[h]	X	X				X								X
Humoral (40 mL at D0, 30 mL at >D0)		X			X	X			X	X	X	X	X	X
CMI (40 ml)		X			X	X			X	X	X	X	X	X
Study Related Procedures														
Vaccination		**X**				**X**								
Distribute Diary		X				X								
Review Diary[i]				X				X						
Phone AEs [j]			X				X							
Blood Volume (mL)	40	105	0	15	85	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 1 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[c] Vital signs will be performed pre and post vaccination at 1 and 29 day visits

[d] FSH (if indicated), HIV-1 serology, Hepatitis B surface antigen, Hepatitis C antibody, WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, Plt, ALT, ALP, AST, Ca, Bicarb, Cl, Gluc, K, Na, Tot Bili, Tot Pr, Alb, BUN, Cr, GFR. Screening laboratory test(s) may be repeated once at the discretion of the principal investigator or designee to investigate any isolated abnormalities

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Hb, Plt, WBC, ALT, AST, Tot Bili, BUN, Cr

[g] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[h] Dipstick with reflex to microscopic urinalysis if blood, protein, or glucose is abnormal

[i] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination. If participant seen on D7 following vaccination, diary entry for D8 will be reviewed at next visit

[j] Detail AEs including injection site reactions via telephone on these days

Table 2b

Group 2 Schedule:

*Visits 2 and 5 are phone calls

Tests and Observations (Days)	D-1	D1 (1)	D4 (4)	D8 (8)	D15 (15) (1)	D18 (4)	D22 (8)	D29 (29) (15)	D43 (29)	D112	D196	D280	D364
Windows	D-56 to -D1		24-72 hrs from visit 1	+/- 1 day	+/- 1 day	24-72 hrs from visit 4	+/- 1 day	+/- 1 day	+/- 5 days	+/- 10 days	+/- 10 days	+/- 10 days	+/- 10 days
Visit	Screen 00	01	02	03	04	05	06	07	08	09	10	11	12
Clinical Assessments													
Consent, Demographics	X												
Medical History Review	X	X			X								X
Inclusion and Exclusion	X	X											
Medications[a]	X	X		X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X		X	X	X	X	X	X	X
Laboratory Assessments													
Screening Labs (40 mL)[d]	X												
Other Flavivirus Serologies[e] (10 mL)		X											
CBC w/diff and Chemistry Panel (15 mL)[f]		X		X	X		X	X	X	X	X	X	X
Pregnancy[g]	X	X			X								
Urinalysis[h]	X	X			X								X
Humoral (40 mL at D0, 30 mL at >D0)		X			X			X	X	X	X	X	X
CMI (40 ml)		X			X			X	X	X	X	X	X
Study Related Procedures													
Vaccination		**X**			**X**								
Distribute Diary		X			X								
Review Diary[i]				X			X						
Phone AEs [j]			X			X							
Blood Volume (mL)	40	105	0	15	85	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 1 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[c] Vital signs will be performed pre and post vaccination at 1 and 15 day visits

[d] FSH (if indicated), HIV-1 serology, Hepatitis B surface antigen, Hepatitis C antibody, WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, Plt, ALT, ALP, AST, Ca, Bicarb, Cl, Gluc, K, Na, Tot Bili, Tot Pr, Alb, BUN, Cr, GFR. Screening laboratory test(s) may be repeated once at the discretion of the principal investigator or designee to investigate any isolated abnormalities

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Hb, Plt, WBC, ALT, AST, Tot Bili, BUN, Cr

[g] Serum pregnancy test at screening and urine pregnancy test at each subsequent vaccination day

[h] Dipstick with reflex to microscopic urinalysis if blood, protein, or glucose is abnormal

[i] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination. If participant seen on D7 following vaccination, diary entry for D8 will be reviewed at next visit

[j] Detail AEs including injection site reactions via telephone on these days

Table 2c

Group 3 Schedule:

*Visit 2 is a phone call

Tests and Observations (Days)	D-1	D1 (1)	D4 (4)	D8 (8)	D15 (15)	D29 (29)	D112	D196	D280	D364
Windows	D-56 to -D1		24-72 hrs from visit 1	+/- 1 day	+/- 1 day	+/- 5 day	+/- 10 days	+/- 10 days	+/- 10 days	+/- 10 days
Visit	Screen 00	01	02	03	04	05	06	07	08	09
Clinical Assessments										
Consent, Demographics	X									
Medical History Review	X	X								X
Inclusion and Exclusion	X	X								
Medications[a]	X	X		X	X	X	X	X	X	X
Physical Exam[b]	X	X		X	X	X	X	X	X	X
Vital signs[c]	X	X		X	X	X	X	X	X	X
Laboratory Assessments										
Screening Labs (40 mL)[d]	X									
Other Flavivirus Serologies[e] (10 mL)		X								
CBC w/diff and Chemistry Panel (15 mL)[f]		X		X	X	X	X	X	X	X
Pregnancy[g]	X	X								
Urinalysis[h]	X	X								X
Humoral (40 mL at D0, 30 mL at >D0)		X			X	X	X	X	X	X
CMI (40 ml)		X			X	X	X	X	X	X
Study Related Procedures										
Vaccination		**X**								
Distribute Diary		X								
Review Diary[I]				X						
Phone AEs [j]			X							
Blood Volume (mL)	40	105	0	15	85	85	85	85	85	85

[a] Prior and new concomitant medications will be recorded at all study visits (Day 1 through study discharge)

[b] Full physical examination performed at screening and study discharge only; perform targeted exam at other visits.

[c] Vital signs will be performed pre and post vaccination at day 1

[d] FSH (if indicated), HIV-1 serology, Hepatitis B surface antigen, Hepatitis C antibody,WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, Plt, ALT, ALP, AST, Ca, Bicarb, Cl, Gluc, K, Na, Tot Bili, Tot Pr, Alb, BUN, Cr, GFR. Screening laboratory test(s) may be repeated once at the discretion of the principal investigator or designee to investigate any isolated abnormalities

[e] Evaluation for serologic evidence of flavivirus infection/vaccination for post-hoc analysis.

