Complete Clinical Protocol

A Double Blind, Randomized, Placebo-Controlled, Phase 1 Dose Escalation Trial to Evaluate the Safety and Immunogenicity of an Inactivated Yellow Fever Virus Vaccine, HydroVax-002 YFV, in Healthy Adults

NCT05172544

24 January, 2023

A Double Blind, Randomized, Placebo-Controlled, Phase 1 Dose Escalation Trial to Evaluate the Safety and Immunogenicity of an Inactivated Yellow Fever Virus Vaccine, HydroVax-002 YFV, in Healthy Adults

Complete Clinical Protocol

Protocol Number: 20-001
IND Sponsor: Najít Technologies, Inc. (NTI)
Version Number: 6.0

STATEMENT OF COMPLIANCE

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research. The IRB/IEC must be registered with OHRP as applicable to the research.

The study will be carried out in accordance with the following as applicable:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), and/or 21 CFR 812 (Investigational Device Exemptions)
- The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6(R2) Good Clinical Practice, and the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- The policies and procedures of National Institutes of Health (NIH) Office of Extramural Research and DMID
- The National Institute of Allergy and Infectious Diseases (NIAID) Terms of Award
- Any additional Federal, State, and Local Regulations and Guidance

The signature below provides the necessary assurance that this study will be conducted according to all stipulations of the protocol including statements regarding confidentiality, and according to local legal and regulatory requirements, US federal regulations, and ICH E6(R2) Good Clinical Practice (GCP) guidelines.

Site Investigator Signature:

Signed:_____ Date:_____

[redacted]

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. PROTOCOL SUMMARY

1.1 Synopsis

1.1.1 Study overview

This study will evaluate the safety and immunogenicity of two different dosages of a novel peroxide-inactivated whole-virus yellow fever vaccine, HydroVax-002 YFV, in healthy adults. The currently available yellow fever vaccine is the live, attenuated YFV-17D vaccine. This live attenuated vaccine is contraindicated in multiple vulnerable populations including infants <9 months of age and immunosuppressed individuals such as those with acquired immunodeficiency syndrome, thymic disease, or persons with hypersensitivity to eggs. Special precautions are also noted for pregnant women, nursing mothers and adults over the age of 60 [1]. These contraindications and precautions stem from known serious adverse events associated with the vaccine including a serious nervous system/neurotropic condition termed yellow fever associated neurotropic disease (YEL-AND). In addition to YEL-AND, vaccination with live YFV-17D can cause severe multiple organ system failure, described as yellow fever vaccine-associated viscerotropic disease (YEL-AVD), a syndrome closely resembling wild-type yellow fever virus (YFV). Because HydroVax-002 YFV is based on inactivated virus that is unable to replicate or spread to the brain or nervous system, we anticipate that these vaccine complications will be eliminated.

1.1.2 Enrollment Period

It is anticipated that enrollment may be completed in 3.5-4 months.

1.1.3 General

This trial will be a randomized, placebo controlled, double-blind (within dosing group), dose escalation Phase 1 trial evaluating dosages of 1 mcg and 5 mcg of HydroVax-002 YFV vaccine given intramuscularly on Day 1 (the day of first vaccination is defined as Day 1) and Day 29 in healthy adults ≥18 and <50 years of age. The study will consist of two dosing groups of HydroVax-002 YFV vaccine to be enrolled sequentially. Each dose group will consist of 10 individuals who receive HydroVax-002 YFV and 2 or 3 who receive placebo. The initial dose of HydroVax-002 YFV to be evaluated in Group 1 will be 1 mcg and the next dose to be evaluated in Group 2 will be 5 mcg. Following assessment of safety and reactogenicity data of Group 1 by the Safety Monitoring Committee (SMC), the vaccine dose will be increased to 5 mcg for Group 2. All participants will be monitored for local and systemic solicited adverse events for 7 days following each vaccination, unsolicited adverse events for 28 days following each vaccination, and for the occurrence of new onset chronic medical conditions and serious adverse events from the time of first vaccination until 6 months following the second vaccination. Safety laboratory tests will be performed prior to vaccination and at days 4 and 15 following vaccination. YFV neutralizing assays will be performed on blood samples collected prior to each vaccine, 15 days following each vaccine, and at days 29, 57, and 180 following the second dose of vaccine.

For each dosing group, the first four subjects enrolled (the sentinel subgroup) will include three HydroVax-002 YFV recipients and one placebo recipient. After the four subjects in the Group 1 sentinel subgroup are enrolled and given their first vaccination, enrollment will then be stopped pending a review of the clinical laboratory, reactogenicity, and safety data collected through the post vaccination Day 8 visit for the last of those subjects.

This review may be conducted by an internal safety review committee (ISRC), consisting of the Blinded medical monitor, clinical program manager, and the PI, or by the SMC, as indicated and detailed in Section 10.1.6. Approval by the reviewing group will allow administration of the second vaccination to the sentinel subgroup and continued enrollment of the remaining 9 Group 1 subjects (expanded group) to resume to complete enrollment of 13 participants (10 vaccine and 3 placebo).

After Group 1 enrollment is completed enrollment will be stopped pending an SMC review of the clinical laboratory, reactogenicity, and adverse event information through the post 2nd vaccination Day 15 visit for all Group 1 subjects. Approval by the SMC will allow escalation to Group 2, and initiation of enrollment of the Group 2 sentinel subgroup. After the four subjects in the Group 2 sentinel subgroup are enrolled and given their first vaccination, enrollment will then be stopped pending a safety review of the sentinel subgroup as specified for the low dose cohort. Approval will allow administration of vaccination 2 to the sentinel subgroup and continued enrollment of Group 2 subjects (expanded group) to complete study enrollment.

This study will accrue 25 participants: (10 receiving 1 mcg of HydroVax-002 YFV; 10 receiving 5 mcg of HydroVax-002 YFV; and 5 receiving placebo).

1.1.4 Study Population

Healthy, male and female adults ≥18 and <50 years of age

1.1.5 Inclusion Criteria

1. Provide written informed consent prior to initiation of any study procedures.
2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Must agree to the collection of venous blood per protocol.
4. Are males or non-pregnant females, ≥18 and <50 years of age, inclusive at time of enrollment.
5. Are in good health[1].
   [1]*As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical or psychiatric diagnoses or conditions, defined as those that have been present for at least 90 days, which would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, emergency room or urgent care for condition, or invasive medical procedure and no adverse symptoms that need medical intervention such as medication change/supplemental oxygen). This includes no change in chronic prescription medication, dose or in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site PI or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening or treatment of continued symptoms of medical diagnosis or condition. Note: Low dose topical, corticosteroids as outlined in the Subject Exclusion Criteria (see Section 5.2) as well as herbals, vitamins and supplements are permitted.*
6. Oral temperature is less than 100.0°F.
7. Pulse is 47 to 100 beats per minute, inclusive.
8. Systolic blood pressure is 85 to 140 mmHg, inclusive.

9. Diastolic blood pressure is 55 to 90 mmHg, inclusive.
10. Screening laboratories (WBC, Hgb, PLTs, Sodium, Potassium, Bicarbonate, Calcium, Cr, non-fasting glucose, ALT, AST, TBIL and urine protein and glucose) are within acceptable parameters[2].
    [2]Hematology, blood chemistry and liver enzymes must be Grade 1 or less at screening (Refer to Table 11, Table 12, and Table 13, respectively); urine glucose negative and urine protein no greater than trace at screening for subjects to qualify for randomization and vaccination.
11. Negative test for HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) at screening blood draw
12. Women of childbearing potential[3] must use an acceptable contraception method[4] from at least 30 days before the first study vaccination until 30 days after the second study vaccination
    [3]Not sterilized via, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year has passed since the last menses if menopausal.
    [4]Includes non-male sexual relationships, full abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more and shown to be azoospermic prior to the subject receiving the study vaccination, effective intrauterine devices, NuvaRing®, tubal ligation, and licensed hormonal methods such as implants, injectables or oral contraceptives (i.e. "the pill").
13. Women of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to each study vaccination.
14. Sexually active males must agree to use a medically acceptable form of contraception[5] in order to be in this study and must agree to continue such use until day 30 after the last vaccination.
    [5]Medically acceptable contraceptives include: (1) surgical sterilization (such as a vasectomy), or (2) a condom used with a spermicide. Contraceptive measures such as Plan B™, sold for emergency use after unprotected sex, are not acceptable methods for routine use.

1.1.6 Exclusion Criteria

1. Have an acute illness[1] or acute febrile illness (oral temperature ≥ 38°C [100.4°F]), as determined by the site PI or appropriate sub-investigator, within 72 hours prior to study vaccination.
   [1]An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.
2. Have any medical disease or condition that, in the opinion of the site PI or appropriate sub-investigator, is a contraindication to study participation[2].
   [2]Including acute, subacute, intermittent or chronic medical disease or condition that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of this trial.
3. Have immunosuppression as a result of an underlying illness or treatment, a recent history or current use of immunosuppressive or immunomodulating disease therapy.
4. Use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.
5. Have known active or recently active (12 months) neoplastic disease or a history of any hematologic malignancy. Non-melanoma, treated, skin cancers are permitted.
6. Known allergy to components of the study product[3].
   [3]Including the following: aluminum hydroxide, sorbitol, potassium chloride, sodium chloride and polysorbate80 (Tween80)

7. Unstable seizure disorder (defined as requiring medication for seizure control or with seizure activity within the past 3 years).
8. Have a history of alcohol or drug abuse within 5 years prior to study vaccination.
9. Have any diagnosis, current or past, of schizophrenia, bipolar disease or other psychiatric diagnosis that may interfere[4] with subject compliance or safety evaluations.
   [4]As determined by the site PI or appropriate sub-investigator.
10. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 5 years prior to study vaccination.
11. Have a history of asthma, other than mild, well-controlled asthma[5]
    [5]Cold or exercise induced asthma controlled with inhaled medications other than inhaled corticosteroids is permissible. Participants should be excluded if they require daily bronchodilator use, or have had an asthma exacerbation requiring oral/parenteral steroid use or have used theophylline or inhaled corticosteroids in the past year.
12. Have a history of diabetes mellitus.
13. Have taken oral or parenteral (including intra-articular) or chronic topical corticosteroids of any dose within 30 days prior to study vaccination[6].
    [6]Corticosteroid nasal sprays for allergic rhinitis are permissible. Persons using a topical corticosteroid for a limited duration for mild uncomplicated dermatitis such as poison ivy or contact dermatitis may be enrolled the day after their therapy is completed.
14. Have taken high-dose inhaled corticosteroids[7] within 30 days prior to study vaccination.
    [7]High-dose defined as per age as using inhaled high-dose per reference chart in the National Heart, Lung and Blood Institute Guidelines for the Diagnosis and Management of Asthma (EPR-3) or other lists published in UPTODATE.
15. Received or plan to receive a licensed, live vaccine within 30 days before or after each study vaccination.
16. Received or plan to receive a licensed, inactivated vaccine or allergy desensitization shot within 14 days before or after each study vaccination.
17. Received an experimental agent[8] within 30 days prior to the study vaccination or expect to receive another experimental agent[9] during the trial-reporting period[10].
    [8]Including vaccine, drug, biologic, device, blood product, or medication.
    [9]Other than from participation in this trial.
    [10]Approximately 7 months after the first study vaccination.
18. Are participating or plan to participate in another clinical trial with an interventional agent[11] that will be received during the trial-reporting period[12].
    [11]Including licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication.
    [12]Approximately 7 months after the first study vaccination.
19. Female subjects who are breastfeeding or plan to breastfeed from the time of the first study vaccination through 30 days after the last study vaccination.
20. Receipt of blood products or immunoglobulin within six months prior to enrollment.
21. Donation of a unit of blood within 60 days prior to enrollment or intends to donate blood during the study period.
22. Body mass index (BMI) ≥ 35.

23. History of a visit to South America, sub-Saharan Africa or Southeast Asia lasting one month or more.
24. Planned travel to areas known to be endemic with Yellow Fever virus during the study period.
25. History of military service.
26. History of vaccination against dengue, yellow fever, tick-borne encephalitis, or Japanese encephalitis.
27. History of vaccination with other flavivirus candidate vaccines.
28. Attended primary (grade) school in Austria, Germany, Japan, South Korea, India, Thailand, Nepal, Vietnam, or Taiwan (locations where the tick-borne encephalitis vaccine is given).
29. History of yellow fever.
30. Plan to have a major change in exercise routine or perform strenuous exercise from 72 hours before any dose of study vaccine/placebo and for 72 hours before any safety laboratories[13].
    [13]*Safety laboratories are obtained on Days 4 and 15 following each dose of study product.*
31. Contraindication to intramuscular vaccination of either upper arm (for example, due to lymphadenectomy or obscuring tattoos).

1.1.7 Study Intervention

Investigational Vaccine: HydroVax-002 YFV Yellow Fever Virus (YFV), inactivated vaccine, alum-adsorbed, administered intramuscularly in a two-dose series on Day 1 and Day 29 at a dose of 1 mcg and at a dose of 5 mcg. Controls will receive sterile 0.9% NaCl placebo intramuscularly at days 1 and 29.

1.1.8 Staged Enrollment

For each dosing group, the first four subjects enrolled (the sentinel subgroup) will include three HydroVax-002 YFV recipients and one placebo recipient (Table 1). After the four subjects in the Group 1 sentinel subgroup are enrolled and given their first vaccination, enrollment will then be stopped pending a review of the clinical laboratory, reactogenicity, and safety data collected through the post vaccination Day 8 visit for the last of those subjects. This review may be conducted by an internal safety review committee (ISRC), consisting of the Blinded medical monitor, clinical program manager, and the PI, or by the SMC, as indicated and detailed in Section 10.1.6. Approval by the reviewing group will allow administration of the second vaccination to the sentinel subgroup and continued enrollment of the remaining 9 Group 1 subjects (expanded group) to resume to complete enrollment of 13 participants (10 vaccine and 3 placebo).

After Group 1 enrollment is completed enrollment will be stopped pending an SMC review of the clinical laboratory, reactogenicity, and adverse event information through the post 2nd vaccination Day 8 visit for all Group 1 subjects. Approval by the SMC will allow escalation to Group 2, and initiation of enrollment of the Group 2 sentinel subgroup. After the four subjects in the Group 2 sentinel subgroup are enrolled and given their first vaccination, enrollment will then be stopped pending a safety review of the sentinel subgroup as specified for the low dose cohort. Approval will allow administration of vaccination 2 to the sentinel subgroup and continued enrollment of Group 2 subjects (expanded group) to complete study enrollment.

Table 1. Study design and dose escalation

Group	Subgroup	HydroVax-002 YFV Dose	No. Subjects	
			HydroVax-002 YFV[1]	Placebo[2]
1. Low Dose	Sentinel[3]	1 mcg	3	1
	Expanded[4]	1 mcg	7	2
2. High Dose	Sentinel[3]	5 mcg	3	1
	Expanded	5 mcg	7	1
		Total	20	5

[1] HydroVax-002 YFV will be given as an intramuscular injection at Day 1 and at Day 29

[2] Placebo will be given as an intramuscular injection at Day 1 and at Day 29

[3] ISRC review of the clinical laboratory, reactogenicity and adverse event data available from enrollment through the post 1st vaccination Day 8 visit for the first 4 sentinel subjects (3 HydroVax-002 YFV and 1 placebo) will be conducted prior to administration of the second vaccination to the sentinel group and prior to initiation of enrollment of the remaining subjects in the Group. If deemed necessary by the ISRC, the SMC will review the safety data.

