

A Phase 1, Double-blinded, Placebo Controlled, Clinical Trial to Evaluate the Safety, Reactogenicity, and Immunogenicity of HEV-239 (Hecolin®) in a Healthy US Adult Population

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The study trial will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 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), 21 CFR 812 (Investigational Device Exemptions)
- International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use E6(R2) Good Clinical Practice (ICH E6 GCP): Integrated Addendum to ICH E6(R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 (2018)), including the latest finalized revision.
- 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
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

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

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

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LIST OF ABBREVIATIONS

aa	Amino acids
AE	Adverse Event/Adverse Experience
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ASCs	Antibody secreting cells
β-hCG	β-human chorionic gonadotropin
BLA	Biologics License Applications
BMI	Body mass index
BP	Blood pressure
CFR	Code of Federal Regulations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CMS	Clinical Material Services
CONSORT	Consolidated Standards of Reporting Trials
Cr	Creatinine
CRO	Contract Research Organization
CSR	Clinical Study Report
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board

DTaP	Diphtheria, tetanus, acellular pertussis vaccine
eCRF	Electronic Case Report Form
ELISA	Enzyme Linked Immunosorbent Assay
e-CRFs	Electronic-Case report forms
e-Memory	Electronic memory aid
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GMCs	Geometric Mean Concentrations
GT	Genotype
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HEV	Hepatitis E virus
HEV-239	Hepatitis E virus vaccine containing a 239 amino acid subfragment of (Hecolin®)
HgB	Hemoglobin
Hib	<i>Haemophilus influenzae</i> type B
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HR	Heart rate
IB	Investigator's Brochure
ICF	Informed Consent Form

ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
Ig	Immunoglobulin
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
kDA	KiloDaltons
mITT	Modified intent-to-treat
MBCs	Memory B cells
MedDRA [®]	Medical Dictionary for Regulatory Activities
µg	Micrograms
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research

ORF	Open reading frames
OTC	Over the counter
P	Protruding (domain) of hepatitis E virus
PBMCs	Peripheral Blood Mononuclear Cells
PE	Physical examination
PFS	Pre-filled syringe
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per protocol
QA	Quality Assurance
QC	Quality Control
RBC	Red blood cell
RNA	Ribonucleic acid
RCD	Reverse cumulative distribution
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States
VLP	Virus Like Particle
VTEU	Vaccine Treatment and Evaluation Unit
WBC	White blood cell count
WFI	Water for Injection

WHO

World Health Organization

PROTOCOL SUMMARY

Title:	A Phase 1, Double-blinded, Placebo Controlled, Clinical Trial to Evaluate the Safety, Reactogenicity, and Immunogenicity of HEV-239 (Hecolin®) in a Healthy US Adult Population
Design of the Study:	This is a Phase I double-blind, placebo controlled trial (4:1 ratio of vaccine to placebo) of HEV-239 in 25 US males and non-pregnant females to assess the safety, reactogenicity, and immunogenicity of HEV-239. Subjects will receive 3 doses of study product on Days 1, 29, and 180, by intramuscular injection (IM)
Study Phase:	1
Study Population:	25 US healthy male or non-pregnant female adults (ages 18 – 45 years inclusive), who meet all eligibility criteria.
Number of Sites:	1
Description of Study Product or Intervention:	This vaccine is a 239 amino acid sub-fragment of the 660 amino acid structural protein of HEV (genotype 1) produced in <i>E. coli</i> . The vaccine consists of 30 micrograms of the purified 239 amino acid protein adsorbed to 0.8 mg aluminum hydroxide, suspended in 0.5 mL buffered saline, and contains thimerosal.
Study Objectives:	Primary: <ul style="list-style-type: none">Assess the safety and reactogenicity of HEV-239 following delivery of each vaccine dose.

-
- Assess the number of subjects with ≥ 4 fold rise in HEV IgG at any time after vaccination.

Secondary:

- Assess the number of subjects with HEV IgM seroconversion at any time after vaccination.
- Assess the number of subjects with HEV IgG seroconversion at any time after vaccination.
- Assess the HEV IgG geometric mean concentrations (GMCs) at any time after vaccination.