[f] Hb, Plt, WBC, ALT, AST, Tot Bili, BUN, Cr

[g] Serum pregnancy test at screening and urine pregnancy test at vaccination day

[h] Dipstick with reflex to microscopic urinalysis if blood, protein, or glucose is abnormal

[i] Record local and systemic AE's at 7 (solicited) and 28 (unsolicited) days following vaccination. If participant seen on D7 following vaccination, diary entry for D8 will be reviewed at next visit

[j] Detail AEs including injection site reactions via telephone on these days

FOR CCI USE ONLY
Approved by the Beth Israel Deaconess Medical Center Committee on Clinical Investigations:

Administrator: Susan Yuen-Lee

Consent Approval Date: _____

Protocol Number: 2016P000268

Study Approval Expiration Date: _____

INFORMED CONSENT FORM TO TAKE PART IN A RESEARCH STUDY

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR: Kathryn E. Stephenson, M.D., M.P.H.
PROTOCOL NUMBER: 2016P000268

INTRODUCTION:

- This is a research study;
- Your participation is voluntary;
- A research study includes only people who choose to take part;
- You may or may not benefit from participating in the study. However, your participation may help others in the future as a result of knowledge gained from the research;
- You may leave the study at any time;
- If you choose not to take part, or if you leave the study, your decision will in no way harm your relationship with any member of the research team or any other individuals at Beth Israel Deaconess Medical Center.

Please read this consent form carefully and ask the investigators or study staff to explain any words or information that you do not clearly understand. Once you read this consent form and understand what your participation in this study will involve, you will be asked to sign this form if you wish to take part. You will be given a signed copy of the form to keep for your records.

DISCLOSURE OF SPECIAL INTERESTS OF BIDMC AND INVESTIGATORS

This study is being conducted by Dr. Kathryn E. Stephenson. Dr. Kathryn Stephenson, an employee of Beth Israel Deaconess Medical Center (BIDMC), is the Regulatory Sponsor-Investigator of the clinical trial at BIDMC. The study is funded by the Walter Reed Army Institute of Research (WRAIR) and is supported by the U.S. Department of Defense (DoD). WRAIR is also providing the vaccine. BIDMC and Dr. Stephenson (as well as Co-Investigators) have no additional interests in this research project.

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PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

WHOM TO CONTACT IF YOU HAVE QUESTIONS OR PROBLEMS

If you have any questions, concerns or complaints about this research or experience any problems, you should contact Dr. Stephenson or her research staff at [617] 735-4610.

PURPOSE

BIDMC is conducting this study to investigate an experimental vaccine that is being developed for the prevention of Zika Virus infection. Zika virus is an emerging disease spread by mosquito bites. In humans, it has been known to cause symptoms such as rash, fever, eye infections, and joint pain. More seriously, it has been linked to microcephaly in neonates. Microcephaly is when a baby's brain does not develop properly during pregnancy, resulting in a smaller than normal head, as well as intellectual and developmental disabilities. Babies born with microcephaly almost always have poor brain function and a reduced life expectancy. There is currently no specific treatment or cure for Zika Virus infection.

The purpose of this study is to test how safe and well tolerated a Zika Virus Purified Inactivated Vaccine (ZPIV) is in human participants. This will be done by looking at the types of side effects participants have as a result of vaccination. Additionally, the study will look at how your immune system responds to and processes the vaccine. Your immune system is your body's defense system. It protects you from disease.

ZPIV is an inactivated vaccine. Inactivated vaccines consist of vaccine particles that are grown in a laboratory and then killed, making them non-infectious. The virus particles in the vaccine are destroyed and therefore cannot replicate in your body. However, the virus particles can be recognized by your immune system, and generate an immune response. Inactivated vaccines are common and many are currently licensed for use. Examples include vaccines for polio, hepatitis, and rabies.

The vaccine being tested in this study is investigational. The Food and Drug Administration (FDA) has reviewed the research study plan and the information about the vaccine and allowed the study to proceed, however the FDA has not yet approved this vaccine for the prevention of Zika Virus infection. It is impossible to get Zika Virus infection from the vaccine.

WHAT IS KNOWN ABOUT THIS VACCINE

This is one of the first studies in humans of the Zika Purified Inactivated Vaccine (ZPIV). ZPIV has been tested in both mice and monkeys. Animals that received the vaccine were protected from ZPIV infection after experimental exposure to the virus. These studies showed that the vaccines generated antibodies to ZPIV that were important for protection. There were no notable side effects within these animals following vaccination.

SUBJECT'S NAME:
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While there is no human clinical experience with ZPIV, two similar vaccines have been evaluated in clinical trials in humans: a vaccine for Dengue infection (an illness characterized by fevers and muscle pain) and Japanese Encephalitis (a rare illness affecting the brain). Studies with both these vaccines were reasonably well tolerated. Local symptoms experienced by participants included pain/tenderness, redness, swelling, a hard spot, and itching at the injection site. The most common general symptoms experienced by the participants included tiredness, headache, muscle pain, nausea/vomiting, experiencing a cold, and fever. Additionally, some subjects experienced a mild elevation in their liver enzymes. No deaths occurred in either of these trials.

STUDY PARTICIPANTS

You have been asked to be in the study because you are a healthy volunteer between the ages of 18-50, willing to attend all study visits for the next 12 months, willing to use appropriate birth control, and willing to provide written informed consent.

Approximately 36 people will be vaccinated in this study at Beth Israel Deaconess Medical Center. Approximately 108 people will be seen in total to enroll these 36 participants.

DESCRIPTION OF STUDY DETAILS

Study Overview:

Each participant is expected to be in the study for 12 months and attend 9-12 scheduled study visits (including 1-2 phone calls). Each participant will be randomly assigned to one of the 3 groups described in the table below.