[4] SMC review of the clinical laboratory, reactogenicity and adverse event data available from enrollment through the post 2nd vaccination Day15 visit for Group 1 (low dose) (n=13) will be conducted prior to initiation of enrollment in Group 2 (high dose).

1.2 Schedule of Assessments (SoA)

Table 2. Schedule of Assessments (SoA)

Study day relative to 1st vaccination	-28 to -1	1	2	4	8	15	29							ET[1]	U[2]
Study day relative to 2nd vaccination							1	2	4	8	15	29	57	180	
Visit window in days			+ 1	+ 1	+ 1	+/- 1	+ 3	+ 1	+ 1	+ 1	+/- 1	+/- 4	+/- 4	+/- 7	
Study visit	00[3]	01	01a	02	02a	03	04	04a	05	05a	06	07	08	09	
Visit type[4]	I	I	P	I	P	I	I	P	I	P	I	I	I	I	I	I
Obtain informed consent[5]	X														
Assessment of eligibility[6]	X	X					X								
Review of medical history	X	X					X								
Concomitant meds	X[7]	X	X	X	X	X	X	X	X	X	X	X			X[8]	X[8]
Vital signs[9]	X	X		X		X	X		X		X	X	X[10]	X[10]	X[8, 10]	X[10]
Perform physical exam[11]	X															
Height and weight[12]	X															
Counseling[13]	X	X		X		X	X		X		X				X[8]	
Review of contraceptive/menstrual history[14]	X	X					X									
Pregnancy test[15, 16]	X	X					X									
HIV antibody ELISA, anti-HCV antibody, HBsAg	X															
Blood for clinical screening labs[17]	X															
Urine for screening lab[18]	X															
Targeted physical exam[19]		X[19]		X[19]		X[19]	X[19]		X[19]		X[19]	X[19]	X[19]	X[19]	X[19]	X[19]
Randomization[20]		X														
Vaccination[21]		X					X									

Study day relative to 1st vaccination	-28 to -1	1	2	4	8	15	29							ET[1]	U[2]
Study day relative to 2nd vaccination							1	2	4	8	15	29	57	180	
Visit window in days			+ 1	+ 1	+ 1	+/- 1	+ 3	+ 1	+ 1	+ 1	+/- 1	+/- 4	+/- 4	+/- 7	
Study visit	00[3]	01	01a	02	02a	03	04	04a	05	05a	06	07	08	09	
Visit type[4]	I	I	P	I	P	I	I	P	I	P	I	I	I	I	I	I
Evaluate vaccination site				X		X			X		X				X[8]	X[8]
Blood for clinical safety labs[16, 17]		X		X		X	X		X		X				X[22]	X[22]
Urine for safety lab[23]		X				X	X				X				X[22]	X[22]
Blood for YFV neutralizing assays[16]		X				X	X				X	X	X	X[26]	X[22]	X[22]
Blood for viremia testing				X					X						X[22]	X[22]
Identification of unsolicited adverse events[24]		X	X	X	X	X	X	X	X	X	X	X			X[8]	X[8]
Identification of medically-attended adverse events[24]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Identification of serious adverse events[24]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Identification of new onset of chronic condition[24]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review memory aid data			X	X	X			X	X	X					X[25]	X[25]

[1]ET=early termination visit.

[2]U=unscheduled visit.

[3]Screening procedures are performed at visit 00.

[4]Visit type: I=In-person, P= Phone.

[5]Consent process must be completed and informed consent form signed before any study-related procedures are conducted.

[6]Review inclusion/exclusion criteria.

[7]Obtain concomitant medications (prescription and over-the-counter drugs taken within the previous 60 days).

Study day relative to 1st vaccination	-28 to -1	1	2	4	8	15	29								
Study day relative to 2nd vaccination							1	2	4	8	15	29	57	180	ET[1]	U[2]
Visit window in days			+ 1	+ 1	+ 1	+/- 1	+ 3	+ 1	+ 1	+ 1	+/- 1	+/- 4	+/- 4	+/- 7		
Study visit	00[3]	01	01a	02	02a	03	04	04a	05	05a	06	07	08	09		
Visit type[4]	I	I	P	I	P	I	I	P	I	P	I	I	I	I	I	I

[8]Perform assessment if before Visit 07.

[9]Vital signs including oral temperature, blood pressure and pulse.

[10]Required at this visit if clinically indicated.

[11]Physical exam assessing general appearance and the following areas/systems: skin, lymph nodes, HEENT, neck, respiratory, cardiovascular, pulmonary, abdomen, extremities, musculoskeletal, and neurological.

[12]Obtain weight and height, calculate BMI.

[13]Counseling for avoidance of pregnancy and, at screening only, for HIV testing.

[14]For women of childbearing potential.

[15]Pregnancy test must be negative and be completed at screening (serum test) and within 24 hours prior to vaccination (urine test) for women of childbearing potential.

[16]On vaccination visits, these items should be completed prior to vaccination.

[17]White blood cell count, hemoglobin, platelets, sodium, potassium, bicarbonate, calcium, creatinine, glucose (non-fasting), ALT, AST, and total bilirubin.

[18]For glucose and protein by dipstick.

[19]Targeted physical exam if indicated by medical history.

[20]Subjects will be randomized to receive HydroVax-002 YFV (varying dosages) at day 1 and day 29 or to receive saline placebo at day 1 and day 29.

[21]All subjects will be observed for a minimum of 30 minutes following vaccination.

[22]Perform procedure if indicated.

[23]For glucose, protein, blood, and leukocyte esterase. If positive for blood or leukocyte esterase perform microscopic urine exam.

[24]Collection of AE, MAAE, SAE and NOCMC begin after first vaccination.

[25]Perform assessment if before Visit 06.

[26]Serum sample collected for exploratory serological analysis.

2. INTRODUCTION

2.1 Study Rationale

Yellow fever virus (YFV) is a mosquito-borne pathogen that is endemic in over 40 countries [2] and clinical presentation often involves severe acute onset with fever, nausea, vomiting, hepatitis, hemorrhage and renal failure. The case fatality rate for severe cases of yellow fever ranges from 20-60% [3, 4]. Prevention or reduction of disease burden is mainly accomplished through immunization with a live-attenuated yellow fever virus (e.g., YFV-17D or YFV-17DD) but these live vaccines are specifically contraindicated in young infants, pregnant or nursing mothers of young infants, people with egg allergies or people with a number of pre-existing medical conditions. In addition, specific warnings are in place for individuals >60 years of age due to the risk of rare, but potentially life-threatening, complications. This means that there is currently no safe vaccine available to protect these vulnerable populations. To address this unmet clinical need, we have developed an advanced yellow fever vaccine formulation in which the virus is inactivated using the proprietary hydrogen peroxide-based HydroVax™ technology.

2.2 Background

2.2.1 Purpose of Study

The Sponsor proposes a Phase 1 double-blind placebo-controlled clinical trial for a first-in-person evaluation of the safety and immunogenicity of a novel peroxide-inactivated whole-virus yellow fever vaccine.

2.2.1.1 Yellow Fever Virus

Yellow fever virus (YFV) represents an important mosquito-borne human pathogen that is endemic in more than 40 countries in sub-Saharan Africa and South America [2]. Although disease may be mild in some instances, clinical presentation often involves severe acute onset with fever, nausea, vomiting, hepatitis, hemorrhage and renal failure. The case fatality rate for severe cases of yellow fever ranges from 20-60% [3, 4]. There are no licensed antiviral drugs to treat yellow fever and prevention or reduction of disease burden is mainly accomplished through vaccination as well as through vector control measures. Though the requirements for protective vaccine-mediated immunity are unknown for many viruses, a correlate of immunity exists for YFV. Protective immunity against YFV was originally defined as a log neutralizing index (LNI) of ≥0.7 [5, 6] based on NHP challenge studies but more recent yellow fever vaccine clinical trials have been updated to use more reliable serum-dilution, constant-virus plaque-reduction neutralization tests (PRNT) [7, 8].

The live, attenuated YFV-17D vaccine was developed in 1936 and although there are two main substrains that are used in commercial manufacturing (17D and 17DD), there appear to be no major differences in safety or immunogenicity between YFV-17D vaccines [3, 9-11]. Yellow fever vaccination is recommended for people living in endemic areas and for travelers who may visit endemic areas, and for many years, YFV-17D has been considered "*one of the safest vaccines in the world*" [12]. However, this live attenuated vaccine is contraindicated in persons with hypersensitivity to eggs as well as several vulnerable populations including infants <9 months of age and immunosuppressed individuals such as those with acquired immunodeficiency syndrome, leukemia, lymphoma, thymic disease, generalized malignancy, and patients who are undergoing drug therapy (e.g., systemic corticosteroids, alkylating drugs, antimetabolites or other immunomodulatory drugs) or radiation therapy. Thymic

disorders associated with abnormal immune cell function (e.g., myasthenia gravis, thymoma) may be an independent risk factor for the development of yellow fever vaccine-associated viscerotropic disease. Live YFV vaccination is also contraindicated in lactating women who are providing breastmilk to infants less than 9 months of age due to the potential for transmission of vaccine virus in breastmilk [13, 14]. Specific warnings are also noted for adults over the age of 60 due to a greatly increased risk of rare but potentially severe adverse events [1]. These contraindications and warnings stem from known serious adverse events associated with the vaccine including a serious nervous system/neurotropic condition termed yellow fever associated neurotropic disease (YEL-AND). The CDC estimates the overall risk of YEL-AND at 8 cases per million vaccinations [15] with some rare fatalities due to encephalitis previously reported [16]. However, these estimates are based mainly on passive surveillance; active surveillance programs indicate an incidence of neurotropic disease occurring at rates as high as 1:10,000 [17] to 1:25,000 vaccinations [18] when closely monitored. Because HydroVax-002 YFV is based on inactivated virus that is unable to replicate or spread to the brain or nervous system, we anticipate that these vaccine complications will be eliminated.

In addition to YEL-AND, vaccination with live YFV-17D can cause severe multiple organ system failure, described as yellow fever vaccine-associated viscerotropic disease (YEL-AVD), a syndrome closely resembling wild-type yellow fever. YEL-AVD occurs at a rate of 4 cases per million vaccinations [15]. For vaccine recipients >60 years of age, the incidence is estimated to be 6- to 20-fold higher [19-22], i.e., 1 case of YEL-AVD per 50,000 doses, with a 50% mortality rate [23]. Because the HydroVax-002 YFV vaccine is inactivated, the virus is unable to replicate or spread to vital organs and we anticipate that the risk of YEL-AVD will be eliminated.

2.2.1.2 Candidate Study Vaccine – HydroVax-002 YFV

The candidate study vaccine, HydroVax-002 YFV, is an inactivated vaccine [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

This is the first human trial of HydroVax-002 YFV.

[redacted]

[redacted]

[redacted]

Common potential side effects resulting from intramuscular injections include stinging, discomfort, redness of skin, or mild bruising at vaccine injection site.

Uncommon side effects include general signs and symptoms associated with administration of the vaccine injection, including fever, chills, rash, aches and pains, nausea, dizziness and fatigue. These side effects will be monitored, but are generally short term, mild to moderate severity and usually do not require treatment.

Rare risks with any injection procedure include infection at the site of injection. Signs of infection at the injection site include: severe pain, erythema, induration, warmth or drainage. There may be side effects from the study products, which may be serious or life threatening that we do not know about yet.

2.3.2 Known Potential Benefits

Participation in this study provides no known direct benefit to the individual subjects.

2.3.3 Assessment of Potential Risks and Benefits

This study may not benefit participants directly. As this is a first-in-person trial, it is unknown whether the study product, HydroVax-002 YFV, will help to protect subjects from yellow fever disease or, if it does, how long that protection may last. The study placebo, saline solution, would offer no known benefit to study participants. As detailed in Section 2.3.1, there are potential risks for any intramuscular injection.

Study risk is minimized by using a dose escalation structure, as well as a sentinel group approach within each dose level. Group safety reviews occur between sentinel and expanded groups, and between dose groups. In addition, each individual study subject is followed closely during acute time periods following test article administration.

While individual subjects may not benefit from involvement in this study, their participation is crucial to the larger societal benefit of improved medical interventions. As noted in Section 2.1, the current live attenuated YFV vaccine is contraindicated in multiple vulnerable populations including infants <9 months of age and immunosuppressed individuals such as those with acquired immunodeficiency syndrome, thymic disease, or persons with hypersensitivity to eggs. By participating in this study, subjects are enabling the development of a potential advancement to address this current unmet medical need.

3. STUDY OBJECTIVES AND OUTCOME MEASURES

The overall objective of the study is to evaluate the safety and immunogenicity of two different dose levels of HydroVax-002 YFV.

OBJECTIVES	ENDPOINTS (Outcome Measures)
Primary	
To assess the safety, reactogenicity, and tolerability of the HydroVax-002 YFV vaccine administered intramuscularly in a two-dose series on Days 1 and 29 at a dose of 1 mcg or a dose of 5 mcg	• Occurrence of all serious adverse events (SAEs) at any time during the study • Occurrence of all Grade 3 unsolicited adverse events (AEs) from first vaccination through Day 29 after the second vaccination • Occurrence of all Grade 3 laboratory toxicities from first vaccination through Day 15 after the second vaccination • Occurrence of solicited local AE and reactogenicity signs and symptoms in the 7 days after each vaccination • Occurrence of solicited systemic AE and reactogenicity signs and symptoms in the 7 days after each vaccination • Occurrence of any AE through Day 29 after the second vaccination
Secondary	
To assess YFV-specific neutralizing antibody responses after a first dose and after a second dose of HydroVax-002 YFV vaccine given at dose levels of 1 mcg and 5 mcg	• Percentage of subjects achieving seroconversion (≥1:10 in plaque reduction neutralizing titer [$PRNT_{50}$] titer) at Day 29 after first vaccination and at Day 29 after second vaccination • Geometric mean neutralizing titers at Days 15 and 29 after first vaccination and at Days 15, 29, and 57 following second vaccination • Reverse cumulative distribution curve of neutralizing titers on Days 15 and 29 after first vaccination and at Days 15, 29, and 57 after the second vaccination for each dose group and for all dose groups combined
Exploratory	

To assess long-term YFV-specific neutralizing antibody responses after a second dose of HydroVax-002 YFV vaccine given at dose levels of 1 mcg and 5 mcg	• Geometric mean neutralizing titers at Day 180 following second vaccination • Reverse cumulative distribution curve of neutralizing titers on Day 180 after the second vaccination for each dose group and for all dose groups combined

4. STUDY DESIGN

4.1 Overall Design

This trial will be a randomized, placebo controlled, double-blind (within dosing group), dose escalation Phase 1 trial evaluating dosages of 1 mcg and 5 mcg of HydroVax-002 YFV vaccine given intramuscularly on Day 1 (the day of first vaccination is defined as Day 1) and Day 29 in healthy adults ≥18 and <50 years of age. The study will consist of two dosing groups of HydroVax-002 YFV vaccine to be enrolled sequentially. Each dose group will consist of 10 individuals who receive HydroVax-002 YFV, as well as 5 total subjects who receive placebo. Each dose-group will include a sentinel subgroup consisting of 3 vaccine and 1 placebo recipient. In each of the two (1 mcg and 5 mcg) dose phases, enrollment is halted after the dose 1 vaccination of the sentinel subgroup. Following assessment of safety and reactogenicity data of Group 1 by the Safety Monitoring Committee (SMC), the vaccine dose will be increased to 5 mcg for Group 2.