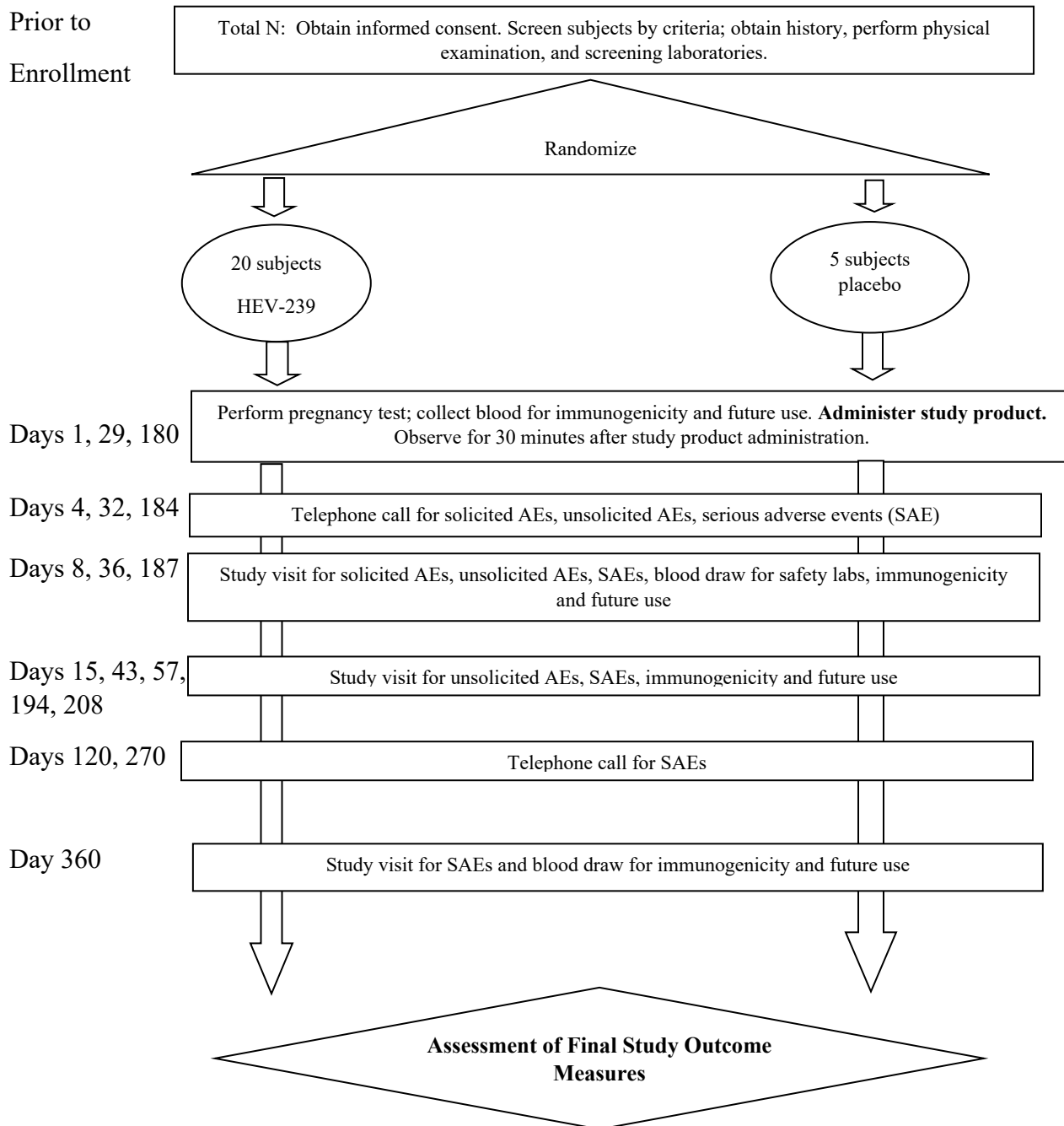
**Duration of Individual
Subject Participation:**

Up to 13 months.

**Estimated Time to Last
Subject/Last Study Day:**

Approximately 15 months.

Figure 1: Schematic of Study Design



1 KEY ROLES

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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis worldwide.¹ Clinical manifestations range from asymptomatic infection to fulminant hepatitis, acute hepatitis, hepatic failure, and death. Although typically self-limited, HEV has been reported to cause chronic hepatitis with progression to liver cirrhosis and hepatocellular carcinoma in immunocompromised hosts.² HEV has an incubation period of 2 – 8 weeks (mean of 40 days).³ The symptoms of acute HEV infection are similar to those of hepatitis A and include fever, vomiting, myalgias, and malaise, followed by progression in some to jaundice, acholic stools, darkened urine, and elevated liver transaminases lasting 1 to 6 weeks.³ Extra-hepatic manifestations are uncommon, but may include Guillain-Barre syndrome, neuralgic amyotrophy, meningoencephalitis, pancreatitis, and glomerulonephritis.^{4,5} Adults are more likely to develop symptomatic disease than children, and pregnant women are most severely affected with mortality rates of 10 – 30% in the third trimester. Mother-to-infant transmission may also occur, resulting in fetal loss and increased perinatal morbidity and mortality.^{6,7} Unfortunately, supportive care is the only medical therapy available for hepatitis E.

Globally it is estimated that HEV causes 20 million infections each year, resulting in 3.3 million cases of symptomatic disease and 70,000 deaths.⁸ Although the virus is globally distributed, it is highly endemic in several countries in Asia and Africa, where the seroprevalence of HEV IgG approaches 50%.⁹ HEV is mainly transmitted via the fecal-oral route due to contaminated water although vertical and iatrogenic transmission from infected blood products can occur. HEV is responsible for sporadic cases and large outbreaks in the developing world and disproportionately affects areas with poor hygiene standards and population crowding such as refugee camps.^{10,11} Although less common, HEV also causes sporadic cases in industrialized nations, and the disease burden may be underestimated.¹²

HEV has traditionally been grouped into four different genotypes (GTs 1-4) with a single known serotype. GT 1 is endemic in many tropical countries and exclusively infects humans via the fecal-oral route. Data about GT 2 is limited,¹³ but also supports exclusive infection of humans via the fecal-oral route.¹⁴ GTs 3 and 4 are known zoonoses responsible for sporadic transmission via consumption of raw or undercooked meat. Common animal reservoirs for HEV include swine, deer, rabbits, cows, sheep, and wild boars.¹⁵ GT3 is the most commonly identified genotype in Europe,¹² while GT4 is most commonly identified in Asian industrialized

countries.^{3,16} The implication is that autochthonous transmission of HEV in the developed world is often as a foodborne zoonosis.