				Injection Schedule			
Group	**Subgroup**	**Number**	**Dose**	**Week 0**	**Week 1**	**Week 2**	**Week 4**
1	A	10	5 micrograms	ZPIV			ZPIV
	B	2		Placebo			Placebo
2	A	10	5 micrograms	ZPIV		ZPIV	
	B	2		Placebo		Placebo	
3	A	10	5 micrograms	ZPIV			
	B	2		Placebo			
Total		36					

SUBJECT'S NAME:
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PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

Participants in Group 1 will receive one injection in the arm of ZPIV at weeks 0 and 4. Participants in Group 2 will receive one injection in the arm of ZPIV at weeks 0 and 2. Participants in Group 3 will receive one injection of ZPIV in the arm at week 0 only.

There are 12 participants in each group. Of these 12, ten will receive the active ZPIV product and two will receive placebo. A placebo is a substance containing no active drug or vaccine. In this study, the placebo is an injection with saline (salt water).

The decision to place you in a particular group is random and based on chance (like rolling a die). Out of the 36 enrolled participants, 30 will receive ZPIV, and 6 will receive placebo. Neither you nor the study staff will know whether you are receiving the study product or the placebo. The pharmacist is the only one who will know which product is being administered. Once every participant has completed their final study visit, you will find out if you received the ZPIV product or the placebo.

If you agree to be in this study, you will be asked to read and sign this consent form. After you sign the consent form, the following things will happen:

1. Screening Procedures:
   The screening visit is your first visit in the clinic and will take about 1 ½ to two hours. It may be completed across multiple visits. The purpose of the screening visit is to tell you about the study, have you read the Informed Consent Form, and determine if you are eligible for participation.

   Before any of the screening procedures are done, the study staff will explain the study to you, and answer your questions. You will then read the Informed Consent Form. If you want to join the study, you will sign this form. You will then take a short test to make sure that you understand the key elements of the study.

   If you sign the Informed Consent Form, we will then:
   - Ask questions about your general health and medical history, and prior and current medications you may be taking (including birth control).
   - Perform a complete physical exam, including measuring your vital signs (temperature, heart rate, respiratory rate, and blood pressure) and your height and weight.
   - Counsel you on how to reduce your risk of getting Zika Virus infection and HIV and how to prevent pregnancy for the duration of this study.
   - Draw blood and collect urine to:
     - Test for anemia (low blood counts) and check kidney and liver function,
     - Screen for HIV, hepatitis B, hepatitis C infection,

SUBJECT'S NAME:
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PROTOCOL #: 2016P000268

- Review whether you are eligible to take part in this study. If anything abnormal is detected on our screening tests, you will be referred to appropriate medical care.

2. Vaccination Visit Procedures:

After it is confirmed that you are eligible for this study, you will be scheduled for your first vaccination visit. You will have either 1 or 2 vaccination visits over the course of the study depending on which group you are assigned to. Vaccination visits will take approximately 3-4 hours to complete. At your first vaccination visit, we will assign you to either Group 1, 2, or 3.

At all vaccination visits we will check for any changes in your health, changes to your medications or recent vaccinations, and how you are feeling that day. We will perform a brief physical exam. We will draw blood samples to test for anemia (low blood counts) and check your kidney and liver function, as well as to look at your immune responses to the vaccines and other research questions. We will also take a urine sample to test for your general health. We will perform a urine pregnancy test if you were born female. We will counsel you on how to reduce your risk of getting Zika virus infection and on preventing pregnancy. We will then confirm that you are still eligible to continue receiving the study vaccine (or placebo) and order the vaccine (or placebo) from the research pharmacy.

When the study product arrives in clinic, we will inject the product into your upper arm (deltoid muscle). We will then monitor you for 30 minutes to check for any possible reactions.

Subject Diary:

At the end of each vaccination visit, we will give you a diary to fill out daily at home, starting from the day of each study injection and for 6 days after. We will ask you to record:

- Your body temperature.
- Any redness of skin, pain/tenderness, itching, or swelling or hard spot at the injection site.
- Feverishness (chills, shivering, sweating), fatigue (tiredness), general unwell feeling, muscle pain, joint pain, headache, nausea, vomiting, diarrhea, abdominal pain, and rash (not at the injection site)
- Any medications (i.e. Tylenol, Advil, etc.) that you took to relieve your symptoms.

You will be provided with a thermometer and a ruler. The study staff will teach you how to correctly fill out your diary and how to use the thermometer and ruler to obtain any measurements.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

3. Monitoring/Follow-Up Procedures.
   All participants in the study will have to return to the clinic for follow-up visits throughout the study. These visits will take approximately 30 minutes to complete. Additionally, you will be asked to participate in 1-2 remote safety visits. During these visits a study staff member will call you to ask how you are feeling. These visits will take approximately 10-15 minutes.

   During the monitoring/follow-up visits we will check for changes in your health, medications, recent vaccinations, and how you are feeling on that day. During certain visits we will also review the diary with you and talk to you in detail about any side effects. We will perform a brief physical exam. We may also collect blood and urine samples to check on your general health, for reactions to the vaccines, and to perform research studies.

   For all participants the monitoring/ follow up procedures schedule is as follows:
   - A phone call 1-3 days after each vaccination
   - A clinic visit one week after each vaccination
   - A clinic visit two weeks after each vaccination
   - A clinic visit four weeks after each vaccination
   - Additional clinic visits 112, 196, 280, and 364 days after the first vaccination
   - Groups 1 and 2 will have one visit that is a combination of vaccine visit and follow up visit.

4. Unscheduled Visits or Telephone/Email Contact:
   We may ask you to return to the clinic for unscheduled visits and/or lab tests. We will ask you to do this if your test results are not normal or as otherwise needed.