For each dosing group, the first 4 subjects enrolled (the sentinel subgroup) will include 3 HydroVax-002 YFV recipients and 1 placebo recipient. After the 4 subjects in the Group 1 sentinel subgroup are enrolled and given their first vaccination, enrollment will then be stopped pending a review of the clinical laboratory, reactogenicity, and safety data collected through the post vaccination one Day 8 visit for the last of those subjects. This review will be conducted by an internal safety review committee (ISRC), consisting of the Blinded medical monitor, clinical program manager and the PI. If deemed necessary by the ISRC, a further review will be conducted by the SMC, as detailed in Section 10.1.6. Approval by the reviewing group will allow administration of the second vaccination to the sentinel subgroup and continued enrollment of the remaining 9 Group 1 subjects (expanded group) to resume to complete enrollment of 13 participants (10 vaccine and 3 placebo).

After Group 1 enrollment is completed enrollment will be stopped pending an SMC review of the clinical laboratory, reactogenicity, and adverse event information through the post 2nd vaccination Day 15 visit for all Group 1 subjects. Approval by the SMC will allow escalation to Group 2, and initiation of enrollment of the Group 2 sentinel subgroup. After the 4 subjects in the Group 2 sentinel subgroup are enrolled and given their first vaccination, enrollment will then be stopped pending a safety review of the sentinel subgroup as specified for the low dose cohort. Approval will allow administration of vaccination 2 to the sentinel subgroup and continued enrollment of Group 2 subjects (expanded group) to complete study enrollment.

All subjects will complete a subject memory aid for 7 days after each study vaccination. Unsolicited, non-serious adverse events and serious adverse events will be collected through 29 days after the second vaccination. Serious adverse events and new onset of chronic medical conditions will be collected from the time of vaccination throughout the study period. Subjects' participation will be approximately 8 months. Table 2 provides a detailed outline of the study schedule.

Blood samples will be obtained at specified time points following each vaccination for safety laboratory assessments and assessment of humoral immune responses.

Table 3 provides the estimated blood volume of collection at each visit and cumulatively throughout the study.

Table 3. Laboratory samples and estimated blood volume (mL) by visit

Days relative to 1st vaccination	-28 to -1	1	4	15	29					
Days relative to 2nd vaccination					1	4	15	29	57	180
Study visit	00	01	02	03	04	05	06	07	08	09
Vaccination visit		1st			2nd					
Laboratory tests and blood volumes (mL)[1]										
Screening labs	22									
Safety labs		12	12	12	12	12	12			
YFV antibody assays		20		20	20		20	20	20	20
Viremia assays			10			10				
Total volume per visit	22	32	22	32	32	22	32	20	20	20
Cumulative total	22	54	76	108	140	162	194	214	234	254

[1]Additional blood draws may be needed as part of follow up of AEs.

4.2 Scientific Rationale for Study Design

For this Phase 1 first-in-human trial, a conservative dose escalation design will be used, in which each dose group of 10 vaccine recipients, and a total of 5 placebo recipients is split into two subgroups. The first subgroup, termed the sentinel subgroup, includes 3 vaccine and 1 placebo recipient. In each of the two (1 mcg and 5 mcg) dose phases, enrollment is halted after vaccination of the sentinel subgroup. This allows the opportunity to identify early vaccine related adverse events in this subgroup prior to exposure of additional subjects, a design which decreases the risk of multiple serious vaccine related adverse events compared to sequential vaccination of the entire dose group without halting [34]. After enrollment of the expanded subgroups, escalation to the next dose group will be delayed until 14 days of safety data following the second vaccination (corresponding to study Day 15) is available from all subjects in the preceding dose group.

4.2.1 Inclusion of Placebo

Placebo recipients are included in order to maintain blinding, provide some safety reference data, and to provide controls for the immunogenicity assays.

4.3 Justification for Dose

A repeat-dose Investigational New Drug (IND) application enabling *in vivo* toxicology study was conducted in the Sprague-Dawley rat model utilizing an accelerated vaccination schedule involving four doses of HydroVax-002 YFV administered by intramuscular injection at weekly intervals. Each 0.5 milliliter (mL) dose was administered in the form of two 0.25 mL intramuscular injections of HydroVax-002 YFV [5 mcg/0.5 mL dose

formulated with 0.1% aluminum hydroxide (Al (OH)3)]. The 3-dose vaccination schedule represents n+1 for the proposed 2-dose schedule to be administered during this Phase 1 clinical trial. This dose of vaccine was well-tolerated in the study animals. These results indicate that the vaccine test article demonstrates an acceptable pre-clinical safety profile at the planned maximum dose level of 5 mcg.

This route and vaccination schedule is commonly used for other inactivated whole-virus vaccines formulated with alum. Analysis of antigen dose used in other inactivated alum-adsorbed, whole-virus vaccines consisting of closely related flaviviruses produced in Vero cells provides information of potential relevance for this dose ranging study of HydroVax-002 YFV. The commercial tick-borne encephalitis virus vaccine, TicoVac, contains 5 mcg/dose, and the commercial Japanese encephalitis virus vaccine, IXIARO, contains 6 mcg/dose. The experimental yellow fever virus vaccine, XRX-001, was tested in Phase I trials at 4.8 mcg/dose and at 0.48 mcg/dose.[8] Antiviral immunity following a single dose of inactivated Japanese encephalitis virus vaccine is low after a single 6 mcg dose,[35] whereas two or three intramuscular injections of a 12 mcg/dose vaccine were tested and found to be safe, well tolerated, and immunogenic [36].

Based on prior results with a closely related inactivated flavivirus vaccine (HydroVax-001 WNV, [37]) we do not anticipate significant safety signals. Based on the animal model evaluations of HydroVax-002 YFV and studies of other inactivated alum-absorbed flavivirus vaccines, the antigen doses to be evaluated in this Phase 1 trial of HydroVax are 1 mcg and 5 mcg.

5. STUDY POPULATION

Only subjects who meet the inclusion criteria and do not meet the exclusion criteria will be eligible for enrollment. Inclusion and exclusion criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. No exemptions are granted to inclusion and exclusion criteria in studies sponsored by NTI.

5.1 Inclusion Criteria

1. Provide written informed consent prior to initiation of any study procedures.
2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Must agree to the collection of venous blood per protocol.
4. Are males or non-pregnant females, ≥18 and <50 years of age, inclusive at time of enrollment.
5. Are in good health[1].
   [1]As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical or psychiatric diagnoses or conditions, defined as those that have been present for at least 90 days, which would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, emergency room or urgent care for condition, or invasive medical procedure and no adverse symptoms that need medical intervention such as medication change/supplemental oxygen). This includes no change in chronic prescription medication, dose or in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site PI or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening or treatment of continued symptoms of medical diagnosis or condition. Note: Low dose topical, corticosteroids as outlined in the Subject Exclusion Criteria (see Section 5.2) as well as herbals, vitamins and supplements are permitted.
6. Oral temperature is less than 100.0°F.
7. Pulse is 47 to 100 beats per minute, inclusive.
8. Systolic blood pressure is 85 to 140 mmHg, inclusive.
9. Diastolic blood pressure is 55 to 90 mmHg, inclusive.
10. Screening laboratories (WBC, Hgb, PLTs, Sodium, Potassium, Bicarbonate, Calcium, Cr, non-fasting glucose, potassium, ALT, AST, TBIL, and urine protein and glucose) are within acceptable parameters[2].
    [2]Hematology, blood chemistry and liver enzymes must be Grade 1 or less at screening (Refer to Table 11, Table 12, and Table 13, respectively); urine glucose negative and urine protein no greater than trace at screening for subjects to qualify for randomization and vaccination.
11. Negative test for HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) at screening blood draw.
12. Women of childbearing potential[3] must use an acceptable contraception method[4] from at least 30 days before the first study vaccination until 30 days after the second study vaccination.
    [3]Not sterilized via, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year has passed since the last menses if menopausal.
    [4]Includes non-male sexual relationships, full abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more and shown to be azoospermic prior to the subject

receiving the study vaccination, effective intrauterine devices, NuvaRing®, tubal ligation, and licensed hormonal methods such as implants, injectables or oral contraceptives (i.e. "the pill").

13. Women of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to each study vaccination.
14. Sexually active males must agree to use a medically acceptable form of contraception[5] in order to be in this study and must agree to continue such use until day 30 after the last vaccination.

[5]*Medically acceptable contraceptives include: (1) surgical sterilization (such as a vasectomy), or (2) a condom used with a spermicide. Contraceptive measures such as Plan B™, sold for emergency use after unprotected sex, are not acceptable methods for routine use.*

5.2 Exclusion Criteria

1. Have an acute illness[1] or acute febrile illness (oral temperature ≥ 38°C [100.4°F]), as determined by the site PI or appropriate sub-investigator, within 72 hours prior to study vaccination.
   [1]An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.
2. Have any medical disease or condition that, in the opinion of the site PI or appropriate sub-investigator, is a contraindication to study participation[2].
   [2]Including acute, subacute, intermittent or chronic medical disease or condition that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of this trial.
3. Have immunosuppression as a result of an underlying illness or treatment, a recent history or current use of immunosuppressive or immunomodulating disease therapy.
4. Use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.
5. Have known active or recently active (12 months) neoplastic disease or a history of any hematologic malignancy. Non-melanoma, treated, skin cancers are permitted.
6. Known allergy to components of the study product[3].
   [3]Including the following: aluminum hydroxide, sorbitol, potassium chloride, sodium chloride and polysorbate80 (Tween80).
7. Unstable seizure disorder (defined as requiring medication for seizure control or with seizure activity within the past 3 years).
8. Have a history of alcohol or drug abuse within 5 years prior to study vaccination.
9. Have any diagnosis, current or past, of schizophrenia, bipolar disease or other psychiatric diagnosis that may interfere[4] with subject compliance or safety evaluations.
   [4]As determined by the site PI or appropriate sub-investigator.
10. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 5 years prior to study vaccination.
11. Have a history of asthma, other than mild, well-controlled asthma[5].
    [5]Cold or exercise induced asthma controlled with inhaled medications other than inhaled corticosteroids is permissible. Participants should be excluded if they require daily bronchodilator use, or have had an asthma exacerbation requiring oral/parenteral steroid use or have used theophylline or inhaled corticosteroids in the past year.
12. Have a history of diabetes mellitus.
13. Have taken oral or parenteral (including intra-articular) or chronic topical corticosteroids of any dose within 30 days prior to study vaccination[6].
    [6]Corticosteroid nasal sprays for allergic rhinitis are permissible. Persons using a topical corticosteroid for a limited duration for mild uncomplicated dermatitis such as poison ivy or contact dermatitis may be enrolled the day after their therapy is completed.
14. Have taken high-dose inhaled corticosteroids[7] within 30 days prior to study vaccination.
    [7]High-dose defined as per age as using inhaled high-dose per reference chart in the National Heart, Lung and Blood Institute Guidelines for the Diagnosis and Management of Asthma (EPR-3) or other lists published in UPTODATE.
15. Received or plan to receive a licensed, live vaccine within 30 days before or after each study vaccination.

16. Received or plan to receive a licensed, inactivated vaccine or allergy desensitization shot within 14 days before or after each study vaccination.
17. Received an experimental agent[8] within 30 days prior to the study vaccination or expect to receive another experimental agent[9] during the trial-reporting period[10].
    [8]*Including vaccine, drug, biologic, device, blood product, or medication.*
    [9]*Other than from participation in this trial.*
    [10]*Approximately 7 months after the first study vaccination.*
18. Are participating or plan to participate in another clinical trial with an interventional agent[11] that will be received during the trial-reporting period[12].
    [11]*Including licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication.*
    [12]*Approximately 7 months after the first study vaccination.*
19. Female subjects who are breastfeeding or plan to breastfeed from the time of the first study vaccination through 30 days after the last study vaccination.
20. Receipt of blood products or immunoglobulin within six months prior to enrollment.
21. Donation of a unit of blood within 60 days prior to enrollment or intends to donate blood during the study period.
22. Body mass index (BMI) ≥ 35.
23. History of a visit to South America, sub-Saharan Africa or Southeast Asia lasting one month or more.
24. Planned travel to areas known to be endemic with Yellow Fever virus during the study period.
25. History of military service.
26. History of vaccination against dengue, yellow fever, tick-borne encephalitis, or Japanese encephalitis.
27. History of vaccination with other flavivirus candidate vaccines.
28. Attended primary (grade) school in Austria, Germany, Japan, South Korea, India, Thailand, Nepal, Vietnam, or Taiwan (locations where the tick-borne encephalitis vaccine is given).
29. History of yellow fever.
30. Plan to have a major change in exercise routine or perform strenuous exercise from 72 hours before any dose of study vaccine/placebo and for 72 hours before any safety laboratories[13].
    [13]*Safety laboratories are obtained on Days 4 and 15 following each dose of study product.*
31. Contraindication to intramuscular vaccination of either upper arm (for example, due to lymphadenectomy or obscuring tattoos).

5.3 Exclusion of Specific Populations

Children and pregnant women are excluded from this first-in-human Phase 1 study because insufficient information is available on the safety of HydroVax-002 YFV to judge potential risks for children and pregnant women.

5.4 Inclusion of Vulnerable Participants

Not applicable.

5.5 Lifestyle Considerations

During this study, participants are asked to:

- Avoid getting pregnant during the study from the time of screening until 30 days after the second vaccination
- Use an acceptable contraception method, if a woman of childbearing potential, from 30 days before the first study vaccination until 30 days after the second study vaccination
- Use a medically acceptable form of contraception, if a sexually active male, until day 30 after the last vaccination
- Refrain from receiving another experimental agent or participating in another clinical trial with an interventional agent
- Refrain from donating blood during the study period
- Refrain from a major change in exercise routine or strenuous exercise from 72 hours before any dose of study vaccine/placebo and for 72 hours before each blood collection for clinical laboratory tests (Vaccination Days and Days 4 and 15 following each dose)
- Abstain from travel to areas known to be endemic with Yellow Fever virus until 6 months after vaccination

5.6 Screen Failures

Following consent, after the screening evaluations have been completed, the investigator or designee is to review the inclusion/exclusion criteria and determine the subject's eligibility for the study. If there is any uncertainty, the PI should make the decision on whether a potential subject is eligible for enrollment.

Only the following information will be collected on screen failures: demographics (age, screen number, sex, ethnicity, and race) and reason for ineligibility. Subjects who are found to be ineligible will be told the reason for ineligibility.

Individuals who do not meet the criteria for participation in this study (screen failure) because of a laboratory value that is outside the range of eligibility, but is thought to be due to an acute condition or due to laboratory error, may be repeated once

5.7 Strategies for Recruitment and Retention

5.7.1 Recruitment

Potential participants will be selected from existing participant registries, through advertising or by word of mouth in the Duke University, Durham, NC area and surrounding communities. Recruitment staff will contact interested individuals to assess their eligibility for this study. It is anticipated that the demographic composition of subjects for this study will be representative of the respective region.