An effective HEV vaccine is needed, particularly for the mitigation of outbreaks and for the prevention of maternal, fetal, and infant morbidity and mortality. Infection with HEV results in cross-protective neutralizing antibodies against all four genotypes,¹⁷ which appear to confer long-term protection against symptomatic disease.¹⁸ Thus, a vaccine that is effective in one country is expected to provide protection against hepatitis E elsewhere.

HEV is a small (27-34 nm) non-enveloped, single-stranded, positive-sense RNA virus of the genus *Hepevirus* within the family *Hepeviridae*. Its genome contains 3 partially overlapping open reading frames (ORFs 1-3) flanked by two short non-coding regions. ORF2 encodes the major HEV capsid protein, a 660-amino-acid (aa) protein which is responsible for virion assembly, target cell attachment, and for viral immunogenicity.¹ Structural analysis studies using cryoelectron microscopy and crystallography predicted that the predominant HEV neutralizing epitope resides in the protruding (P) domain (aa 459-607) of ORF2.¹⁹

Vaccine Development:

Two HEV vaccine candidates have been evaluated in clinical trials to date although HEV-239 (Hecolin®) is the only vaccine currently commercially available. The first vaccine candidate was a 56 kDa recombinant HEV protein expressed in insect cells that was highly efficacious in a phase II trial of Nepalese army recruits, but was not further developed by the sponsoring commercial entity.²⁰ The second vaccine HEV-239 is a 26kDa protein expressed in *Escherichia coli* as a non-fusion virus-like particle (VLP). It was licensed in 2012 for use in China and is produced and marketed by Xiamen Innovax Biotech Co. Ltd. HEV-239 encompasses the neutralizing epitope within the (P) domain (aa 368-606; 239 amino acids in length). The purified protein is adsorbed to aluminum hydroxide in buffered saline and then packaged into a pre-filled syringe for IM use.

Preclinical testing of HEV-239 was performed in Balb/c mice, Kunming (KM) mice, Sprague Dawley rats, and rhesus monkeys, which demonstrated safety and immunogenicity eliciting both HEV-specific antibodies and T-cell mediated responses.^{21,22} HEV-239 provided 100% protection against hepatitis E virus challenge (both homologous and heterologous) in naïve rhesus monkeys.²²

An initial Phase I study was conducted in an unpublished study of 44 healthy individuals, 16 – 55 years of age.²¹ Immunogenicity data are not available in the IB for this study, safety data are outlined in [Section 2.3.1](#) below.²¹

In a randomized, placebo-controlled phase II clinical trial in healthy seronegative adults, HEV-239 was tested in 16 – 55 year old adults in China.²³ In this study, several different administration schedules and doses were tested against placebo (hepatitis B vaccine). Group A received a 20µg dose in a 0/1/6 month schedule; Group B received a 20µg dose in a 0/6 month schedule; Group C received placebo (hepatitis B vaccine) in a 0/1/6 month schedule; and Groups E – H received an escalating dose (10 – 40 µg doses) on a 0/1/6 month schedule. In this study, rates of Grade 3 local or systemic reactions were similar (see [Section 2.3.1](#) for additional details) between HEV-239 and placebo recipients. All subjects receiving 3 doses of HEV-239 seroconverted after the 3rd dose of vaccine. Group A serum HEV IgG Geometric Mean Concentrations (GMCs) were 5.95 U/mL one month after the second dose. These were higher than the HEV serum IgG GMCs of 0.8 U/mL observed in Group B one month after the first dose of the vaccine. Group A titers also remained higher than Group B at 6 months after enrollment (3.12 U/mL versus 0.5 U/mL).²³ Both groups boosted to similar levels after the final dose was administered at 6 months. The GMC observed in the lowest dose (10 µg dose) was lower than that observed for the other doses (20 – 40 µg doses). Subjects in Groups A and B were followed with serology and an efficacy of 85.2 – 88.7% was observed in prevention of HEV in comparison to placebo recipients through 13 months. The 30µg dose was chosen for the subsequent Phase III clinical trial of HEV-239.

This study was followed by a landmark Phase III clinical trial of HEV-239 in healthy Chinese adults, which formed the foundation for vaccine approval and licensure in China. The study was a randomized, double-blinded, placebo-controlled (1:1 ratio) trial which evaluated the safety, immunogenicity, and efficacy of HEV-239 in subjects from 11 townships in the Jiangsu Province of China from August 2007 to June 2009.²⁴ Healthy adults ages 16-65 years were randomized to receive three doses of either HEV-239 (30 µg of purified recombinant hepatitis E antigen adsorbed to 0.8 g aluminum hydroxide suspended in 0.5 mL buffered saline) or placebo (a licensed hepatitis B vaccine) administered intramuscularly at 0, 1, and 6 months. The primary endpoint was prevention of hepatitis E during 12 months following the 31st day after the third vaccine dose. Overall 56,302 were randomized in both the vaccine and placebo groups to achieve this primary endpoint. Smaller groups were followed for immunogenicity (over 5,500 in both vaccine and placebo groups) and reactogenicity (over 1,300 in both vaccine and placebo groups). Overall, vaccine was well tolerated with similar systemic AEs but slightly higher rates of swelling and pain in HEV-239 recipients in comparison to placebo (See [Section 2.3.1](#) for additional details). SAEs and deaths were similar between the groups. Overall, 100% protection

against pre-specified hepatitis E infection was observed after administration of either 2 or 3 doses of vaccine.²⁴ In those that received at least one dose of HEV-239, efficacy was 93.8% (59.8 – 99.9%).