   While you are in this study, the study doctors or study staff members may contact you between visits to remind you about upcoming appointments, to see how you are feeling, to tell you any information that may affect your health, and for other reasons as appropriate. We may contact you by telephone or email, which may take about 5-10 minutes of your time.

5. Stopping the Study Early:
   If you decide to leave this study, please tell the study staff. We will ask you to come back to the clinic at least once. This visit will take about 30 minutes. At this visit, we will perform the same procedures as a follow-up visit.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

Total Blood Volume:
At each visit we will collect a blood sample. Between 1 tablespoon (15ml) and 10 tablespoons (135ml) will be taken, depending on which blood tests are scheduled for the visit. In total, this means approximately 44-57 tablespoons (660-845 ml) of blood over the entire course of this research study will be drawn. Your body will make new blood to replace the blood we draw. Additionally, the doctor can always decide to draw a smaller volume if it is in your best interest.

Sample Use:
The blood drawn from you as part of this study will be used to look for side effects of the vaccine, and to study the immune responses generated by the vaccine. For example, we will test your blood to see if the vaccine generates antibodies and white blood cells against Zika virus, and to measure how long those immune responses last. As part of this research, antibodies may be tested to see if they can neutralize (or inactivate) Zika virus in mice or other animals. Your cells may also be mixed with other human cells, mixed with animal cells, or grown in lab animals like mice. These tests help us understand if the vaccine might work to protect against Zika. In addition, some of the blood drawn from you as part of this study will be used for a genetic test of HLA (human leukocyte antigen) type. HLA is made up of proteins that play an important role in how the immune system responds to foreign organisms. For research, HLA testing is used to try to identify factors associated with response to a vaccine, progression of a disease or related conditions. Your cells may also be used for other genetic tests, such as whole genome or whole exome analysis, or the creation of a cell line. These tests may be done to identify genetic factors that predict how someone responds to vaccination, or to help produce antibodies for further studies.

YOUR RESPONSIBILITIES AS A RESEARCH PARTICIPANT

If you join the study, it is important that you:

- Come to all study visits and follow the study instructions.
- Be informed about the study and ask questions if you don't understand something.
- Give staff complete and accurate study-related information including your medical history and any medications you are taking.
- Update the staff about any symptoms or illnesses you are experiencing.
- Inform the staff of any problems or discrimination you experience because of your participation in the study.
- Use appropriate contraception methods as outlined in this document and explained to you by the study doctor.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

- Talk to us if you feel the study is taking too much of your time. We will try to make changes to help you. Talk to us if you don't like how you are being treated. We will try to fix the problem.
- Stay in touch with us. Tell us if your address, phone number, or email address changes; if you are moving away; or if you want to leave the study.

You should **NOT**:

- Donate blood.
- Donate body parts, body fluids, or body tissues without talking to the study staff first.
- Travel to areas where Zika Virus transmission is currently ongoing (as defined by the Centers for Disease Control Zika Travel Notices, or at the discretion of the investigator)
- Receive any experimental medication (including experimental vaccines other than the study vaccine).
- Receive any live-attenuated vaccines within 60 days of receiving ZPIV.
    - Note: You should not receive inactive influenza or pneumococcal vaccines, allergy treatments with injections, or tuberculin skin tests within 14 days of receiving ZPIV.
- Join another research study without talking to the study staff first.
    - Note: while you are taking part in this study, you cannot take part in another research study where you will receive a study product such as an experimental medication.
- Leave the study without telling the study staff first.

RISKS AND DISCOMFORTS

As a result of your participation in this study, you are at risk for side effects listed in this section. You should discuss these with the investigator and with your regular doctor if you choose.

General Risks Associated with Vaccination:

The most common risks associated with vaccination include stinging, arm discomfort, pain or soreness, redness, itching, bruising and/or swelling/hardness at the site of injection. These side effects commonly occur with any injection, but they do not usually last long (24-48 hours)
It is less common, but also possible that you will experience a fever, chills, rash, general unwell feeling (malaise), muscle aches, joint aches and pains, nausea, lack of appetite, headache, and/or fatigue (feeling tired). The side effects may occur in the first few days after vaccination. Rarely, people may experience more severe side effects that limit their normal activities or make them go to the doctor.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

It is rare, but you could have an allergic reaction to a vaccine. Signs of allergic reaction include rash, hives, itchiness, difficulty breathing, and/or swelling of lips, tongue or face.

Allergic reactions can be life-threatening; therefore, the study staff will observe you for at least 30 minutes after each injection. You should tell your study doctor if you have ever had a bad reaction to any injection or vaccine. Medications are available in the clinic to treat serious allergic reactions. If you think you are having a severe allergic reaction after you leave the study site, seek medical attention immediately and contact the emergency study number. This emergency number is listed on the participant identification card you will be given at your first vaccination visit, and will connect you or your treating physician to a study investigator 24 hours/day.

You may take medications to help with pain control and inflammation after the injection (i.e. Tylenol, Advil, etc.), but please report their use to the study staff.

RISKS AND POSSIBLE SIDE EFFECTS FROM ZPIV:

ZPIV Risks:
While there is no human clinical experience with ZPIV, two other similar inactivated vaccines against viruses similar to Zika virus have been evaluated in clinical trials in humans: a dengue vaccine and a Japanese encephalitis vaccine (manufactured as IXIARO®). Studies with both vaccines indicate that this type of vaccine (formalin inactivated) is reasonably well tolerated. For example, in a study of two doses of an inactivated dengue vaccine, few side effects were detected among the 20 adult subjects. Side effects at the site of injection consisted of pain and tenderness with one report of swelling. The most common reported systemic side effects included fatigue, headache, muscle aches, nausea, vomiting, and fever with the last three side effects occurring after the 2nd administration of the higher dose. Other more rare side effects included a mild to moderate elevation in liver enzymes, and mild chills and joint pain. In addition, the inactivated Japanese encephalitis vaccine (IXIARO®) has been licensed for use in the US since 2009. Following vaccination, the most common side effects reported are headache, muscle aches, influenza-like illness, and fatigue.