The IRB will approve the recruitment process and all materials provided prior to any recruitment to prospective subjects directly.

Screening will begin with a brief discussion with study staff. Some people will be excluded based on demographic data and medical history (i.e., pregnant, prior travel, prior vaccination, etc.). Information about the study will be presented to potential subjects and questions will be asked to determine potential eligibility. Screening procedures can begin only after informed consent is obtained.

5.7.2 Retention

Retention of subjects in this trial is especially important for determining the primary and secondary endpoints. As such, after vaccination, participating subjects will be reminded of subsequent study visits and every effort will be made to accommodate the subject's schedule to facilitate follow-up within the specified visit window. Additionally, there are many circumstances that influence the ability to obtain outcome information after vaccination. Follow-up visits may be conducted by phone if in person visits are not feasible. Lastly, compensation strategies will be implemented to encourage subjects to return for follow-up assessments and sample collections.

5.7.3 Compensation Plan for Subjects

Compensation will be determined locally at the study site and in accordance with local IRB regulations and approval.

5.7.4 Costs

There is no cost to subjects for the research tests, procedures/evaluations, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party.

6. STUDY PRODUCT

6.1 Study Product and Administration

In this dose escalation study, the first 13 subjects will be randomized (10:3) to receive two doses of either 1 mcg HydroVax-002 YFV IM injection or placebo IM injection 28 days apart. The second 12 subjects will be randomized (5:1) to receive two doses of 5 mcg HydroVax-002 YFV IM injection or placebo IM injection 28 days apart.

6.1.1 Study Product Description

6.1.1.1 Vaccine

The HydroVax-002 YFV vaccine is a yellow fever virus, Vero cell tissue culture-derived vaccine inactivated with hydrogen peroxide, formulated with 0.1% aluminum hydroxide. HydroVax-002 YFV drug product contains 5 mcg purified whole virus YFV formulated in a volume of 0.5 mL/dose with 0.1% aluminum hydroxide and 10% sorbitol, 0.001% polysorbate 80 in phosphate-buffer [pH = 7.5] with 350 mM sodium chloride. A single 0.5 mL dose of HydroVax-002 YFV may also contain residual amounts of Vero host cell DNA (<1 ng/0.5 mL) or Vero host cell protein (<300 ng/0.5). HydroVax-002 YFV does not contain antibiotics or preservatives. The stoppers used for single-dose vials do not contain latex.

6.1.1.2 Placebo and Diluent

Sodium Chloride Injection USP, 0.9% (NaCl 0.9%, Normal Saline) will be used as the placebo and diluent.

6.1.2 Dosing and Administration

Based on dosing group assignment, subjects will receive either 1 mcg, 5 mcg, or placebo per vaccination dose administered in the non-dominant arm as a 0.5 mL intramuscular (IM) injection on days 1 and 29. Following vaccination, subjects will be observed for a minimum of 30 minutes.

6.1.3 Dose Escalation

For this Phase 1 first-in-human trial, a conservative dose escalation design will be used, in which each dose group of 10 vaccine recipients, and a total of 5 placebo recipients, is split into two subgroups. The first subgroup, termed the sentinel subgroup, includes 3 vaccine and 1 placebo recipient. In each of the two (1 mcg and 5 mcg) dose phases, enrollment is halted after vaccination of the sentinel subgroup. This allows the opportunity to identify potential early vaccine related adverse events in this subgroup prior to exposure of additional subjects, a design which decreases the risk of multiple serious vaccine related adverse events compared to sequential vaccination of the entire dose group without halting [34]. After enrollment of the expanded subgroups, escalation to the next dose group will be delayed until 14 days of safety data following the second vaccination (corresponding to study Day 15) is available from all subjects in the preceding dose group.

6.1.4 Dose Modifications

Not applicable.

6.1.5 Overdosage

Overdosage is not anticipated in the context of a clinical trial. Subjects will be given intramuscular injections on Days 1 and 29. No study product will be administered outside the clinical setting.

6.2 6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

[redacted]

Sodium Chloride Injection USP 0.9% for placebo and diluent will be sourced by the Duke Investigational Drug Service.

After receipt of the study product, the principal investigator is responsible for distribution and disposition of these study products and has ultimate responsibility for drug accountability. The principal investigator may delegate to the research pharmacist responsibility for study vaccine accountability. The research pharmacist must maintain and document logs of receipt, accountability, vaccine dilution and dispensation, storage conditions, and disposal. These study product accountability and dispensing logs must be maintained in the study file.

Upon completion of the study and the final monitoring visit, used and unused vials of HydroVax-002 YFV and Placebo/diluent (sterile 0.9% NaCl) will be retained until monitored and released for disposition. For detailed information regarding final disposition of study vaccine and placebo/diluent see the protocol-specific MOP.

6.2.2 Formulation, Appearance, Packaging, and Labeling

HydroVax-002 YFV vaccine will be supplied to the investigative site in crystal zenith 2 mL vials containing 5 mcg of purified, inactivated whole YFV virus formulated with alum per 0.5 mL with stopper, crimp seals, and flip-off cap. Each vial will contain a fill volume of 0.6 mL. The study vaccine is a white, opaque, non-uniform suspension that becomes homogeneous upon shaking.

Sodium Chloride Injection USP 0.9% is a sterile, nonpyrogenic, isotonic solution of sodium chloride and WFI. It will be used to dilute the vaccine and will be used as the placebo.

Further details regarding formulation, packaging and labeling are included in the protocol-specific Manual of Procedures (MOP) and the Investigator's Brochure for the HydroVax-002 YFV vaccine.

6.2.3 Product Storage and Stability

Study vaccine and the normal saline placebo/diluent will be shipped and stored refrigerated at 2°C to 8°C. Study vaccine and the placebo/diluent should not be frozen.

During storage, HydroVax-002 YFV vials should be kept in a lightproof container. 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.

6.2.4 Preparation

Study vaccine preparation, including vaccine dilutions for the various dosing groups, will be performed by the site research pharmacist on the same day of study vaccine administration. Detailed information regarding the handling of vaccine, including preparation of the dilutions, labeling, storage, and administration of study vaccine will be provided in the protocol-specific MOP.

Aseptic technique will be used for the administration of each dose of study product using a sterile needle appropriate in length for each participant, as described in the protocol-specific MOP.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Treatment Assignment Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the clinical site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Coordinating Center's (CC) Advantage eClinical® (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for the trial, the subject will be assigned to a treatment arm within each dose group (low dose and high dose) subjects will be randomly assigned to receive the study test product or placebo. Subjects will be block randomized so that 1 of the first 4 sentinel subjects will receive placebo and the other 3 active vaccine. In the expanded groups, 7 of the 8 or 9 subjects will be randomized to vaccine and 1 or 2 to placebo. Subjects will receive the same treatment for both vaccinations.

6.3.2 Randomization

Enrollment of subjects will be done online using the enrollment module of Advantage eClinical®. The randomization code will be prepared by statisticians at the CC and included in the enrollment module for the trial. Advantage eClinical® will generate a treatment assignment for each subject after the demographic and eligibility data have been entered into the system. A designated individual at each site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the Advantage eClinical® User's Guide. Manual back-up procedures and instructions are provided for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

6.3.3 Blinding and Masking Procedures

This is a partially-blinded clinical trial.

Study staff and investigators will not be blinded to dose group but, within dose groups, subjects, investigators, and study staff other than unblinded site research pharmacist (or other designated unblinded personnel administering study product) will be blinded as to the subject's treatment assignment (vaccine vs. placebo). Laboratory personnel performing antibody assays will be blinded to dose group and treatment assignment.

The randomization scheme will be generated by the CC and provided to unblinded study personnel (i.e., research pharmacists performing study vaccination preparations and unblinded study vaccine administrators) at the clinical site.

An unblinded site research pharmacist will prepare the study product and an unblinded research nurse will inject the study product per the randomization assignment. The pharmacist will conceal the contents of the syringe by wrapping the syringe barrel with an overlay, opaque tape, or other equivalent material. In addition, the subject will be asked to look away when the vaccine is being administered.

The unblinded site research pharmacist and unblinded research nurse will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

6.3.3.1 Unblinding

A copy of subject treatment assignments will be retained at the site in a secure file by the unblinded staff. In the event of a medical emergency when knowledge of the treatment assignment will influence subject's care, the Principal Investigator may be provided with the treatment assignment. The Principal Investigator must contact NTI and document the event with information regarding the reasons for unblinding. After discussion with NTI, the Principal Investigator may receive the treatment assignment from the data coordinating center or if necessary, from the on-site secure file by the unblinded staff.

6.4 Study Intervention Compliance

Each dose of study product will be administered by a member of the clinical research team who is qualified and licensed to administer study product. Administration site, date, and time will be documented on the case report form (CRF) and entered into the eCRF.

6.5 Concomitant Therapy

All concomitant medications taken within 60 days prior to signing the ICF will be reviewed with subjects to determine stability of chronic diseases and eligibility. Medications reported in the eCRF are limited to those taken

within 30 days prior to the first dose of study vaccine. Concomitant medications will be reviewed at every study visit through Visit 07 (29 days following receipt of the second dose of study product).

Women of childbearing potential in a heterosexual relationship must agree to use true abstinence or use at least one acceptable primary form of contraception through 30 days following the second dose of study vaccine.

Prohibited medications:

- Medications that may be associated with impaired immune responsiveness including immunosuppressive or immunomodulating therapies.
- Anticancer chemotherapy within 3 years prior to vaccination.
- Medications used to control seizures within the past 3 years.
- Daily bronchodilators, oral or parental steroids, theophylline, or inhaled corticosteroids to treat an asthma exacerbation within the past year.
- Oral, parenteral, intra-articular, chronic topical steroids or high-dose inhaled steroids within 30 days.
- Investigational drug, vaccine, biologic, blood product, or medication within 30 days prior to receipt of the first dose of study product or during the study period.
- A live vaccine within 30 days of receipt of study product.
- An inactivated vaccine or allergy desensitization shot within 14 days of receipt of study product.
- Blood products or immunoglobulin during the six months prior to enrollment.

6.5.1 Rescue Medicine

Not applicable.

7. Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

7.1 Halting Criteria and Discontinuation of Study Intervention

7.1.1 Study Halting Criteria

Additional enrollment and study interventions/administration of study products in this trial will be halted for SMC review/recommendation if any of the following are reported:

- Any subject experiences ulceration, abscess, or necrosis at the injection site related to study product administration.
- Any subject presents with laryngospasm, bronchospasm, hypotension or anaphylaxis occurring within 24 hours following product administration that is considered related to study product.
- Two or more subjects experience generalized urticaria (defined as occurring at more than two body parts) within 72 hours after administration of study product that is considered related to study product.
- Any subject experiences an SAE after administration of study product and prior to the subject's last visit that is considered related to study product.
- Two or more subjects who received at least one dose of study vaccine to date, cumulative to all study vaccine administrations, across all treatment arms, experience the same Grade 3 (unsolicited AE or safety laboratory), in the same HLT by MedDRA coding, considered related to study product.

This trial will also be halted for SMC review/recommendation if, within 7 days after administration of either study vaccination, any of the following occurs:

- Three or more subjects who received at least one dose of study vaccine to date, cumulative to all study vaccine administrations, across all treatment arms, experience the same severe (Grade 3) study vaccine-related injection site reaction. Erythema and induration (hardness)/edema (swelling) will also be measured in mm but size will not be used as halting criteria.
- Three or more subjects who received at least one dose of study vaccine to date, cumulative to all study vaccine administrations, across all treatment arms, experience the same severe (Grade 3) study vaccine-related subjective systemic reaction, for which the severity (grade) is corroborated by study personnel.
- Three or more subjects who received at least one dose of study vaccine to date, cumulative to all study vaccine administrations, across all treatment arms, experience the same severe (Grade 3) study vaccine-related quantitative systemic reaction.

Grading scales for solicited injection site and systemic (subjective and quantitative) reactions are included in Section 8.5.1.1.

Grading scales for clinical safety laboratory AEs are included Appendix A.

If any of the halting rules are met following any subject receipt of any study vaccination, then this trial will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the SMC to proceed.

NTI retains the authority to suspend additional enrollment and study interventions/administration of study products during this trial, as applicable.

NTI is empowered to stop enrollment and study vaccinations if AEs that meet the halting criteria are reported.

7.1.2 Individual Halting Criteria

Individual Halting Criteria must be reviewed by the site PI prior to administration of the second study vaccination for each subject. It is the responsibility of the site to ensure that any subject meeting the below criteria are discontinued from receiving study product; however, the Emmes BMM or safety monitor may be consulted as needed.
The second study vaccination will not be administered to a subject if any of the following criteria are met, as determined by the site PI:

- Serious adverse event associated with study vaccination.
- Pregnancy in a female subject.
- Type 1 hypersensitivity associated with study product.
- Grade 3 systemic reaction associated with study product.
- Grade 3 local reaction (with the exception of erythema/induration) associated with study product (e.g., pain).
- Grade 2 or higher fever within the first 24 hours following the initial vaccination.
- Medical condition for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would pose a risk to the subject or would be likely to confound interpretation of the results.
- Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity. For subjects with injection site or systemic signs or symptoms, or with an acute illness, including but not limited to an oral temperature greater than or equal to 100.4°F, the second study vaccination should be postponed/deferred until signs, symptoms, or acute illness have resolved and still within the acceptable protocol-specified window for that visit. If outside this window, the Blinded Medical Monitor must first approve the second study vaccination and the documentation of approval should be filed in the subject's chart.
- Any unresolved or continuing solicited or unsolicited Grade 3 adverse event after first vaccination. An unresolved or continuing Grade 1 or Grade 2 adverse event is permissible unless, in the opinion of the site principal investigator or appropriate subinvestigator, it would render study vaccination unsafe or interfere with the evaluation of responses.
- Grade 2 or higher clinical safety laboratory result that does not decrease to Grade 1 or below prior to the second study vaccination. For a subject to receive the second study vaccination, the most recently evaluated clinical safety laboratory values obtained prior to the second study vaccination must be Grade 1 or less. The

second study vaccination should be scheduled to occur within the acceptable protocol-specified window for that visit. If outside this window, the Blinded Medical Monitor must first approve the second study vaccination and the documentation of approval should be filed in the subject's chart.

- Grade 3 adverse event that occurs without alternative etiology in the 7 days following the first study vaccination.
- Severe or sustained reaction or disability related to the first study vaccination.
- New information becomes available that makes further participation unsafe.

7.2 Participant Withdrawal from the Study and Replacement

7.2.1 Participant Withdrawal

Subjects may voluntarily withdraw their consent for trial participation at any time and for any reason, without penalty or loss of benefits to which they are otherwise entitled.

The site PI or appropriate sub-investigator may also withdraw a subject from receiving the study vaccine for any reason.

A subject may withdraw or be withdrawn from the study for the following reasons:

- Medical disease or condition, or any new clinical finding for which continued participation, in the opinion of the site PI or appropriate sub-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of this trial, or would interfere with the evaluation of responses (for example, has baseline significant laboratory abnormalities).
- The subject withdraws consent.
- Subject no longer meets eligibility criteria (see Sections 5.1 and 5.2). Note: Medication changes in the 60 days prior to enrollment, as specified in Subject Inclusion Criterion (See Section 5.1, Point Number 5), are exclusionary for receipt of the first study vaccination only.
- As deemed necessary by the site PI or appropriate sub-investigator for noncompliance or other reasons.
- The subject is lost to follow-up.
- The study is terminated.
- Any reason that, in the opinion of the investigator, precludes the subject's participation in the study.
- New information becomes available that makes further participation unsafe.