A follow-up study was subsequently conducted through 4.5 years for evidence of hepatitis E infection.²⁵ In this study, 7 cases of hepatitis E were identified in vaccine recipients versus 53 in placebo recipients (vaccine efficacy 86.8%, 95% CI of 71 – 94%).²⁵ In those enrolled in the immunogenicity subset, among those that were seronegative prior to vaccination, 87% of those who received 3 doses of HEV-239 maintained antibodies against hepatitis E through 4.5 years.

2.2 Scientific Rationale

2.2.1 Purpose of Study

A vaccine against HEV that has undergone safety and immunogenicity testing outside of China therefore would be very beneficial. Such a vaccine could provide protection for travelers or be used in outbreaks to prevent continued transmission of disease. It also would be highly beneficial in preventing maternal, fetal, and infant deaths. The vaccine, though licensed in China, is neither FDA approved nor WHO prequalified. Although vaccine safety is established in a Chinese population, safety in other population cannot be presumed. Data are needed establishing its safety and immunogenicity in other populations. Such safety and immunogenicity data from this study could enhance efforts to use this important vaccine in other countries around the world. This Phase I study will use the dose (30 µg), dosing schedule (0, 1, and 6 months), and route of administration (IM) that was used in the Phase 3 study in China.

The purpose of this Phase 1 randomized-controlled double-blind study is to assess the safety, reactogenicity, and immunogenicity of HEV-239 in healthy adults. After screening and determining their eligibility, 25 subjects will be randomized (4 vaccine: 1 placebo) to receive vaccine (n = 20) or placebo (n = 5). Vaccine will be administered intramuscularly (IM) in 0.5 mL buffered saline containing 30 µg of purified antigen adsorbed to 0.8 mg aluminum hydroxide. The vaccine is provided as single dose units in prefilled syringes (Innovax Biotech); the placebo subjects will receive normal saline. Each subject will receive 3 injections, either vaccine or placebo at Days 1, 29, and 180. Subjects will be followed through 12 months for safety and durability of the immune responses observed.

2.2.2 Study Population

This study will enroll 25 healthy adults in the US population and will include male or non-pregnant females ages 18-45 (inclusive). They will have no significant medical illness or clinically significant physical exam findings including vitals upon enrollment. They will need to meet all eligibility criteria. The subjects will be recruited from the general community surrounding Emory University in Atlanta that has substantial experience conducting Phase I studies.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential risks of participating in this trial are those associated with having blood drawn, the IM injection and possible reactions to the HEV-239 vaccine with aluminum adjuvant, and breach of confidentiality.

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down and elevate his/her legs. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure to the blood draw site for a few minutes after the blood is taken. IM injection may also cause transient discomfort and fainting. Drawing blood and IM injection may cause infection. The use of aseptic (sterile) technique will make infection at the site where blood will be drawn or where the study vaccination will be given extremely unlikely.

There is a small amount of risk to subjects who report that they are in good health but who may have an unknown health problem at the time of screening. This trial will screen by physical exam, history, and vital signs. The following baseline laboratories will be obtained at screening on all subjects to determined eligibility.

Baseline Laboratories:

- Hematology
 - Complete blood count with differential (which will include white blood cells, the absolute neutrophil count (ANC), the absolute eosinophil count (AEC), hemoglobin, and platelets).
 - A WBC evaluation may include any abnormal cells, which will then be reported by the laboratory. A hematology evaluation may include abnormalities in the red blood cell (RBC) count and/or RBC parameters

and/or RBC morphology, which will then be reported by the laboratory. In addition, any other abnormal cells in a blood smear will also be reported.

- Chemistry (fasting or non-fasting)
 - Creatinine
 - Glucose
 - Alanine aminotransferase (ALT)
- Serology
 - Hepatitis B surface antigen
 - Hepatitis C antibody
 - HIV types 1 and 2 antigen/antibody
 - Hepatitis E virus IgM and IgG antibodies
- Serum pregnancy test (in women of childbearing potential)
- Urine
 - A urine drug screen will be obtained

Safety laboratories will be obtained on Day 8 after each vaccine dose and also before the Day 180 vaccination. To minimize risks, subjects will not receive the subsequent study vaccinations if a related Grade 3 laboratory abnormality occurs. The following safety laboratories will be obtained on all subjects to determined safety.

Safety Laboratories:

- Hematology
 - Complete blood count with differential (which will include white blood cells, ANC, AEC, hemoglobin, and platelets).
 - A WBC evaluation may include any abnormal cells, which will then be reported by the laboratory. A hematology evaluation may include abnormalities in the red blood cell (RBC) count and/or RBC parameters and/or RBC morphology, which will then be reported by the laboratory. In addition, any other abnormal cells in a blood smear will also be reported. Only clinically significant abnormal WBC, abnormal RBC, or any other abnormal cells in a blood smear will be reported as AEs. Relatedness and clinical significance for abnormal results will be entered on the Local Laboratory Results eCRF.
- Chemistry (fasting or non-fasting)
 - Creatinine
 - Alanine aminotransferase (ALT)

Expected risks include pain, soreness, redness, warmth, swelling, firmness and itching at the site of injection. Other reactions may include fever, chills, rash, myalgias, nausea, headache, fatigue, malaise and arthralgias. As with other vaccines, an allergic reaction including anaphylaxis is possible. There may be other side effects from the study vaccinations, even serious ones that are not yet known.