Natural Zika virus infection has been associated with rare brain and nervous system complications in adults, including the autoimmune disease Guillain-Barre syndrome. Guillain-Barre is a disease in which your body's immune system attacks the nervous system, often leading to paralysis. It is not yet clear whether Guillain-Barre syndrome and other neurologic complications are a result of autoimmune disease (a distorted reaction of the immune system) or because the virus is infecting the nervous system directly. The potential for a Zika vaccine to induce Guillain-Barre syndrome is not known.

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PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

In summary, other inactivated flavivirus vaccines appear to be reasonably well tolerated. Typical side effects include arm pain, tenderness, redness, swelling, and itching, and mild to moderate systemic side effects including headache, muscle ache, fatigue, influenza-like illness, nausea and fever. The potential for a Zika vaccine to induce Guillain-Barre syndrome or other neuroinflammatory diseases is not known.

Aluminum Phosphate Risks:
Aluminum phosphate is an "adjuvant" which will be mixed with ZPIV in the same injection. A vaccine adjuvant is a substance that is added to the vaccine to increase the body's immune response to the vaccine. Aluminum is used as an adjuvant in many commercial vaccines.

Aluminum is one of the most common metals found in nature. Aluminum phosphate has been used safely in vaccines for more than 70 years. Aluminum-containing vaccines have been associated with severe local reaction such as redness, lumps under the skin, contact allergy or irritation, and swelling at the site of injection. There have also been reports, especially in patients with impaired renal function, of systemic accumulation of aluminum, which has been associated with nervous disorders and bone disease. Nonetheless, aluminum intake from vaccines is far less than that received from the diet or certain medications.

Unknown Vaccine Risks:
There may be risks with the use of ZPIV that are not yet known. It is important that you tell the study staff immediately about any alarming or unexpected signs or symptoms.

Risk of Genetic Tests:
If you consent, some of the blood drawn as part of this study will be used for a test called the human leukocyte antigen test (also called HLA typing) or for other genetic tests on DNA. Genetic tests like HLA typing look at your genes (units of DNA that are responsible for human traits and characteristics). In medicine, HLA typing is used to identify a matching donor for organ transplants. For research, HLA typing is used to try to identify factors associated with response to therapies or vaccines, or to the progression of a disease or related conditions. Your participation in HLA typing/genetic testing is voluntary. Your decision to agree or disagree to HLA typing/genetic testing will not impact your ability to be in the study.

The greatest risk associated with genetic testing is to your privacy. Test results can be used to provide information about how susceptible you are to certain diseases. Used inappropriately, this information could be discriminatory (for example, by insurance companies). HLA typing can also be used to determine who the true parent of a child is. The blood samples that you donate will not be used for these purposes; they will be used only to provide study investigators information about your

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

immune system. You should also know that a Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.
- All health insurance companies, group health plans, and employers with 15 or more employees must follow this law as of November 21, 2009. All health insurance companies and group health plans follow this law as of May 21, 2010.

GINA's health insurance protections do not apply to members of the military who receive their healthcare through Tricare and for veterans who receive their healthcare through the Veterans' Administration. While GINA's employment protections do not apply to military members and Federal employees presently, an Executive Order protects federal employees from genetic discrimination in employment and the military has its own policies in place that may protect against genetic discrimination. GINA's protections should apply for a military member once he or she leaves the service and enters the private sector.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

The risk of your genetic information being used inappropriately is extremely low. This is because any results from these tests will not be part of your medical record and will not be given to you or your doctor. Your samples and genetic information will be coded and not connected to your name or any other personal information.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

Please check the appropriate box:

☐ Yes, I agree to have HLA typing/genetic testing on my blood samples.

☐ No, I do not agree to have HLA typing/genetic testing on my blood samples.

Signature of Subject or
Legally Authorized Representative

Date

Relationship of Legally Authorized Representative to Subject

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

Risks Associated with Pregnancy:
If you are pregnant or breastfeeding or planning to become pregnant you will not be allowed to take part in the study. In addition, it is important that you do not get pregnant during the study. You will be provided with condoms by the study staff.

The effect of the study vaccine on an unborn child or a breastfed infant has not been studied and there is currently no information on the possible effects of the study vaccines in these cases. The effect of the study vaccine on sperm is also unknown. If you are pregnant or planning on becoming pregnant, these study products may involve risks to the embryo or fetus that are currently unforeseeable. In all cases, the type of birth control methods that you and your partner will use during the study must be discussed with the study doctor and he/she must approve the method(s) before you can enter the study.

For participants who were born female:
If you were born female and are sexually active in a way that could lead you to get pregnant, you must agree to use effective birth control from the day of first vaccination until three months after your final vaccination. Effective birth control means using any of the following methods:

- Condoms (male or female) with or without spermicide.
- Diaphragm or cervical cap with spermicide.
- Intrauterine device.
- Hormonal contraception (i.e. birth control pill).
- Successful vasectomy in the male partner
- Not be of reproductive potential (i.e. uterus or ovaries removed, having tubes tied, not having sex with men, not having sex with anyone).

For participants who were born male:
If you were born male and are sexually active in a way that could lead to getting your partner pregnant, you must agree to use an effective method of contraception (such as consistent condom use) from the day of first vaccination until 3 months following final vaccination.

LOSS OF CONFIDENTIALITY
There is the potential for loss of confidentiality by participating in this study. Every effort will be made to protect the confidentiality of your identifiable information. However, if your participation becomes known, it could create a problem or hardship for you depending upon the type of information disclosed. There may also be damage to your future financial standing, health care, employment, professional standing or ability to get access to health or other insurance.