7.2.2 Subject Replacement

Subjects who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after signing the informed consent form, randomization, and receipt of study vaccine will not be replaced. Subjects who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after signing the informed consent form and randomization but before receipt of study vaccine may be replaced.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff.

7.4 Follow up for subjects that discontinued study intervention

The primary reason for withdrawal from this trial will be recorded on the Study Status data collection form (DCF). Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 1.2.

Although subjects are free to withdraw at any time or may be withdrawn by the site PI or appropriate sub-investigator at any time (see Section 7.2.1), those subjects who receive only one dose of study vaccine will be encouraged to remain in this trial for follow-up safety assessments (may be conducted by phone call rather than in person) continuing through approximately 6 months after their last study vaccination. These subjects will also be encouraged to provide a venous blood sample for serological assays after their last study vaccination. See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Every attempt will be made to follow all AEs, including solicited injection site and systemic reactions, unsolicited non-serious AEs, SAEs, and NOCMCs ongoing at the time of early withdrawal through resolution as per applicable collection times defined for the specific type of AE.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject's study records.

The site PI or appropriate sub-investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws or is withdrawn from this study.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening and Immunogenicity Assessments

8.1.1 Screening Procedures

Screening procedures may be done up to 28 days prior to enrollment (Day-28 to Day -1). After informed consent the following assessments are performed to determine eligibility and obtain baseline data:

1. Take a focused medical history, including the following information:

- History of any chronic medical conditions related to inclusion and exclusion criteria.
- History of any medication allergies including history of hypersensitivity to any components of the study product.
- Concomitant medications taken within 60 days prior to signing the ICF will be reviewed with subjects to determine stability of chronic diseases and eligibility.
- Ask if they are participating in another clinical trial or plan to enroll in another clinical trial during the study period.

2. Women of childbearing potentials should be counseled to either practice abstinence or use at least one primary form of contraception from 30 days prior to receipt of study product until 30 days after receipt of the last dose of study vaccine.
3. Sexually active men should be counseled to use contraception from the date of receipt of the first dose of study vaccine until one month after receipt of the second dose of study vaccine.
4. Women of childbearing potential must have a negative serum HCG pregnancy test at screening and a negative urine HCG pregnancy test prior to each dose of study product.
5. Physical exam (standard exam at screening and targeted exam at enrollment).
6. Height and weight measurement (Calculation of Body Mass Index).
7. Vital signs measurement (including HR, BP, oral temperature).
8. Blood for laboratory evaluations.

- Serology at screening: HIV, Hepatitis B surface antigen, Hepatitis C antibody.
- Chemistry at screening: sodium, potassium, bicarbonate, calcium, creatinine, glucose [non-fasting], ALT, AST, and total bilirubin.
- Hematology at screening: white blood cells (WBCs), hemoglobin, and platelet count.
- Urine for glucose and protein.

Clinical screening laboratory evaluations will be performed locally by the Duke University Health System Clinical Laboratories. Urine pregnancy tests will be performed by trained research staff. The volume of venous blood to be collected is presented in Table 3.

The overall eligibility of the subject to participate in the study will be assessed once all screening values are available. Study subjects who qualify will be randomized into the Advantage eClinical system and all others will be registered as screen failures. Results of screening testing will be discussed with the subject. If a positive result for HIV, hepatitis C or hepatitis B occurs or there is a laboratory result deemed to be clinically significant by the PI, the subject will be referred for appropriate follow-up. Laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to laboratory error may be repeated once.

8.1.2 Immunogenicity Assessments

8.1.2.1 Humoral Immunogenicity Tests

Neutralizing YFV-specific antibody responses following vaccination will be assessed by $PRNT_{50}$ assays performed on blood samples obtained at Baseline (Day 1) and Days 15 and 29 after the first vaccination and at Days 15, 29, 57, and 180 following the second vaccination.

8.1.2.2 Specimen Preparation, Handling, and Shipping

Blood specimens for YFV neutralizing assays will be processed as specified in the MOP and shipped monthly to Fisher BioServices. They will then be sent to OHSU (Beaverton, OR) for testing.

8.2 Safety and Other Assessments

Study procedures are specified in the SoA. A study physician licensed to make medical diagnoses and listed on Form 1572 will be responsible for all trial-related medical decisions.

8.2.1 Physical examination

A targeted physical exam will be performed at baseline and at subsequent in-person visits as indicated by changes in medical history.

8.2.2 Safety Clinical Laboratory Tests

Safety labs will be conducted on blood samples obtained on Days 4 and 15 after each vaccination and on the day of, but prior to, administration of the first and second vaccination (day 29 after first vaccination). Safety labs include hematology tests (hemoglobin, WBC, and platelet count) and blood chemistry tests (sodium, potassium, bicarbonate, calcium, creatinine, glucose [non-fasting], ALT, AST, and total bilirubin).

Urine will be tested by dipstick to evaluate glucose, protein, blood, and leukocyte esterase on days 1, 15 and 29 after first vaccination and day 15 after second vaccination.

Safety labs will be tested locally by Duke University Health System Clinical Laboratories.
For laboratory values outside the site's reference ranges, the site PI will document clinical significance in the eCRF. Any result that is deemed clinically significant but does not meet the protocol's grading criteria (Appendix A) should either be repeated by the study site, or the subject referred for appropriate clinical follow-up.

For any result that meets the protocol's grading criteria (whether or not it is deemed clinically significant), the PI will also assess relationship to study product, action taken with product, and whether the subject was discontinued due to the event. Graded laboratory abnormalities should be followed until either a return to normal or are judged to be stable, as determined by the PI.

In the absence of a diagnosis, any clinical safety laboratory test result that meets the definition of an SAE as determined by the investigator (e.g., life-threatening) must be reported as an SAE using the AE/SAE form set.

8.2.3 Viremia Detection Tests

Blood samples will be obtained on Day 4 after each vaccination for YFV viremia testing. Viremia testing will include plaque assay and/or RT-PCR. Positive results may be followed by additional testing (e.g., blind passage/amplification). Frozen serum specimens for viremia testing will be shipped to Fisher BioServices. They will then be sent to OHSU (Beaverton, OR) for testing as needed.

8.2.4 Additional Sera Samples

Additional sera samples may be shipped to Fisher BioServices for storage.

8.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

If a physiologic parameter, (e.g., vital signs, or laboratory value) is outside of the protocol-specified range, then the measurement may be repeated once if, in the judgment of the investigator, the abnormality is the result of an acute, short-term, rapidly reversible condition (e.g., stress, anxiety, or "white coat syndrome"). A physiologic parameter may also be repeated if there is a technical problem with the measurement caused by malfunctioning, or an inappropriate measuring device (i.e., inappropriate-sized blood pressure cuff).

8.4 Unscheduled Visit

If the investigator deems the reaction warrants further evaluation or intervention, the investigator will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation at an unscheduled visit if appropriate.

8.5 Adverse Events and Serious Adverse Events

8.5.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

Adverse events can be further divided into solicited adverse events and unsolicited adverse events. Solicited adverse events are those for which the study team will specifically query the participant whether they occurred. Unsolicited adverse events are those events that the subject report occurring without being queried about the specific event.

All AEs will be assessed for severity and relationship to study intervention (see Section 8.5.3.2).

All AEs, solicited and unsolicited, will be captured on the appropriate data collection form. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the FDA Form 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. In addition, all medically-attended adverse events (MAAEs) (i.e., events for which medical care from a healthcare provider is sought) will be collected through Study Visit 09.

8.5.1.1 Solicited Adverse Events/Reactogenicity

Solicited adverse events are anticipated local and systemic adverse events for which consistent collection of information is desired. This may include:

- Common and expected events according to the available knowledge about the product, or
- Specific events of concern that should be ascertained on every participant.

All subjects will be observed for at least 30 minutes after each vaccination to detect and treat any immediate adverse reactions. Subjects will be provided with a memory aid, digital, oral thermometer, and ruler for recording daily maximum oral temperature and systemic and local AEs beginning with the day of vaccination and continuing for the next 7 days. Subjects will be encouraged to take their temperature around the same time each day and also if they feel feverish, beginning with the day of vaccination. Subjects will be instructed on how to use the memory aid and how to rate any adverse events. The subject will record temperature and the presence and intensity of post vaccination local reactogenicity events, including signs of redness and swelling and symptoms of pain and tenderness. The subjects will measure redness and swelling at the vaccination site with the measuring device provided. Systemic solicited symptoms will include feverishness, fatigue, headache, chills, nausea, new muscle pain (exclusive of the injection sites), aggravated muscle pain (increase of existing pain, exclusive of the injection site), new joint pain, and aggravated joint pain (increase of existing pain). The subjects will measure temperature with the thermometer provided. Subjects will be instructed to notify the study center if they develop any severe reactions and/or fever equal to or exceeding 101.3°F.

Subjects will also be asked to record any medications taken and any emergency room or physician visits (other than routine check-ups). The subject memory aid will be reviewed with the subject at subsequent clinic visits.

After vaccination, antipyretics or analgesics should not be given routinely. Administration of an antipyretic or analgesic is to be recorded in the memory aid and entered into the CRF.

Injection site symptoms (pain or tenderness) and swelling will be graded as indicated in Table 4.

Table 4. Table of injection site symptom grading

Local Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site	Subject is aware of pain but it does not interfere with daily activity and no pain medication is taken	Subject is aware of pain; there is interference with daily activity or requires use of non-narcotic pain medication	Subject is aware of pain and it prevents daily activity, or any use of narcotic pain reliever
Tenderness – hurts only when injection site is touched	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Induration/Swelling[1]	Does not interfere with activity	Interferes with activity	Prevents daily activity

[1]Will be also measured but size will not be used as halting criteria

Erythema/redness and induration/swelling as analyzed by measurement will be graded as indicated in Table 5.

Table 5. Table of erythema/redness and induration/swelling grading

Parameter	Grade 1	Grade 2	Grade 3
Erythema/Redness[1]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
Induration/Swelling[1]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm

[1]Will not be used as halting criteria

Note: Induration/Swelling should be evaluated and graded using the symptom scale as well as the actual measurement.

Solicited systemic symptoms will be graded by the subject using the scale in Table 6.

Table 6. Table of systemic symptom grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Fatigue	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Headache	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Chills	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Nausea	No interference with activity	Some interference with activity	Significant interference, prevents daily activity

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
New muscle pain (exclusive of the injection site)	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Aggravated muscle pain (increase in existing pain, exclusive of the injection site)	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
New joint pain	No interference with activity	Some interference with activity	Significant interference, prevents daily activity
Aggravated joint pain (increase in existing pain)	No interference with activity	Some interference with activity	Significant interference, prevents daily activity

Fever will be graded as shown in Table 7.

Table 7. Table of fever grading

Oral Temperature	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
°C °F	≥ 37.8 and < 38.5 ≥ 100.0 and < 101.3	≥ 38.5 and < 39.0 ≥ 101.3 and < 102.0	≥ 39.0 ≥102.0

Any symptoms still present on Day 8 after the second vaccination will continue to be followed by the subject on the memory aid until two days after symptom resolution.

If a subject experiences an AE or an injection site reaction that is still present at the subject's EOS visit, the subject will be followed until either resolution of the event/reaction or until the event/reaction is stable, as determined by the site PI. Follow-up procedures, evaluations, and outcomes will be recorded on the subject's case report forms.

8.5.1.2 Unsolicited Adverse Events

All AEs spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures must be recorded in the source document and on the appropriate page of the case report form. All reported unsolicited AEs are graded in accordance with the protocol toxicity tables.

8.5.1.3 Unexpected

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unapproved investigational medicinal product).

8.5.2 Definition of Serious Adverse Events (SAE)

An adverse event 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 (21 CFR 312.32 (a)):

- Death.
- A life-threatening adverse event.
- Inpatient hospitalization or prolongation of existing hospitalization.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that do not result in inpatient hospitalization, etc.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the FDA Form 1572 or by the Institution as the site Principal Investigator or Sub-Investigator.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study physician (for investigational new drug application [IND] studies, a physician listed on the FDA Form 1572 as the site Principal Investigator or Sub-Investigator).

Reviewed and evaluated by the sponsor, the Safety Monitoring Committee (SMC) (periodic review unless related), and the IRB/IEC.

8.5.2.1 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is any SAE where a causal relationship with the study product is at least reasonably possible but is not listed in the Investigator Brochure, Package Insert, and/or Summary of Product Characteristics.

8.5.3 Classification of an Adverse Events

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose, provide a medical evaluation of adverse events, and classify adverse events based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

8.5.3.1 Seriousness and Severity of Event

Event seriousness will be determined according to the protocol definition of an SAE (Section 8.5.1). All AEs or SAEs will be assessed for severity, according to the toxicity grading scales in Appendix A.

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

8.5.3.2 Relationship to Study Intervention

The licensed study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In general, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.5.4 Time Period and Frequency for Event Assessment and Follow-up

For this study all solicited AEs will be documented for the 8 days following study product administration.

For this study, all unsolicited, non-serious AEs will be documented from Study Visit 01 (Day 1) through Study Visit 07 (Day 29 post second vaccination).

All MAAEs, SAEs and new onset chronic medical conditions (NOCMC) will be documented from Study Visit 01 (Day 1) through Study Visit 09 (final visit).

Resolution of an AE is defined as the return to pre-treatment status or as stabilization of the condition, with the expectation that it will remain chronic. Site PIs are required to follow all reported AEs until either resolution of the AE or until the event is stable, as determined by the site PI; all SAEs will be followed until either resolution

of the SAE or until the SAE is stable, as determined by the PI. Subjects who have an unresolved SAE at either the final study visit or at discontinuation of study participation will continue to be followed, with the subject's permission, until the SAE is stable/deemed chronic, as determined by the site PI.

8.5.5 Adverse Event Reporting

8.5.5.1 Investigators Reporting of AEs

Adverse Events, including local and systemic reactions not meeting the criteria for "serious adverse events," will be captured on the appropriate case report form. Information to be collected for unsolicited adverse events includes event description, date of onset, investigator assessment of severity, relationship to study product, date of resolution of the event, seriousness, and outcome.

8.5.6 Serious Adverse Event Reporting

8.5.6.1 Investigators Reporting of AEs

Serious adverse events will be collected throughout the study until the final visit is completed.

The following procedures will apply to all serious adverse events:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed physician listed on the FDA Form 1572 as the principal investigator or subinvestigator.
- Recorded on the appropriate serious adverse event report form and electronic case report form (eCRF).
- Reviewed and evaluated by an Independent Safety Monitor, the SMC (periodic review unless related), NTI and the Institutional Review Board (IRB) (as indicated).
- Reviewed and followed to resolution or stabilization by a licensed physician listed on the FDA Form 1572 as the principal investigator or subinvestigator.