Pre-Clinical and Phase I-III Data:

Pre-clinical toxicology studies included acute single dose toxicity in mice, repetitive injection toxicity and immunogenicity in rats, teratogenic and developmental toxicity studies in mice and rats, a local tolerability study in rabbits, and a systemic anaphylaxis study in guinea pigs with HEV-239.²¹ Administration of HEV 239 protein at multiples of clinically relevant doses in a relevant clinical formulation was well tolerated in mice, rats, and rabbits. No significant adverse effects were identified in single dose and short-term repeat dose studies. In a developmental study in the mouse there was a possible dose-related trend towards higher numbers of fetal resorptions and abnormal ossification was observed at the highest doses. However, in a second study in rats at a higher dose, no adverse effects of treatment were identified.²¹ No unexpected local tolerance issues were identified in any of the preclinical studies.

Undesirable effects were assessed in a Phase I study in 44 subjects, Phase II (dose-ranging and dose scheduling) studies in 505 subjects and a double-blinded, placebo-controlled Phase 3 study in which 56,302 subjects were assigned to receive recombinant hepatitis E test vaccine by the intramuscular route at 0, 1 and 6 months.

- An initial Phase I study was conducted in an unpublished study of 44 healthy individuals 16 – 55 years of age.²¹ Two 20 µg doses (0 and 1 month) were administered IM in an open label study in 2005. No SAEs were observed and local reactions of swelling, itching, and/or pain were observed in 3/44 subjects after the first dose and 3/42 subjects after the second dose. These symptoms resolved within 48 – 72 hours after inoculation except for one subject who had swelling and pain on Day 7. Three subjects had a systemic increase in body temperatures. A single subject had a temperature rise to 38.5°C at 72 hours after the second vaccine dose. No subjects had clinically significant changes in kidney or liver function 30 days after the second dose of vaccine.
- In the randomized placebo-controlled Phase II clinical trial in healthy seronegative adults, Grade 3 local reactions were observed only in Group A (20 µg dose x 3 doses) and H (40 µg dose x 3 doses) subjects, (rates of 1.6% and 1.7% respectively per dose). Grade 3 systemic reactions were observed only in Group H (1.7% per dose).²³ The rates of Grade 3 local or

systemic reactions were similar in vaccine groups A, B, E – H and those receiving placebo (hepatitis B vaccine). The rates of local AEs were similar in vaccine groups, but higher rates of itching (6.0% versus 1.6%), swelling (4.3% versus 0.7%), and any local reactions (8.5% versus 2.0%) were observed with vaccine group A versus placebo. Pain was also slightly more common in all HEV-239 recipients than in placebo (2.4% versus 0.2%). The rates of total systemic AEs were also similar between vaccine groups and the placebo group. Rates of AEs were the same with different vaccine doses (range 10 – 40 µg).

- Phase III safety data was obtained from 56,302 subjects who received HEV-239 30 µg dose at 0, 1, and 6 months and a similar placebo group (hepatitis B vaccine)^{21,24} who were followed up to 4.5 years.²⁵ The population was 16-65 years of age predominantly female (56.5%) and Asian. Most of the adverse events were Grade 1 adverse events that generally resolved spontaneously without special treatment. Overall, vaccine was well tolerated with similar systemic AEs (20.3% versus 19.8%) to placebo. The most common systemic events included fever (18.6% vs 18.0%), fatigue and asthenia (2.1% versus 1.5%), and headache (1.1% versus 0.6%) in vaccine versus placebo. Higher rates of local adverse events were observed with vaccine versus placebo (13.5% versus 7.1%) although ≥Grade 3 events were similar. These local reactions were primarily increased pain (10.3 versus 5.5%) and swelling (2.3% versus 0.6%) in comparison to placebo. Unsolicited AEs (12% versus 11.9%), SAEs within 30 days of any dose (0.4% versus 0.4%), SAEs after the 30 day window periods (2.5% versus 2.5%), and deaths (0.2% versus 0.2%, none were considered related) were similar between vaccine and placebo recipients respectively. SAEs that were reported in the Phase 3 study were considered unrelated to study vaccine by the Data Safety Monitoring Board (DSMB).
- Retrospective data about women that became pregnant during the Phase III vaccine trial have been published.^{21,26} 37 pregnant women in the vaccine group received 53 doses of vaccine versus 31 pregnant women in the placebo group receiving 46 doses of placebo. The rates of adverse reactions were similar between the two groups and no SAEs reported. Importantly, fetal outcomes (rates of prematurity, body length, and weight) were similar in HEV-239 and placebo recipients suggesting the vaccine is safe for both the mother and fetus.
- Data have also been published regarding the safety of HEV-239 vaccination in hepatitis B surface antigen carriers.^{21,27} Local and systemic AEs overall and those ≥Grade 3 were similar in vaccine recipients and those that received placebo.

Post-Licensure Data:

After the vaccine was launched in China in October 2012,²⁸ Inovax implemented a series of post-marketing clinical studies but data are limited. A post-marketing surveillance database supports the safety of the vaccine based upon an estimated >279,000 administrations of HEV-

239 to nearly 93,000 people.²¹ Post-licensure safety data are limited but include a systemic rash that occurred 2 days after vaccination in a 28-year-old man.²¹ This resolved within 3 weeks of onset.