Confidentiality
Information learned from your participation in this study may be reviewed and photocopied by the

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

Food and Drug Administration (FDA) and/or other federal and state regulatory agencies, and by the study Sponsor-Investigator, the Department of Defense research monitor, accreditation agencies, the Committee on Clinical Investigations, the Human Subjects Protection Office and others involved in research administration of Beth Israel Deaconess Medical Center with protection of confidentiality so far as permitted by applicable law. Information resulting from this study may be used for research purposes and may be published; however, you will not be identified by name in such publications.
Beth Israel Deaconess Medical Center Committee on Clinical Investigations and other persons charged with monitoring the way in which the research is conducted may have access to data bearing your identifying information, but persons having such access will provide an assurance of confidentiality to Beth Israel Deaconess Medical Center. Such persons include an independent research monitor appointed by the U.S. Department of Defense, who is providing the funding for this study.

Certificate of Confidentiality
A Certificate of Confidentiality has been obtained from the Department of Health and Human Services. This will help further protect information that may identify you. The Certificate prevents the investigator from being forced to disclose information that may identify you for use in court.

A Certificate of Confidentiality does not prevent you or anyone you tell from voluntarily releasing information about yourself or your involvement in this research. The investigator may not withhold information if you give your health insurance company or employer permission to receive information about your participation in this research. This means that you and your family must also actively protect your own privacy.

Finally, the investigator can take steps, including reporting to authorities as required by law, to prevent serious harm to yourself or others.

There is a possibility that information that identifies you will be given to the Beth Israel Deaconess Medical Center oversight officials or to officials of the Department of Health and Human Services (i.e. mandatory disease reporting). This Information may be used for audits or evaluations, or to ensure that research work is being done correctly. However, these officials are also obliged to protect your privacy.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

Mandatory Reporting:
If you decide to participate in this study and agree to be screened for HIV, hepatitis B, and hepatitis C, the results of this testing will be entered into your study record and your BIDMC chart. Your physician is required by law to report positive HIV, hepatitis B, and hepatitis C test results to health officials, including the Massachusetts Department of Public Health (MDPH) HIV/AIDS Surveillance Program. Reports to the MDPH will include your full name, address, medical record number, social security number, date of birth and gender. These reports may also include information about your medical history, health condition, and treatment.

Your written approval is not required for this mandatory reporting, assuming you agree to participate in this study and receive these screening tests as a result of that participation. In general, releases of your HIV and hepatitis B/C test results outside BIDMC (with the exception of required reporting to public health officials) require your written informed consent. By signing this form, you agree that those individuals who need to see your medical information, including the results of your HIV and hepatitis B/C test results, in order to conduct the study may receive the results of your test.

By signing here you acknowledge that all of your questions have been answered and you consent to the release of your HIV test results by BIDMC as described above.

Subject Name (Sign) _____ Date _____

Subject Name (Print) _____

If you get infected with HIV, hepatitis B or hepatitis C during the study, the study staff will help you to get care and support. You will not be able to stay in the study. We will counsel you about your infection and about telling your partner(s), if appropriate. We will tell you where you can get support and medical care.

POSSIBLE BENEFITS
There is no direct benefit to you from being in this study. No one knows if the vaccine against Zika Virus infection will work. Also, you may get the inactive placebo. However, you and others may benefit in the future from the information that will be learned from the study. The results of this study could play a role in future vaccine development.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

OTHER AVAILABLE OPTIONS

Taking part in this study is voluntary. Instead of being in this study, you have the alternative option to not enroll in the study. Remember, this research study is not meant to diagnose or treat medical problems. Participation in this research study does not take the place of routine physical examinations or visits to your regular doctor.

If you choose to participate, you have the right to leave the study at any time. Your decision to not participate will not result in any penalties or loss of benefits to you. The investigators will tell you about new information that may affect your willingness to stay in this study. If you decide not to participate in the study or decide to leave the study early, your decision will also not affect your relationship with the research team or any other individual at Beth Israel Deaconess Medical Center.

If you decide to leave the study early we will ask you to come back to the clinic one last time for a physical exam, and we may ask to take some blood and urine samples. We will also ask about any personal problems or benefits you have experienced from being in the study. We believe these steps are important to protecting your health, but it is up to you whether to complete them.

INVESTIGATORS RIGHT TO STOP THE STUDY

The investigators have the right to end your participation in this study if they determine that you are no longer eligible to take part, or if it would be dangerous for you to continue, or if you do not follow study procedures as directed by the investigators. For example, you will be withdrawn from the study if you enroll in a different research study where you receive another study product, you become pregnant, or you need treatment for a medical problem. The study Sponsor-Investigator, Beth Israel Deaconess Medical Center, DoD Research Monitor, or the funding sources may stop the study at any time.

We may stop your injections or take you out of the study even if you want to continue and even if you were scheduled for additional injections. If we stop your injections, we may ask you to stay in the study to complete other study procedures. If you are unable to continue participation in the study, but you do not withdraw consent, an exit visit will be conducted. If you withdraw consent, no further data will be collected after consent has been withdrawn.

COSTS AND/OR PAYMENTS TO YOU

Payments to You

BIDMC will pay $50 per visit to reimburse you for your time, and for your travel/parking expenses to and from office visits and tests. Each participant will attend 9-12 clinic visits and be compensated a maximum of $600 (paid at $50 per visit). The breakdown of compensation is as follows:

- If you are assigned to Group 1 you will have 12 clinic visits and be compensated a total of

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

$600

- If you are assigned to Groups 2, you will have 11 clinic visits and be compensated a total of $550.
- If you are assigned to Group 3 you will have 9 clinic visits and be compensated a total of $450.

Any payments made to you may be taxable income to you. We are required to obtain your name and social security number for preparation and submission of Internal Revenue Service (IRS) Form 1099-Misc. You may receive a Form 1099 from BIDMC if you receive $600 or more in one calendar year for taking part in one or more research studies at BIDMC. Questions about your own tax status should be referred to your personal tax advisor.