The Blinded Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

8.5.6.2 Regulatory Reporting of AEs

Following notification from the site Principal Investigator or appropriate sub-investigator, NTI, as the IND sponsor, will review the suspected unexpected serious adverse event (SUSAR) and ensure it is reported as an IND safety report to the FDA. Submissions of IND safety reports will be performed by Emmes on behalf of NTI. Emmes will notify all participating site Principal Investigators (i.e., all Principal Investigators to whom the sponsor is providing drug under its IND(s)) of potential serious risks from clinical studies or any other source, as soon as possible. Emmes, on behalf of NTI, will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. If the event is not fatal or life-threatening the IND safety report will be submitted within 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, NTI (or Emmes, on NTI's behalf) will submit to the FDA any

additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

SAEs that are not SUSARs will be reported to the FDA at least annually in a summary format which includes all SAEs.

8.5.7 Adverse Events of Special Interest

This study will also assess New-Onset Chronic Medical Conditions (NOCMCs) from the day of receipt of the first dose of study product until study completion. NOCMCs are defined as any new ICD-10 diagnosis (10th revision of the International Statistical Classification of Diseases and Related Health Problems) that is applied to the subject during the course of the study, after receipt of the study agent, that is expected to continue for at least 3 months and requires continued health care intervention. NOCMCs will be assessed and recorded as unsolicited AEs.

8.5.8 Reporting of Pregnancy

Pregnancy is not an adverse event. Pregnancies occurring in study subjects will be reported via Advantage eClinical® on the Pregnancy Report form. No further study vaccinations will be administered to pregnant subjects, but if the subject has received a study vaccination and with the subject's permission all study mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome pending the subject's permission.

8.6 Unanticipated Problems

8.6.1 Definition of Unanticipated Problems (UP)

The Department of Health and Human Services Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.6.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Coordinating Center (CC). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number.
- A detailed description of the event, incident, experience, or outcome.
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP.
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the CC/study sponsor within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the CC/study sponsor within 5 days of the investigator becoming aware of the problem.

8.6.3 Reporting Unanticipated Problems to Participants

Not applicable.

9. STATISTICAL CONSIDERATIONS

9.1 Study Hypotheses

The goal of this study is to assess the safety, reactogenicity, and tolerability of the HydroVax-002 YFV vaccine administered intramuscularly in a two-dose series at a dose of 1 mcg and at a dose of 5 mcg. Other goals are to assess YFV-specific neutralizing antibody responses after a first dose and after a second dose of HydroVax-002 YFV vaccine given at doses of 1 mcg and 5 mcg. This study, like other Phase 1 studies, is exploratory rather than confirmatory. Its purpose is to estimate event rates and patterns of immune responses rather than to test formal statistical hypotheses. For the purposes of collecting pilot data and planning of potential future trials, comparisons of secondary outcomes will be made among the groups, although the study is not powered to detect small to moderate differences.

9.2 Sample Size Considerations

A total of 25 subjects, age ≥18 and <50 years of age, will be enrolled, where 10 subjects will receive 1 mcg of HydroVax-002 YFV, 10 will receive 5 mcg of HydroVax-002 YFV, and 5 will receive placebo. The study will consist of two dosing groups of HydroVax-002 YFV vaccine to be dosed sequentially. The initial dose of HydroVax-002 YFV to be evaluated in Group 1 will be 1 mcg and the next dose to be evaluated in Group 2 will be 5 mcg. The first 4 subjects of each group (3 receiving HydroVax-002 YFV and 1 receiving placebo) will serve as a sentinel subgroup. Randomization will be allocated in a 3:1 ratio between HydroVax-002 YFV and placebo in the sentinel subgroups. Subjects in each expanded subgroup will be randomized between HydroVax-002 YFV and placebo using a permuted-block design.

9.2.1 Safety

The sample size of 10 vaccinated subjects in each dosage group with 5 subjects that receive placebo is small given the early stage (Phase 1) of the product's development, thus the precision of estimate for AEs is limited. Rare adverse events are not demonstrable in a clinical study of this size, however the probabilities of observing one or more AEs given various true event rates and sample sizes are presented in Table 8. If there are adverse events associated with HydroVax-002 YFV in this population, this study will have approximately 80% power to observe at least one such event in a dosage group size of 10 if the true rate is 15%. Comparisons will primarily be made between each vaccine dose group and placebo.

Table 8. Probability of observing one or more adverse events in one dosage group (n=10) given particular true event rates

"True" Unknown Event Rate	Probability of Observing an Event (%)
0.1%	1.0
0.5%	4.9
1.0%	9.6
2.0%	18.3
3.0%	26.3
4.0%	33.5

"True" Unknown Event Rate	Probability of Observing an Event (%)
5.0%	40.1
10.0%	65.1
15.0%	80.3

If there are no observed adverse events among the 10 vaccinated subjects in a dosage group associated with the investigational product, the upper 95% exact confidence bound for the incidence of adverse events will be 30.8%.

9.2.2 Immunogenicity

This study is not powered to detect small to moderate individual or pair-wise differences in immunogenicity outcomes between treatments.

9.3 Populations for Analyses

The Safety Analysis population includes all eligible subjects who received at least one dose of study vaccine.

The evaluable population includes all eligible subjects who received at least one dose of study vaccine and contributed both pre- and at least one post-study vaccination blood samples for testing for which valid results were reported.

The per protocol (PP) population excludes subjects who did not receive both doses of study vaccine or who had major protocol deviations, such as receipt of non-study vaccines during the time frame prohibited by the protocol or receipt of the second study vaccination substantially out of window.

In the case of mis-randomization subjects will be analyzed according to the study product actually received for all analysis populations.

9.4 Statistical Analyses

9.4.1 General Approach

The analyses of safety data will be primarily descriptive. Data will be represented to show difference in reactogenicity signs and symptoms between the vaccine candidate and dose groups.

9.4.2 Analysis of Primary Outcome Measures (Safety)

Solicited AEs and laboratory toxicities will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none or mild versus moderate or severe) and using exact confidence intervals to summarize the reactogenicity and toxicity rates. Tabular and graphical summaries of events will be presented for each solicited symptom, by type (local/systemic/toxicity), severity (none or mild, moderate or severe), and time point post-vaccination.

Unsolicited AEs will be coded by the Medical Dictionary for Regulatory Activities (MedDRA) for preferred term and system organ class. The rate and exact 95% confidence intervals of AEs in aggregate, and by MedDRA categories, will be computed. SAEs will be reported by a detailed listing showing the type, MedDRA coding, relevant dates (vaccination and adverse event), severity, relatedness, and outcome for each event. Primary outcome measures include:

- Occurrence of all serious adverse events (SAEs) at any time during the study.
- Occurrence of all Grade 3 unsolicited adverse events (AEs) from first vaccination through day 29 after the second vaccination.
- Occurrence of all Grade 3 laboratory toxicities from first vaccination through day 15 after the second vaccination.
- Occurrence of solicited local AE and reactogenicity signs and symptoms in the 7 days after each vaccination.
- Occurrence of solicited systemic AE and reactogenicity signs and symptoms in the 7 days after each vaccination.
- Occurrence of any AE through day 29 after the second vaccination.

9.4.3 Analysis of Secondary and Exploratory Outcome Measures (Immunogenicity)

Basic descriptive statistics, such as point estimates and 95% confidence intervals, and graphical summaries, such as the reverse cumulative distribution, of seroconversion rates and geometric mean titer (GMT) will be computed by intervention at baseline, 15 and 29 days after the first vaccination, and 15, 29, 57, and 180 days after the second vaccination. YFV-specific neutralizing antibody titers may be measured through a standard plaque reduction neutralizing titer [$PRNT_{50}$] assay, or utilizing a Log_{10} Neutralizing Index (LNI) assay [5, 38]. For the purposes of this study, and in line with recent yellow fever vaccine clinical trials [7, 8], antibody titers will be assessed using the $PRNT_{50}$ assay, and seroconversion will be defined as a $PRNT_{50}$ neutralizing antibody titer of ≥1:10. The limit of detection in the neutralizing assay will be a titer of <10. For the purposes of determining seroconversion rates and GMT, antibody titers of <10 will be assumed to be 5 (one dilution step below the assay limit value).

Rates of seroconversion will be summarized by tabulating the frequency of positive responses by treatment group at Day 29 after first vaccination and Day 57 after second vaccination. Response rates for each treatment group will be presented with their corresponding 95% confidence interval estimates at each time point. The relationship between the proportion of responders and dosage will be examined using logistic regression. The distribution of antibody titers will also be graphically summarized using the reverse cumulative frequency distribution of titers for each treatment group.

The $PRNT_{50}$ titers at days 15 and 29 after first vaccination and at days 15, 29, and 57 following second vaccination will be summarized graphically for each treatment group. The geometric mean titer (GMT) and pair-wise differences of each treatment group will also be presented with 95% confidence intervals. The relationship between GMT and dosage will be examined using standard linear regression methods. Secondary outcome measures include:

- Percentage of subjects achieving seroconversion (≥1:10 in plaque reduction neutralizing titer [$PRNT_{50}$] titer) at day 29 after first vaccination and at day 29 after second vaccination.

- Geometric mean neutralizing titers at days 15 and 29 after first vaccination and at days 15, 29, and 57 following second vaccination.
- Reverse cumulative distribution curve of neutralizing titers on Days 15 and 29 after first vaccination and at days 15, 29, and 57 after the second vaccination for each dose group and for all dose groups combined.

Exploratory outcome measures include:

- Geometric mean neutralizing titers at Day 180 following second vaccination.
- Reverse cumulative distribution curve of neutralizing titers on Day 180 after the second vaccination for each dose group and for all dose groups combined.
- Additional exploratory immunogenicity analyses utilizing the LNI assay may be conducted for comparison to historical datasets

9.4.4 Timing of Analyses

9.4.4.1 Interim Safety Analyses

The blinded medical monitor, clinical program manager, unblinded medical monitor and PI of the clinical laboratory will review data through the Day 15 visit after vaccination 1 for the 4 subjects in the sentinel groups. After the completion of Group 1 enrollment, the SMC will then review the data on all Group 1 subjects through the post 2nd vaccination Day 15 visit. These reviews, however, may not involve any hypothesis testing and will not be considered in estimating the precision of any estimates made at the conclusion of the study.

9.4.4.2 Final Analyses

Clinical, safety, and reactogenicity data through approximately Day 57 after last study vaccination will represent the primary clinical database for the trial. Once all subjects have completed the Day 57 post dose 2 visit and the data are entered in the database, validated and monitored according to the clinical monitoring plan, the primary clinical database will be locked. Unblinded analyses of the primary and secondary safety, reactogenicity, and immunogenicity outcomes (Neutralization and viremia) and any supporting analyses of these outcomes described in the SAP will be performed by the SDCC and provided to the sponsor and PI. Individual subject listings will not be generated at this point. The laboratory staff that run the assays for the Day 180 post dose 2 visit will remain blinded, and the assessment of relationship to study product of any SAEs that may be reported at the Day 180 post dose 2 visit will be delegated to blinded sub-investigators at the clinical site. While the results will not be used to make any decisions concerning the conduct of this trial, they may be used to make decisions on activities external to this trial such as the design of future trials of this vaccine. Since this early analysis of the data is not intended to impact the conduct of the trial, it has no impact on Type I error and adjustments are not planned.

The remaining analyses, including generation of all individual subject listings, will be completed and the CSR generated after the last subject's last visit is completed, and the final clinical database, including all long-term safety follow-up data, is cleaned, monitored and locked.

A formal statistical analysis plan (SAP) will be developed and finalized prior to unblinding for any analysis, which defines the analyses to be included in the CSR.

9.4.5 Sub-group Analyses

Sub-group analysis (e.g, analysis based on age, sex, race/ethnicity or other demographic characteristics) is not planned due to the limited size of this Phase 1 clinical study.

9.4.6 Tabulation of Individual participant Data

Individual participant data will be listed by measure and time point after the last subject's last visit is completed and the final clinical database, including all long-term safety follow-up data, is cleaned, monitored and locked.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical and Study Oversight Considerations

Each site principal investigator will obtain IRB approval for this protocol and any amendments to be conducted at his/her research site(s).

NTI must receive the documentation that verifies IRB/IEC-approval for this protocol, informed consent, and associated documents prior to the recruitment, screening, and enrollment of subjects.

10.1.1 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the trial, verbally and with a written ICF. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate. The ICF must not include any exculpatory statements.

The site PI or their designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the trial, the procedures and experimental aspects of the trial, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research and a detailed summary of the proposed study procedures and study interventions/study products. This will include aspects of the trial that are experimental, the probability for random assignment to treatment arms, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, alternative treatment/procedures that may be available, and the important potential benefits and risks of these available alternative treatment/procedures.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the site PI) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitors, auditors, IRB, NTI, and regulatory authorities will be granted direct access to the subject's original medical records for verification of trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written ICF, the subject is authorizing such access.

Subjects will be informed that records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or samples/specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in the trial and have the opportunity to discuss the trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

ICFs will be IRB-approved and subjects will be asked to read and review the ICF. Subjects must sign the ICF prior to starting any study procedures being done specifically for the trial.

Once signed, a copy of the ICF will be given to the subjects for their records. The subject(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from the trial.

New information that significantly impacts the subject's risk of receiving the study interventions/study products will be communicated by the site PIs or their designees to the subjects who consent to participate in the trial in accordance with IRB requirements. The ICF will be updated and subjects will be re-consented in accordance with IRB requirements, if necessary. Subjects will be given a copy of all ICFs that they sign.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, Clinical Staff may pre-screen via chart review and refer potential subjects to the Research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

10.1.1.1 Requirements for Permission by Parents/Guardians and Assent by Children (in case of a minor)

Not applicable.

10.1.1.2 Other Informed Consent Procedures

Not applicable.

10.1.1.3 Ethical Standard

The PI will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [April 18, 1979]) and codified in 45 CFR 46, 21 CFR 50 and 21 CFR 56, as applicable. The site PIs will also ensure conformity with ICH E6 GCP and applicable federal regulations, guidance and guidelines for GCP and Clinical Trials with humans.

10.1.1.4 Institutional Review Board

The institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office for Human Research Protections (OHRP) for federally funded research. The IRB must be registered with OHRP [OHRP-only or OHRP/FDA] as applicable to the research. The IRB FWA number will be provided to NTI.

The site PI will obtain IRB approval for this protocol to be conducted at his/her research site(s) and send supporting documentation to NTI before initiating recruitment of subjects. The site PI will submit applicable information to the IRB on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP guidelines, and as applicable, 21 CFR 56 (Institutional Review Boards), 21 CFR 50 (Protection of Human Subjects), and other federal, state and local regulations and guidance. NTI must receive the documentation that verifies IRB approval for this protocol, associated informed consent documents, and upon request, any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB before they are implemented. IRB review and approval will occur at least annually throughout the enrollment and follow-up of subjects and may cease if annual review is no longer required by applicable regulations. The site PI will notify the IRB of protocol deviations and reportable SAEs in accordance with IRB requirements.

10.1.2 Study Termination and Closure

This study may be prematurely terminated if there is sufficient reasonable cause, including but not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Regulatory authorities

If the study is prematurely terminated, the Principal Investigator (PI) will promptly inform study participants and the Institutional Review Board (IRB) and regulatory authorities as applicable. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule. The PI will assure appropriate follow-up for the subjects, as necessary.

The sponsor will notify regulatory authorities as applicable.

10.1.3 Confidentiality and Privacy

Subject confidentiality is strictly held in trust by the site PIs, other study personnel, the sponsor, and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in this trial. No information concerning this trial, or the data generated from this trial, will be released to any unauthorized third party without prior written approval of the subject and NTI.