Summary Data:

Clinical trials of HEV-239 were conducted in China and included both hepatitis E immune and susceptible individuals. A vaccine dose of 30 µg absorbed to aluminum hydroxide was selected for the Phase III study. Increased local reactions (primarily pain and swelling) have been observed with HEV-239 in comparison to placebo (a hepatitis B vaccine). Systemic AEs, unsolicited AEs, SAEs, and deaths have been similar between HEV-239 and placebo recipients.

Extensive experience exists with the adjuvant used in this study (aluminum hydroxide). Aluminum is currently used in hepatitis A, hepatitis B, diphtheria-tetanus-pertussis (DTaP, Tdap), Haemophilus influenzae type b (Hib), human papillomavirus (HPV) and pneumococcal vaccines. Aluminum hydroxide was used as the adjuvant in the Phase I-III studies of HEV-239 and so the safety profile is anticipated to be incorporated into the overall vaccine safety profile. HEV-239 also contains small amounts of thimerosal. Although concern has been raised about thimerosal and risk of autism in children, multiple studies have demonstrated no increase in risk.²⁹⁻³¹ Thimerosal was also used in the Phase I-III studies of HEV-239, so the safety profile is also anticipated to be incorporated into the overall vaccine safety profile.

Although limited data are available from the Phase III study,²⁶ it is not really known whether this vaccine poses risks to an unborn child. Therefore, women of childbearing potential must agree to use an effective method of birth control for at least 28 days prior to first study vaccination and for at least 3 months following the third vaccination.

Subjects will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subject's PHI. All study records will be kept in a locked file cabinet or maintained in a locked room at the participating VTEU sites. Electronic files will be password-protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this trial will be allowed access to the PHI that is collected. Any publications from this trial will not include information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating VTEU sites for quality assurance and data analysis include groups such as the local Institutional Review Board (IRB), NIAID, and the FDA.

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by US Law. This web site will not include information that can identify subjects.

There may be other risks, discomforts, or side effects that are unknown at this time.

2.3.2 Potential Benefits

It is unknown whether there are direct benefits attributable to the receipt of the HEV-239 vaccine. Vaccination using the HEV-239 vaccine may or may not provide protection against a serious disease with the hepatitis E virus, should the participant be exposed. Exposure is uncommon in the US, but occurs particularly with travel outside the US to outbreak or endemic areas. The duration of any such protection against hepatitis E is currently unknown although it is thought to be at least 5 years after receipt of the vaccine.²⁵ There may be preparedness benefits to society if HEV-239 evaluated in this clinical trial proves to be sufficiently safe and immunogenic that it could be employed if an outbreak of hepatitis E virus occurred or as immunoprophylaxis for travelers.

In addition to endemic areas, HEV infection is also a real threat to the displaced populations. Outbreaks are common in refugee camps (ref. <http://www.who.int/mediacentre/factsheets/fs280/en/>). Medical providers, military personnel, and peacekeeping force personnel dispatched could also benefit from the protection afforded by a HEV vaccine.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

This is a Phase I double-blind, randomized, placebo controlled trial (1:4 ratio of placebo to vaccine) of HEV-239 in 25 US males and non-pregnant females ages 18 – 45 (inclusive) to assess the safety, reactogenicity, and immunogenicity of HEV-239. Subjects will receive 3 doses of study product on Days 1, 29, and 180 ([Table 1: Study Groups](#)). Subjects will remain in the study for up to 13 months (including screening). Subjects and study staff will be blinded to a subject's study vaccine assignment. This vaccine is a 239 amino acid sub-fragment of the 660 amino acid HEV structural protein that is produced in *E. coli*.

Table 1: Study Groups

Study Group	Number of Subjects	First Dose (Day 1, Visit 01)	Second Dose (Day 29, Visit 05)	Third Dose (Day 180, Visit 11)
HEV-239 Vaccine	20	HEV-239	HEV-239	HEV-239
Placebo	5	Placebo	Placebo	Placebo
Total	25			

- The HEV-239 vaccine dose contains 30 micrograms of purified protein adsorbed to 0.8 mg aluminum hydroxide suspended in 0.5 mL buffered saline, and thimerosal.
- The placebo will be 0.9% Sodium Chloride Injection, USP (Normal Saline) is a sterile, nonpyrogenic, isotonic solution of sodium chloride and Water for Injection (WFI). A total of 0.5 mL will be administered which contains sodium chloride 4.5 mg. It contains no bacteriostatic, antimicrobial agent, preservatives, or added buffer and may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3 [4.5 to 7.0]).

Thorough medical history, physical examination (PE), and screening laboratory tests (see [Sections 7.1](#) and [7.2](#)) will be obtained at screening after informed consent is obtained. Each subject may be screened beginning 28 days prior to the prime vaccination. At Day 1, the inclusion and exclusion criteria will be reviewed prior to randomization. Subjects who sign an informed consent and meet all inclusion criteria and no exclusion criteria will be enrolled in the study.