Costs of Research-Related Injury

If you are injured as a direct result of your participation in this study, you should contact the Investigator at the number provided under the section "Who to Call if You Have Questions" in this form. You will be offered the necessary care to treat your injury. Information may be collected by the Sponsor-Investigator, directly from you or other health care providers who treated your problem or injury. We reserve the right to bill your insurance company, if appropriate, for the care you get for the injury. We will try to get these costs paid for, but you may be responsible for some of them. You may be responsible for all co-payments and deductibles required under your insurance. At this time there is no plan to reimburse you for items such as lost wages or lost time from work. By signing this consent form you have not given up any legal rights.

LONG-TERM STORAGE OF BIOLOGICAL SPECIMENS

We would like to store your samples and potentially use them for future research – which might include DNA research. These samples will be managed by BIDMC. Samples will be de-identified so that they are not linked to you. Samples will be kept indefinitely for future research involving vaccine development, immune responses, and other diseases. You will not be compensated for this use of your samples, and future use provides no benefit to you. The results from these future studies will never be discussed with you, your family, or your doctor, nor will the results ever become a part of your medical record.

If you do not agree to the long-term storage of your samples or their use in future research, this will have no influence on your participation in the study. In that case, your samples will be destroyed after they are no longer needed for the study.

If you allow your samples to be stored and used for future research you can withdraw your consent for your samples to be used for future research at any time. In this case your samples will be destroyed after they are no longer needed for the main study. You would need to tell your study

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

doctor that you are withdrawing your consent for your samples to be used for future research. This can be done at any time, for any reason. This will not affect your access to the care, medicine, and equipment you would otherwise be getting. Your study doctor will then tell the Sponsor-Investigator to destroy all your samples when they are no longer needed for the main study.

CONSENT FOR LONG TERM STORAGE OF BIOLOGICAL SPECIMENS*:*
I have read the above information about the storage of my biological specimens for potential future research use. I understand that this is completely voluntary, and that I may withdraw my consent at any time. I understand that my decision does not influence my participation in the study.

Please check the appropriate box:

☐ Yes, I allow for my samples to be stored and used for future research.

☐ No, I do not allow for my samples to be stored and used for future research

_____ _____

Signature of Subject or
Legally Authorized Representative

Date

_____

Relationship of Legally Authorized Representative to Subject

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

OTHER IMPORTANT INFORMATION

Clinicaltrials.gov

A description of this clinical trial will be available on www.ClinicalTrials.gov as required by U.S. law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Contacting Your Other Doctors

If the study doctor needs additional information about your medical history, or needs to share information that could affect your health, they may want to contact your other doctors and inform them of your participation in the study. We would like to mail/fax your doctor(s) a letter stating you are participating in our study, and that they may contact us if they have questions.

I have been informed that my study doctor will inform my other doctors, if any, about my participation in this study, and I agree to this. (*You may still be in this study even if you do not agree*).

Yes	No	NA, I have no other doctors
☐	☐	☐

Signature of Subject or
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Date

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SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

USE OF YOUR TISSUE FOR COMMERCIAL DEVELOPMENT

As part of this research program, samples of your tissue and information about your medical history may be provided to other researchers and/or outside collaborators without identifying you by name. They may use your tissue and information in other scientific research, product testing or commercial development. It is unknown whether a product will ultimately be developed from any such work that may be performed. In signing this consent form, you are acknowledging and voluntarily consenting to the possibility that your tissue may be used for commercial purposes. You also understand and agree that tissue obtained from you in this research may be used to establish a cell line that could be patented and licensed. Beth Israel Medical Deaconess Medical Center has no program to compensate you in the event product testing or commercial development takes place.

AUTHORIZATION FOR USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION

As part of this study, we will be collecting, using and sharing with others information about you. Please review this section carefully as it contains information about the federal privacy rules and the use and disclosure of your information.

The information about you collected for the study will be stored on paper and computer records, without identifying you by name. The information collected by the study doctor or study staff as part of the study may be sent to other members of WRAIR or NIH collaborators, to contractors and consultants working for these companies and to regulatory authorities (Food and Drug Administration).

Some of this information, called Protected Health Information (“PHI”), is protected by federal privacy laws. By signing this consent form, you give your permission to have your PHI collected, used and disclosed for purposes of this study. After the Study staff or the Study doctor discloses your PHI to others, it could be re-disclosed and no longer protected by federal privacy laws.
You may decide not to give permission for the use or disclosure of your protected health information for the study. In that case, you will not be able to participate in the study. This is because the study staff and/or the study doctor would not be able to collect the information needed to evaluate the study drug.

By signing this informed consent document, you are allowing the investigators and other authorized personnel to use [internally at BIDMC] and disclose [to people and organizations outside the BIDMC workforce identified in this consent] health information about you. This may include information about you that already exists (for example: your medical records and other sources of health information, demographic information, the results of any laboratory tests, and mental health records

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

if applicable), as well as any new information generated as part of this study. This is your Protected Health Information.

People/Groups at BIDMC Who Will Share and Use Your Protected Health Information
Your Protected Health Information may be shared with and used by investigators working on this study, including the supporting research team (such as research assistants and coordinators, statisticians, data managers, laboratory personnel, pharmacy personnel, and administrative assistants), and may also be shared and used by other health care providers at BIDMC who have treated you in the past and have information relevant to the research, or who provide services to you in connection with the research. Your Protected Health Information may also be shared with the members and staff of the Committee on Clinical Investigations of Beth Israel Deaconess Medical Center, which is responsible for reviewing studies for the protection of the research subjects.