Subject confidentiality will be maintained when trial results are published or discussed in conferences and is extended to cover testing of samples/specimens. The study monitor or other authorized representatives of NTI as well as governmental regulatory agencies, such as the FDA, may inspect all documents and records required to be maintained by the site PI. This includes, but is not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this trial. The participating site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password-protected systems. All non-clinical samples/specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number and will not be identified by the subject's name.

As this research is funded by the NIH, it is covered by NIH policy which effectively issues the research a Certificate of Confidentiality. By this policy, researchers cannot be forced to disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, *or* for the purposes of other

research that the release is in compliance with applicable Federal regulations governing the protection of human subjects in research.

10.1.4 Future Use of Stored Specimens for Secondary Research

Subjects will be asked to consent to use of any remaining specimens for possible use in future secondary research studies as a condition of their study participation. A subject's decision can be changed at any time prior to the end of the study by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood has already been used for research purposes, the information from that research may still be used.

Some samples may be stored at the local site and some at a central clinical storage facility. Samples may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. No genetic testing will be performed on stored specimens.

Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality. There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will not be kept in their health records.

10.1.5 Key Roles and Study Governance

Table 9. Table of Key Roles

Principal Investigator	[redacted]
Blinded Medical Monitor	[redacted]
Unblinded Medical Monitor	[redacted]

Scientific Lead	[redacted]
Clinical Program Manager	[redacted]
Coordinating Center	[redacted]

10.1.6 Safety Oversight

10.1.6.1 Safety Monitoring Committee (SMC)

The study oversight will be conducted by a SMC which is an independent group of experts that will be established by NTI to monitor subject safety and advise NTI. SMC members will be separate and independent of study personnel participating in this study and should not have scientific, financial or other conflicts of interest related to the study. The SMC will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

The primary responsibilities of the SMC are to:

- Review and evaluate the accumulated study safety data in a blinded fashion for participant safety after the low dose cohort prior to proceeding to the high dose cohort, study conduct and progress, and
- Make recommendations to the sponsors concerning the continuation, modification, or termination of the trial.

The operating rules of the SMC will be established in conjunction with NTI guidelines.

The SMC will review the safety laboratory results, reactogenicity data, and adverse event information collected through day 15 following Dose 2 for the Low Dose Cohort. SMC approval is required prior to initiation of enrollment in the High Dose Cohort.

The SMC may also be asked to review safety information on the low dose or high dose sentinel groups as documented in Section 10.1.6.2.

NTI or the SMC may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. Any AE/SAE that triggers a study halting rule will be reviewed by the Emmes BMM within 24 hours of its being reported, and, after confirming that a study halting rule was met, Emmes will alert the SMC chair to the situation and will provide a written narrative of the safety event. In addition, Emmes will notify the site PI that a halting rule has been triggered, and, upon approval by the SMC chair, will instruct the study site to suspend both study enrollment and study interventions (i.e., administering vaccine to subjects) until NTI provides explicit directions for resuming the suspended study activities. Concurrent with notification to the study site, Emmes will inform NTI that a halting rule has been triggered and that study activities, excepting follow-up with subjects for safety monitoring, have been paused pending the SMC's review of the inciting event. The SMC will conduct an expeditious review of all relevant information, and a written summary of the committee's discussion, to include any abiding concerns for subject safety, as well as any recommendations by the SMC regarding study continuation, will be submitted by Emmes to NTI. Any decision to suspend study enrollment and/or study interventions, either temporarily or permanently, is exclusively NTI's.

The SMC will review SAEs on a regular basis and ad hoc during the study.

10.1.6.2 Internal Safety Review Committee (ISRC)

An internal safety review committee (ISRC), consisting of the blinded medical monitor, clinical program manager and the PI may be convened to provide an option for a rapid review of safety data collected through the day 8 visit following dose one administered to the sentinel cohort of the low dose group and the sentinel cohort of the high dose group. If within the sentinel group during the period from first vaccination through the day 8 visit, no individual halting rule was met, and no more than one unique Grade 3 adverse event (i.e., no same adverse event in more than one subject) was observed then the study will continue with administration of the second vaccination to the sentinel group and initiation of enrollment in the expanded dose cohort. The ISRC may be convened to assist with this objective determination. If the criteria above will not be met, or at the request of the PI and/or NTI, the SMC will be convened to review the sentinel safety data. If the SMC is convened for any reason for review of the sentinel data, the ISRC will not be convened for the cohort and the SMC will determine whether administration of the second vaccination to the sentinel cohort and initiation of enrollment in the expanded dose cohort in order for additional study vaccinations is to be given.

10.1.7 Clinical Monitoring/Site Monitoring Plan

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study interventions/administration of study products, and data collection processes are of high quality and meet sponsor and ICH E6 GCP guidelines and applicable federal regulations, and that this trial is conducted in

accordance with the protocol, protocol-specific MOP and applicable sponsor SOPs. NTI or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan. NTI-designated clinical monitors will verify that this trial is conducted, and data are generated, documented (recorded) and reported in compliance with the protocol, ICH E6 GCP guidelines and applicable regulatory requirements. Clinical monitoring reports will be submitted to NTI.

Site visits will be made at standard intervals as defined by NTI and may be made more frequently as directed by NTI. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating clinical site, study personnel and all study documentation according to the NTI-approved site monitoring plan. Study monitors will meet with site PIs to discuss any problems and actions to be taken and document visit findings and discussions.

10.1.8 Quality Control and Quality Assurance

Following a written NTI-accepted site quality management plan, the site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site PI will provide direct access to all study-related sites, source data/DCFs and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site PI will ensure all study personnel are appropriately trained and applicable documentation is current and maintained on site.

The CC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

10.1.9 Data Handling and Record Keeping

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the electronic case report form and provided by the CC to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with all source documents or the discrepancies should be explained.

The sponsor will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

10.1.9.1 Data Collection and Management Responsibilities

Data for this study will include safety, laboratory (safety and immunologic) and outcome measures (e.g., reactogenicity and immunogenicity).

Clinical data (including AE/SAEs, concomitant medications, physical assessments, and reactogenicity data) will be entered into the CC's Advantage eClinical® web-based 21 CFR Part 11-compliant Internet Data Entry System (IDES). The data system includes password protection and internal quality checks, such as automatic range checks to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will primarily be entered directly from the data collection forms.

All data collection forms must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

The CC will be responsible for data management, quality review, analysis, and reporting of the study data.

A final clinical study report will be prepared following the last subject visit and upon completion of assays related to the immunogenicity endpoints.

10.1.9.2 Study Records Retention

Study records and reports, including, but not limited to, eCRFs, source documents, ICFs, and study drug disposition records shall be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the drug, until 2 years after the investigation is discontinued and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. ICFs for future research use will be maintained as long as the sample/specimen exists.

No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the site PI when these documents no longer need to be retained. The participating site must contact NTI for authorization prior to the destruction of any study records.

10.1.9.3 Source Records

The site will maintain appropriate medical and research records for this clinical trial, in compliance with ICH E6 GCP Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit the study monitor or other authorized representatives of NTI or its representative as well as governmental regulatory agencies, such as the FDA, to examine (and when required by applicable law, to copy) clinical trial records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after

verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files, and records kept at the pharmacy, at the laboratories and medico-technical departments involved in this clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the clinical trial.

Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, GCP or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site PI or other study personnel. As a result of protocol deviations, corrective actions are to be developed by the site and implemented promptly.
These practices are consistent with ICH E6 GCP guidelines:

- 4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2 and 4.5.3
- 5.1 Quality Assurance and Quality Control, Section 5.1.1
- 5.20 Noncompliance, Sections 5.20.1 and 5.20.2

It is the responsibility of the site PI and other study personnel to use continuous vigilance to identify and report protocol deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. Protocol deviations must be promptly reported to NTI, via the CC's Advantage eClinical® system.

Protocol deviations, as defined above, must be addressed on the appropriate DCF. A completed copy of the Protocol Deviation DCF must be maintained in the regulatory file as well as in the subject's chart. Protocol deviations must be sent to the IRB in accordance with IRB requirements. The site PI and other study personnel are responsible for knowing and adhering to IRB requirements.

10.1.11 Publication and Data Sharing

10.1.11.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

10.1.11.2 Genomic Data Sharing Plan

Not applicable.

10.1.11.3 Publication

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires all investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting, a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan, will be posted on ClinicalTrials.gov.

For this clinical trial, the responsible party is NTI which will register this trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

10.1.12 Conflict of Interest Policy

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

10.2 Additional Considerations

10.2.1 Research Related Injuries

Immediate necessary medical care is available at Duke University Medical Center in the event that a subject is injured as a result of participation in this research study. However, there is no commitment by Duke University, Duke University Health System, Inc., or Duke physicians to provide monetary compensation or free medical care in the event of a study–related injury. In addition, no long term medical care or financial compensation for research-related injuries will be provided by Najít Technologies, Inc. or the Federal government.

10.3 Abbreviations

Abbreviation	Definition
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BA	Bile acids
BMI	Body mass index
BMM	Blinded Medical Monitor
BP	Blood pressure
CC	Coordinating Center (The Emmes Company, LLC)
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
Cr	Creatinine
eCRF	Electronic case report form
DCF	Data collection form
dL	Deciliter
DMID	Division of Microbiology and Infectious Diseases
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EPR	Electrophrenic respiration
ET	Early termination visit
FDA	Food and Drug Administration
FEV	Forced expiratory volume
FNV	French neurotropic vaccine
FWA	Federalwide assurance
GCP	Good clinical practice
GMT	Geometric mean titer
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HEENT	Head, eyes, ears, nose, and throat
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
HLGT	High level group term
HR	Heart rate
ICD-10	International Statistical Classification of Diseases and Related Health Problems, Revision 10
ICF	Informed consent form
ICH	International Conference on Harmonisation

Abbreviation	Definition
IDES	Internet data entry system
IEC	Independent ethics committee
IM	Intramuscular
IND	Investigational new drug application
IRB	Institutional review board
ISRC	Internal safety review committee
IU	International units
LD_{50}	Lethal dose, 50%
LLC	Limited liability company
LLN	Lower limit of normal
LNI	Log_{10} neutralizing index
LOD	Limit of detection
MAAE	Medically-attended adverse event
mcg	Microgram
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
MOP	Manual of procedures
ng	Nanogram
NIAID	National Institute of Allergy and Infectious Diseases
NIBSC	National Institute for Biological Standards and Control
NIH	National Institutes of Health
NOCMC	New onset chronic medical condition
NTI	Najít technologies, Inc.
OER	Office of Extramural Research
OHRP	Office of Human Research Protection
OHSU	Oregon health & science university
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PFU	Plaque forming unit
PI	Principal investigator
PLT	Platelets
PP	Per protocol
$PRNT_{50}$	Plaque reducing neutralization test 50% reduction
QA	Quality assurance
QC	Quality control
RBC	Red blood cell
RM	Rhesus macaque
RT-PCR	Reverse transcriptase PCR
SAE	Serious adverse event

Abbreviation	Definition
SMC	Safety monitoring committee
SoA	Schedule of assessments
SOP	Standard operation procedure
SUSAR	Suspected unexpected serious adverse reactions
TBIL	Total bilirubin
TCID50	Tissue culture infectious dose, 50%
ULN	Upper limit of normal
UMM	Unblinded Medical Monitor
UP	Unanticipated problems
USP	United States Pharmacopeia
WBC	White blood cell
WFI	Water for injection
WHO	World Health Organization
WNV	West Nile virus
YEL-AND	Yellow fever associated neurotropic disease
YEL-AVD	Yellow fever vaccine-associated viscerotropic disease
YF	Yellow fever
YFV	Yellow fever virus

10.4 Protocol Amendment History

Table 10. Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
2.0	05Apr2021	• Addition of electrolytes (sodium, potassium, bicarbonate and calcium) to list of screening and safety evaluations. • Additions to toxicity table for grading of laboratory adverse events for electrolytes (sodium, potassium, bicarbonate and calcium). • Removal of 7 day limitation to the following study halting criterion in Section 7.1.1. ○ Two or more subjects who received at least one dose of study vaccine to date, cumulative to all study vaccine administrations, across all treatment arms, experience the same Grade 3 (unsolicited AE or safety laboratory), in the same HLT by MedDRA coding, considered related to study product. • Revision to state that all medically-attended events (MAAEs) are collected through Study Visit 09. • Minor correction to note that solicited adverse events followed through Day 8 following each dose of study product in Section 8.5.4.	Clinical Protocol changes made in response to CBER requests from March 29, 2021.
3.0	09Apr2021	• Revision to change screening window from "day -45 to -1", relative to 1st vaccination, down to "day -28 to -1". Specific edits were made in Tables 2, 3 and Section 8.1.1. • Primary study endpoints were expanded to encompass all serious adverse events, Grade 3 adverse events and laboratory toxicities, and any adverse event, regardless of assessed relatedness to the vaccine. Specific edits were made in Sections 3 and 9.4.2.	Clinical Protocol changes made in response to non-hold CBER requests from April 8, 2021.
4.0	18Nov2021	• Minor edits throughout to correct typographical errors.	During review of the Data Collection Forms (DCFs) associated with

		• Section 5.1, Inclusion Criteria, Point 10 – Removed BUN (blood urine nitrogen) from the list of screening laboratories to harmonize with the Schedule of Assessments, Table 2. • Section 6.1.2, Dosing and Administration – Clarified that the non-dominant arm will be the injection site for all administrations. • Section 7.1.2, Individual Halting Criteria – Included a preface statement indicating that the site PI must review Individual Halting Criteria for each subject prior to the administration of the second study vaccination. • Section 8.1.1, Screening Procedures – Removed SpO2 from the screening procedure list to harmonize with the Schedule of Assessments, Table 2. • Section 8.1.1, Screening Procedures – Added sodium, potassium, bicarbonate and calcium to the list of enrollment chemistry screens to harmonize with the Schedule of Assessments, Table 2. • Section 8.2.2, Safety Clinical Laboratory Tests – Added sodium, potassium, bicarbonate and calcium to the list of safety chemistry screens to harmonize with the Schedule of Assessments, Table 2. Also clarified procedure for safety laboratory retests. • Section 8.5.1.1, Solicited Adverse Events/Reactogenicity – Updated temperature for instructing subjects to notify the study center from 101.1°F to 101.3°F to harmonize with Table 7, Table of Fever Grading, and updated Fahrenheit temperature from 101.1°F to 101.3°F in Table 7 to match associated Celsius reading of 38.5°C. Also Also updated the grading scale language in Table 4, Table of injection site symptom grading. • Sections 8.5.1.1 and 8.5.4 – Clarified process for following an AE that is still present at EOS visit.	this protocol several inconsistencies were observed between the Schedule of Assessments (SoA) shown in Table 2, and the narrative description of screening and safety procedures in Section 8. The indicated subsections in Section 8 were updated to harmonize with the SoA Table. In addition, inconsistencies between Celsius and Fahrenheit values were discovered in Table 7. Fahrenheit values were updated to match their Celsius counterparts. Further review by Emmes led to additional safety monitoring suggestions as indicates.