Subjects will be observed for 30 minutes after vaccination. The occurrence of solicited injection site and systemic reactogenicity events will be measured from the time of study vaccination through Day 8 after each vaccination. These will be ascertained through use of an electronic Memory (e-Memory) aid, a telephone call on Day 4 after each dose of vaccine, a Day 8 clinic visit, and potentially at the Day 15 clinic visit after each dose of vaccine. Unsolicited AEs will be collected from vaccination through D29 after each vaccination. SAEs will be collected from the time of the first study vaccination through the last study visit (Day 360).

The study includes multiple phlebotomy time points for immunogenicity and blood collection for future use at Visit 1 and Days 8, 15, and 29 after each vaccination. The durability of the immune response and future use collection will be assessed at 5 months after the first boost (Day 180) and at 6 months after the second boost (Day 360).

Note: The study day and the day after boost vaccination may not always align due to the study windows (See [Appendix A](#)) for the appropriate windows of time for each study visit.

Schematic of Study Design: See [Figure 1](#).

3.2 Study Objectives

3.2.1 Primary

- Assess the safety and reactogenicity of HEV-239 following delivery of each vaccine dose.
- Assess the number of subjects with ≥ 4 fold rise in HEV IgG at any time after vaccination.

3.2.2 Secondary

- Assess the number of subjects with HEV IgM seroconversion at any time after vaccination.
- Assess the number of subjects with HEV IgG seroconversion at any time after vaccination.
- Assess the HEV IgG geometric mean concentrations (GMCs) at any time after vaccination.

3.3 Study Endpoints or Outcome Measures

3.3.1 Primary

- Determine the number of solicited local and systemic reactogenicity events from the time of study vaccination through D8 after each study vaccination.
- Determine the number of vaccine-related unsolicited adverse events (AE) from the time of each study vaccination through D29 after each study vaccination.
- Determine the number of clinical safety laboratory AEs at Day 8 after each study vaccination.
- Determine the number of vaccine-related serious adverse event (SAE) from the time of first study vaccination through 6 months after the last dose of vaccine.
- Determine the number of subjects showing ≥ 4 fold rise in serum HEV IgG concentration by ELISA from the baseline at Days 8, 15, 29, 36, 43, 57, 180, 187, 194, 208, and 360.

3.3.2 Secondary

- Determine the number of subjects with HEV IgM seroconversion by ELISA at Days 8, 15, 29, 36, 43, 57, 180, 187, 194, 208, and 360.
- Determine the number of subjects with HEV IgG seroconversion by ELISA at Days 8, 15, 29, 36, 43, 57, 180, 187, 194, 208, and 360.
- Determine the HEV IgG GMCs by ELISA in subjects at Days 1, 8, 15, 29, 36, 43, 57, 180, 187, 194, 208, and 360.

4 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

4.1 Study Product Description

HEV-239 (Hecolin®)

The HEV-239 is a recombinant protein vaccine for the prophylaxis of HEV group 1. The Chinese group 1 HEV strain was used to provide the open reading frame 2, which encodes a 239 amino acid long sub-fragment of the HEV capsid protein. The HEV 239 protein is then expressed in *E.coli* ER2566 using the expression vector pTO-T7 containing the HEV 239 gene.³² The pTO-T7 prokaryotic vector was derived by inserting the T7 promoter and ribosome binding site of pET11a (New England Biolabs) and a plant-derived enhancer upstream of the promoter (Xiamen University).³³

The plasmid pGEX-20T-E2 with the *HEV ORF2* gene (gift from Hong Kong University) was subcloned into the pMD 18-T vector using a TA cloning kit (Takara, Dalian, China) and then digested with *NdeI* and *EcoRI*.³⁴ The *HEV 239* gene fragment was purified by gel electrophoresis and a gel extraction kit (Watson Inc., Shanghai, China) and then ligated to the pTO-T7 vector previously linearized with *NdeI* and *EcoRI*.

The recombinant plasmids were transfected into *E.coli* ER2566 and one isolate selected based on kanamycin resistance and DNA sequencing for the inserted fragment. The protein assembles into virus like particles (VLP). The purified, transformed *E. coli* (vaccine) is added to an aluminum adjuvant and sterilely packaged in a pre-filled syringe (PFS).

Placebo (Sterile Normal Saline)

0.9% Sodium Chloride Injection, USP (Normal Saline) is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection (WFI). This product is used as the placebo and is packaged in single use containers. Each mL contains sodium chloride 9mg and may contain HCl or NaOH for pH adjustment (pH 5.3 [4.5 – 7.0]). It contains no bacteriostatic, antimicrobial agent, preservative or added buffer.

4.1.1 Formulation, Packaging, and Labeling

HEV-239 (Hecolin®)

HEV-239 is a PFS preparation for injection containing the active ingredient, the HEV-239 protein that assembles into a VLP. HEV-239 is a truncated HEV capsid protein manufactured by fermentation in recombinant *E. coli*. The recombinant hepatitis E vaccine (*E. coli*) finished product is manufactured by mixing HEV-239 bulk with aluminum adjuvant. The formulation also includes the excipients sodium chloride, dibasic sodium phosphate, monobasic potassium phosphate, potassium chloride, thimerosal and water for injection. The finished product is a white suspension that is sterile filled into PFS for IM injection. Sedimentation of the HEV-239 bulk may occur, which can be re-suspended with gentle inversion of the PFS several times.

Each single-dose PFS contains 30 µg (60 µg/mL) of the recombinant hepatitis E virus antigen in a sterile 0.5 mL volume. See details of the vaccine in [Table 2: Composition of Recombinant \(*E. coli*\) Hepatitis E Vaccine / HEV-239](#).