People/Groups Outside of BIDMC With Whom Your Protected Health Information Will Be Shared
We will take care to maintain confidentiality and privacy about you and your Protected Health Information. We may share your Protected Health Information with the following groups so that they may carry out their duties related to this study:

- BIDMC and other funding sources of this study that to oversee, administer, or conduct the research. For example, clinical research organizations are companies that are sometimes hired by research sponsors to help manage and run a clinical research study.
- Any external health care providers who provide services to you in connection with this research.
- Laboratories not affiliated with BIDMC that are involved in conducting tests related to the research.
- Statisticians and other data monitors not affiliated with BIDMC.
- The members and staff of any other IRBs (beyond the BIDMC Committee on Clinical Investigations) that oversee the research.
- Centralized data collectors.
- Your health insurance company.
- The Food and Drug Administration [FDA], the Department of Health and Human Services [DHHS], the National Institute of Health [NIH], the Office for Human Research Protections [OHRP], and other federal and state agencies that may have jurisdiction over the research.
- Hospital and Clinical Research Accrediting Agencies.
- Data and Safety Monitoring boards that oversee this study.
- Department of Defense [DoD] research monitor and representatives who need access to research records as a part of regulatory oversight.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

Those who receive your Protected Health Information during the course of the research may not be required by the federal privacy regulations to protect it, and they may make further disclosures to others and use your information without being subject to penalties under those laws.

Why We Are Using and Sharing Your Protected Health Information
The main reason for using and sharing your Protected Health Information is to conduct and oversee the research as described in this Informed Consent Document. There are many other reasons beyond the research for which BIDMC may use or disclose your Protected Health Information. Not all of these reasons require your express written authorization. For example, we will use and share your Protected Health Information to ensure that the research meets legal, institutional and accreditation requirements and to conduct public health activities. The various ways in which BIDMC may use and disclose your protected health information without your authorization are explained in a document called the Notice of Privacy Practices. If you have not received a copy of BIDMC's Notice of Privacy Practices, please ask us for one and review it before signing this form. In addition to signing this document, you may also be asked to sign a BIDMC General Agreement form acknowledging that you have received the BIDMC Notice of Privacy Practices.

No Expiration Date- Right to Withdraw Authorization
Your authorization for the use and disclosure of your Protected Health Information in this Study shall never expire. However, you may withdraw your authorization for the use and disclosure of your Protected Health Information at any time provided you notify the Principal Investigator in writing. If you would like to take back your authorization so that your Protected Health Information can no longer be used in this study, please send a letter notifying the Principal Investigator of your withdrawal of your authorization to Dr. Kathryn Stephenson at 330 Brookline Ave., E/CLS 1036, Boston, MA 02215. Please be aware that the investigators in this study will not be required to destroy or retrieve any of your Protected Health Information that has already been used or disclosed before the Principal Investigator receives your letter, and they are permitted to continue to use and disclose your previously collected information as necessary to complete the research.

Refusal to Sign
Your clinical treatment may not be conditioned upon whether you sign the Authorization for Research. However, if you choose not to sign this informed consent document and authorization for the use and disclosure of your Protected Health Information, you will not be allowed to take part in the research study.

Right to Access and Copy your PHI
If you wish to review or copy your Protected Health Information as it is made part of your medical record, you may do so after the completion or termination of the study by sending a letter to the Principal Investigator requesting a copy of your Protected Health Information. You may not be

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

allowed to inspect or copy your Protected Health Information until this study is completed or terminated.

ADDITIONAL CONTACT FOR QUESTIONS OR CONCERNS
If you have any questions about the study, please contact:

Jessica Ansel, Clinical Research Nurse Coordinator
Telephone: (617) 735-4610

If you feel that this study has caused you harm, please contact:

Kathryn E. Stephenson, Principal Investigator and Assistant Professor of Medicine
Telephone: (617) 735-4556

Please contact the Human Subjects Protection Office at (617) 667-0469 in the event that you would like to obtain information or to offer input about the research study. This office is independent of the investigator or investigator's research staff and can also assist with questions relating to your rights as a participant in research, which may include questions, concerns or complaints about your participation in the study.

ICF REVISION DATES:

CCI Submission	*9/26/2016*
CCI Amendment	*10/26/2016*
CCI Amendment	*12/05/2016*
CCI Amendment	*2/05/2017*
CCI Amendment	*2/21/2017*

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268

CONSENT FORM FOR CLINICAL RESEARCH

I have read the previous page[s] of the consent form and the investigator has explained the details of the study. I understand that I am free to ask additional questions.

If I wish additional information regarding this research and my rights as a research subject, or if I believe I have been harmed by this study, I may contact the Human Subjects Protection Office (HSPO) at (617) 667-0469.

I am aware that this is a research project and that unforeseen side effects may occur.

I understand that the Beth Israel Deaconess Medical Center has no formal program for compensating patients for medical injuries arising from this research. Medical treatment will be provided for injuries at the usual charge to me or to my insurer unless payment is otherwise provided for in this consent form.

I understand that participation in this study is voluntary and I may refuse to participate or may discontinue participation at any time without penalty, loss of benefits, or prejudice to the quality of care which I will receive.

I acknowledge that no guarantees have been made to me regarding the results of the treatment involved in this study, and I consent to participate in the study and have been given a copy of this form.

_____ _____

Signature of Subject or Legally Authorized Representative — Date

_____

Relationship of Legally Authorized Representative to Subject

The subject has been given the opportunity to read this consent form and to ask questions before signing, and has been given a copy.

_____ _____

SIGNATURE OF INVESTIGATOR/Co-Investigator — Date

_____

PRINT NAME OF INVESTIGATOR/Co-Investigator

A signing co-investigator must be listed on the study's approved Research Staffing Form at the time of consent.

SUBJECT'S NAME:
TITLE OF RESEARCH PROTOCOL: A Phase 1, Randomized, Double-Blind Placebo-Controlled Clinical Trial to Evaluate the Safety and Immunogenicity of an Accelerated Vaccination Schedule with a Zika Virus Purified Inactivated Vaccine plus Alum Adjuvant in Healthy Adults
PRINCIPAL INVESTIGATOR'S NAME: KATHRYN E. STEPHENSON, M.D., M.P.H.
PROTOCOL #: 2016P000268