		• 8.5.6.2, Regulatory Reporting of AEs – Provided clarifying language. • Table 9, Key Roles – Updated the Medical Monitor to Blinded Medical Monitor (BMM) and Medical Officer to Unblinded Medical Monitor (UMM). Clarified the specific roles for each throughout the protocol. • 10.1.6.1, Safety Monitoring Committee – Updated language on halting rule notification process.	
5.0	27May2022	• Section 1.1.5, Inclusion Criteria, Point 10 – Updated urine screening to specify that trace protein is not exclusionary at screening. • Section 5.1, Inclusion Criteria, Point 10 – Updated urine screening to specify that trace protein is not exclusionary at screening. • Appendix A, Table 14. Urine dipstick toxicity table. Updated table to indicate that trace urine protein is not exclusionary at screening	Trace protein on urine screening may be commonly found and not be indicative of any underlying pathology. Exclusion modified to exclude only those with 1+ or greater amounts of protein on urine screening.
6.0	12Jan2023	• Immunogenicity analysis of the day 180 serum sample (Visit 09) was converted to exploratory analysis. Changes were made in Sections 1.2, 3.0, 9.4.3, 9.4.4 and 9.4.6.	This change streamlined the analysis and reporting timeline for the clinical study without impacting any planned safety assessments.

11. REFERENCES

1. Monath, T. P., Review of the risks and benefits of yellow fever vaccination including some new analyses. Expert Rev Vaccines 2012, 11 (4), 427-48.
2. Russell, M. N.; Cetron, M. S.; Eidex, R. B., The US-Certified Yellow Fever Vaccination Center Registry: a tool for travelers, state health departments, and vaccine providers. J Travel Med 2006, 13 (1), 48-9.
3. Monath, T. P.; Vasconcelos, P. F., Yellow fever. J Clin Virol 2015, 64, 160-73.
4. Camara, F. P.; de Carvalho, L. M.; Gomes, A. L., Demographic profile of sylvatic yellow fever in Brazil from 1973 to 2008. Trans R Soc Trop Med Hyg 2013, 107 (5), 324-7.
5. Mason, R. A.; Tauraso, N. M.; Spertzel, R. O.; Ginn, R. K., Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. Appl Microbiol 1973, 25 (4), 538-44.
6. Amanna, I. J.; Messaoudi, I.; Slifka, M. K., Protective immunity following vaccination: how is it defined? Hum Vaccin 2008, 4 (4), 316-9.
7. Lang, J.; Zuckerman, J.; Clarke, P.; Barrett, P.; Kirkpatrick, C.; Blondeau, C., Comparison of the immunogenicity and safety of two 17D yellow fever vaccines. Am J Trop Med Hyg 1999, 60 (6), 1045-50.
8. Monath, T. P.; Fowler, E.; Johnson, C. T.; Balser, J.; Morin, M. J.; Sisti, M.; Trent, D. W., An inactivated cell-culture vaccine against yellow fever. The New England journal of medicine 2011, 364 (14), 1326-33.
9. Monath, T. P.; Nichols, R.; Archambault, W. T.; Moore, L.; Marchesani, R.; Tian, J.; Shope, R. E.; Thomas, N.; Schrader, R.; Furby, D.; Bedford, P., Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YF-VAX) in a phase III multicenter, double-blind clinical trial. Am J Trop Med Hyg 2002, 66 (5), 533-41.
10. Camacho, L. A.; Freire Mda, S.; Leal Mda, L.; Aguiar, S. G.; Nascimento, J. P.; Iguchi, T.; Lozana Jde, A.; Farias, R. H., Immunogenicity of WHO-17D and Brazilian 17DD yellow fever vaccines: a randomized trial. Rev Saude Publica 2004, 38 (5), 671-8.
11. Nascimento Silva, J. R.; Camacho, L. A.; Siqueira, M. M.; Freire Mde, S.; Castro, Y. P.; Maia Mde, L.; Yamamura, A. M.; Martins, R. M.; Leal Mde, L., Mutual interference on the immune response to yellow fever vaccine and a combined vaccine against measles, mumps and rubella. Vaccine 2011, 29 (37), 6327-34.
12. Engel, A. R.; Vasconcelos, P. F.; McArthur, M. A.; Barrett, A. D., Characterization of a viscerotropic yellow fever vaccine variant from a patient in Brazil. Vaccine 2006, 24 (15), 2803-9.
13. Sanofi Pasteur Inc., YF-VAX® Package Insert. Swiftwater, PA, 2019.
14. Staples, J. E.; Gershman, M.; Fischer, M.; Centers for Disease, C.; Prevention, Yellow fever vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2010, 59 (RR-7), 1-27.
15. Lindsey, N. P.; Schroeder, B. A.; Miller, E. R.; Braun, M. M.; Hinckley, A. F.; Marano, N.; Slade, B. A.; Barnett, E. D.; Brunette, G. W.; Horan, K.; Staples, J. E.; Kozarsky, P. E.; Hayes, E. B., Adverse event reports following yellow fever vaccination. Vaccine 2008, 26 (48), 6077-82.
16. Centers for Disease Control and Prevention, Fatal viral encephalitis following 17D yellow fever vaccine inoculation. Report of a case in a 3-year-old child. Jama 1966, 198 (6), 671-2.
17. Guimard, T.; Minjolle, S.; Polard, E.; Fily, F.; Zeller, H.; Michelet, C.; Tattevin, P., Short report: Incidence of yellow fever vaccine-associated neurotropic disease. Am J Trop Med Hyg 2009, 81 (6), 1141-3.
18. Fernandes, G. C.; Camacho, L. A.; Sa Carvalho, M.; Batista, M.; de Almeida, S. M., Neurological adverse events temporally associated to mass vaccination against yellow fever in Juiz de Fora, Brazil, 1999-2005. Vaccine 2007, 25 (16), 3124-8.
19. Martin, M.; Tsai, T. F.; Cropp, B.; Chang, G. J.; Holmes, D. A.; Tseng, J.; Shieh, W.; Zaki, S. R.; Al-Sanouri, I.; Cutrona, A. F.; Ray, G.; Weld, L. H.; Cetron, M. S., Fever and multisystem organ failure associated with 17D-204 yellow fever vaccination: a report of four cases. Lancet 2001, 358 (9276), 98-104.

20. Khromava, A. Y.; Eidex, R. B.; Weld, L. H.; Kohl, K. S.; Bradshaw, R. D.; Chen, R. T.; Cetron, M. S., Yellow fever vaccine: an updated assessment of advanced age as a risk factor for serious adverse events. Vaccine 2005, 23 (25), 3256-63.
21. Lawrence, G. L.; Burgess, M. A.; Kass, R. B., Age-related risk of adverse events following yellow fever vaccination in Australia. Commun Dis Intell 2004, 28 (2), 244-8.
22. Monath, T. P.; Cetron, M. S.; McCarthy, K.; Nichols, R.; Archambault, W. T.; Weld, L.; Bedford, P., Yellow fever 17D vaccine safety and immunogenicity in the elderly. Hum Vaccin 2005, 1 (5), 207-14.
23. Barrett, A. D.; Monath, T. P.; Barban, V.; Niedrig, M.; Teuwen, D. E., 17D yellow fever vaccines: new insights. A report of a workshop held during the World Congress on medicine and health in the tropics, Marseille, France, Monday 12 September 2005. Vaccine 2007, 25 (15), 2758-65.
24. Tesh, R. B.; Guzman, H.; da Rosa, A. P.; Vasconcelos, P. F.; Dias, L. B.; Bunnell, J. E.; Zhang, H.; Xiao, S. Y., Experimental yellow fever virus infection in the Golden Hamster (Mesocricetus auratus). I. Virologic, biochemical, and immunologic studies. J Infect Dis 2001, 183 (10), 1431-6.
25. Theiler, M., Studies on the action of yellow fever in mice. Ann Trop Med Parasitol 1930, 24, 249-272.
26. Thibodeaux, B. A.; Garbino, N. C.; Liss, N. M.; Piper, J.; Blair, C. D.; Roehrig, J. T., A small animal peripheral challenge model of yellow fever using interferon-receptor deficient mice and the 17D-204 vaccine strain. Vaccine 2012, 30 (21), 3180-7.
27. Zisman, B.; Wheelock, E. F.; Allison, A. C., Role of macrophages and antibody in resistance of mice against yellow fever virus. J Immunol 1971, 107 (1), 236-43.
28. Reed, L. J.; Muench, H., A simple method of estimating fifty percent endpoints. Am J Hyg 1938, 27 (3), 493-497.
29. Monath, T. P.; Brinker, K. R.; Chandler, F. W.; Kemp, G. E.; Cropp, C. B., Pathophysiologic correlations in a rhesus monkey model of yellow fever with special observations on the acute necrosis of B cell areas of lymphoid tissues. Am J Trop Med Hyg 1981, 30 (2), 431-43.
30. Hammarlund, E.; Amanna, I. J.; Dubois, M. E.; Barron, A.; Engelmann, F.; Messaoudi, I.; Slifka, M. K., A flow cytometry-based assay for quantifying non-plaque forming strains of yellow Fever virus. PLoS One 2012, 7 (9), e41707.
31. Offit, P. A.; Jew, R. K., Addressing parents' concerns: do vaccines contain harmful preservatives, adjuvants, additives, or residuals? Pediatrics 2003, 112 (6 Pt 1), 1394-7.
32. Baylor, N. W.; Egan, W.; Richman, P., Aluminum salts in vaccines--US perspective. Vaccine 2002, 20 Suppl 3, S18-23.
33. Lindblad, E. B., Aluminium compounds for use in vaccines. Immunology and cell biology 2004, 82 (5), 497-505.
34. Saul, A., Models of Phase 1 vaccine trials: optimization of trial design to minimize risks of multiple serious adverse events. Vaccine 2005, 23 (23), 3068-75.
35. Dubischar-Kastner, K.; Eder, S.; Buerger, V.; Gartner-Woelfl, G.; Kaltenboeck, A.; Schuller, E.; Tauber, E.; Klade, C., Long-term immunity and immune response to a booster dose following vaccination with the inactivated Japanese encephalitis vaccine IXIARO, IC51. Vaccine 2010, 28 (32), 5197-202.
36. Lyons, A.; Kanesa-thasan, N.; Kuschner, R. A.; Eckels, K. H.; Putnak, R.; Sun, W.; Burge, R.; Towle, A. C.; Wilson, P.; Tauber, E.; Vaughn, D. W., A Phase 2 study of a purified, inactivated virus vaccine to prevent Japanese encephalitis. Vaccine 2007, 25 (17), 3445-53.
37. Woods, C. W.; Sanchez, A. M.; Swamy, G. K.; McClain, M. T.; Harrington, L.; Freeman, D.; Poore, E. A.; Slifka, D. K.; Poer DeRaad, D. E.; Amanna, I. J.; Slifka, M. K.; Cai, S.; Shahamatdar, V.; Wierzbicki, M. R.; Amegashie, C.; Walter, E. B., An observer blinded, randomized, placebo-controlled, phase I dose escalation trial to evaluate the safety and immunogenicity of an inactivated West Nile virus Vaccine, HydroVax-001, in healthy adults. Vaccine 2019, 37 (30), 4222-4230.
38. Amanna, I. J.; Slifka, M. K., Questions regarding the safety and duration of immunity following live yellow fever vaccination. Expert Rev Vaccines 2016, 15 (12), 1519-1533.

APPENDIX A

Toxicity Tables

ABBREVIATIONS: Abbreviations utilized in the Table:
ULN = Upper Limit of Normal
LLN = Lower Limit of Normal
NOTE: For any of the lab values listed below if the Grade 1 value conflicts with the ULN or LLN value, the ULN or LLN value takes precedence and the event will not be considered a Grade 1 Adverse Event.

1. Laboratory Values

Hematology

Table 11. Hematology toxicity table

Parameter	Unit of measure	Abnormality	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC	cell/mm^3	High	> 9,800 and ≤ 15,000	> 15,000 and ≤ 20,000	> 20,000
		Low	≥ 2,500 and < 3,200	≥ 1,500 and < 2,500	< 1,500
HgB (female)	g/dL	Low	≥ 11.0 and < 12.0	≥ 9.5 and < 11.0	< 9.5
HgB (male)	g/dL	Low	≥ 12.0 and < 13.7	≥ 10.0 and < 12.0	< 10.0
Platelets	cell/mm^3	Low	≥ 120,000 and < 150,000	≥ 100,000 and < 120,000	< 100,000

Blood Chemistries

Table 12. Blood chemistries toxicity table

Parameter	Unit of Measure	Abnormality	Grade 1	Grade 2	Grade 3
Sodium	mmol/L	High	146 to < 150	150 to < 154	≥ 154
		Low	130 to < 135	125 to < 130	< 125
Potassium	mmol/L	High	5.1 to < 6.0	6.0 to < 6.5	≥ 6.5
		Low	3.0 to < 3.5	2.5 to < 3.0	< 2.5
Bicarbonate	mmol/L	Low	16 to < 21	11 to < 16	<11
Calcium	mg/dL	High	10.3 to < 11.5	11.5 to < 12.5	≥ 12.5
		Low	7.8 to < 8.7	7.0 to < 7.8	< 7.0
Random glucose	mg/dL	High	> 140 and ≤ 160	> 160 and ≤ 200	> 200
		Low	≥ 60 and < 70	≥ 55 and < 60	< 55
Bilirubin	n/a	High	> 1.5 and ≤ 2.0 x ULN	> 2.0 and ≤ 2.5 x ULN	> 2.5 x ULN
Creatinine[1] (female)	mg/dL	High	> 1.0 and < 1.8	≥ 1.8 and < 2.0	≥ 2.0

Parameter	Unit of Measure	Abnormality	Grade 1	Grade 2	Grade 3
Creatinine[1] (male)	mg/dL	High	> 1.3 and < 1.8	≥ 1.8 and < 2.0	≥ 2.0

[1]Creatinine below the LLN is not considered an abnormality.

Liver Enzymes

Table 13. Liver enzymes toxicity table

Parameter	Unit of Measure	Abnormality	Grade 1	Grade 2	Grade 3
AST	n/a	High	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN
ALT	n/a	High	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN

Urine dipstick

Table 14. Urine dipstick toxicity table

Parameter	Unit of Measure	Abnormality	Grade 1	Grade 2	Grade 3
Protein[1]	n/a	High	1+	2+	> 2+
Glucose	n/a	High	1+	2+	> 2+
Blood[2]	n/a	High	1+	2+	>2+
Leukocyte esterase[2]	n/a	High	1+	2+	>2+

[1]Trace protein not considered exclusionary at screening.

[2]Not included as screening labs, only as safety labs.

2. Clinical Adverse Events

Cardiovascular

Table 15. Cardiovascular toxicity table

Parameter	Unit of Measure	Abnormality	Grade 1	Grade 2	Grade 3
Systolic blood pressure	mm Hg	Hypertension	141-150	151-160	> 160
Diastolic blood pressure	mm Hg	Hypertension	91-95	96-100	>100
Heart rate	Beats per minute	Tachycardia	101-115	116-130	> 130
		Bradycardia	35-46	30-34	< 30

Respiratory

Table 16. Respiratory toxicity table

Parameter	Grade 1	Grade 2	Grade 3
Cough	transient- no treatment	persistent cough; treatment responsive	Severe cough (interferes with daily activities)
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV1	requires treatment; normalizes with bronchodilator; FEV1 60% - 70%	no normalization with bronchodilator; FEV1 < 60%
Dyspnea	Does not interfere with usual and social activities	Interferes with usual and social activities; no treatment	Prevents daily and usual social activity or requires treatment