Table 2: Composition of Recombinant (<i>E. coli</i>) Hepatitis E Vaccine / HEV-239		
	Ingredient	Quantity (per 1000 doses)
Active ingredient	HEV239 protein	0.030 mg
Inactive ingredient	Aluminum hydroxide	0.800 mg
	Sodium chloride	4.250 mg
	Thimerosal	0.025 mg
	Dibasic sodium phosphate	0.144 mg
	Monobasic potassium phosphate	0.057 mg
	Potassium chloride	0.010 mg
	Water for injection	0.5 mL

This study product will be labeled according to manufacturer specifications and include the statement “Caution: New Drug – Limited by Federal Law to Investigational Use.”

Further details are included in the package insert for HEV-239 and the Investigator’s Brochure.

Placebo (Sterile Normal Saline)

The placebo consists of physiological saline and is a clear, colorless solution. It will be provided to the pharmacy in vials. The pharmacist will, using sterile technique, fill the syringe with 0.5 mL volume of normal saline. A label with the statement “Caution: New drug -Limited by Federal Law to investigational use” will be placed on the immediate package.

4.1.2 Product Storage and Stability

HEV-239 (Hecolin®)

HEV-239 30µg PFS must be stored between 2°C to 8°C (36°F to 46°F), protected from light and not frozen. In stability studies, the degree of alum adsorption and the antigen content of the vaccine at 4°C, 25°C and 37°C remained above 95% for up to 3 months. In addition, in animal studies the immunogenicity of the vaccine drug product, when stored at 37°C for 5 weeks, showed no significant change from storage at 4°C. HEV-239 is considered stable for at least 36 months when maintained under 2°C to 8°C.

Placebo (Sterile Normal Saline)

Sterile Normal Saline vials are stored at 20°C to 25°C (68°F to 77°F) [excursions between 15°C and 30°C (59°F and 86°F) are permissible].

The temperature of the storage unit must be manually recorded daily (excluding non-business days and holidays, as applicable) and continuously monitored and recorded during the course of this trial per the participating VTEU site standard operating procedures (SOPs), and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as ‘Do Not Use’ (until further notice). The participating VTEU site’s research pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, including accidental deep-freezing or disruption of the cold chain, the affected study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information

collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

4.2 Acquisition/Distribution

HEV-239 will be supplied by Xiamen Innovax Biotech Co., Ltd.. Upon request by DMID, HEV-239 will be transferred to the following address:

DMID Clinical Materials Services (CMS)

Fisher BioServices

20439 Seneca Meadows Parkway

Germantown, MD 20876

Phone: 240-477-1350

Fax: 240-477-1360

Email: DMID.CMS@ThermoFisher.com

Study products (HEV-239 and the normal saline placebo) for study vaccine preparation will be provided through the DMID CMS to the participating VTEU site prior to the start of this trial upon request and with prior approval from DMID. Should the site principal investigator require additional HEV-239, placebo, or sterile empty syringes or needles during this trial, further instructions are provided in the protocol-specific MOP.

4.3 Dosage/Regimen, Preparation, Dispensing and Administration of Study Intervention/Investigational Product

Visually inspect the study products upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain(s) visible particulate matter, or if there are any concerns regarding the integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined at 2°C to 8°C (36°F to 46°F) for HEV-239 and at 20°C to 25°C (68°F to 77°F) [excursions between 15°C and 30°C (59°F and 86°F) are permissible] for sterile normal saline and labeled as 'Do Not Use' (until further notice). The site principal investigator or responsible person should immediately contact the DMID

Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If the affected study product(s) cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy the affected study product(s) on site. If the study product is unusable, study personnel will use another syringe or vial from the study supply. Replacement syringe or vial may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

HEV-239 (Hecolin®)

HEV-239 is a pre-filled syringe (PFS) preparation for injection. The finished product is a white suspension that is sterile filled into PFS for intramuscular (IM) injection. Sedimentation of the HEV bulk may occur, which can be re-suspended with gentle inversion of the PFS several times. Each single-dose PFS contains 30 µg (60 µg/mL) of the recombinant hepatitis E virus antigen in a sterile 0.5 mL volume.

Placebo (Sterile Normal Saline)

0.9% Sodium Chloride Injection, USP (Normal Saline) is a sterile, nonpyrogenic, isotonic solution of sodium chloride and WFI. Each mL contains sodium chloride 9 mg. It contains no bacteriostatic, antimicrobial agent, preservatives, or added buffer and may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3 [4.5 to 7.0]). This product will be used as the placebo and is supplied as a single-dose vial. The sterile Normal Saline vials are stored at 20°C to 25°C (68°F to 77°F) [See USP Controlled Room Temperature; excursions between 15°C and 30°C (59°F and 86°F) are permitted]. The placebo will be prepared by withdrawing sufficient volume to deliver a dose of 0.5 mL of 0.9% Sodium Chloride, USP.

Administration of Vaccine

Based on dosing group assignment, subjects will receive study vaccine (HEV-239) or Placebo as a 0.5 mL IM injection into the central and thickest portion of the deltoid muscle. An unblinded study vaccine administrator will vaccinate the subject. Study product will be distributed from the pharmacy with a sleeve (e.g., tape) over the syringe to prevent unintentional unblinding of blinded personnel. The choice of the arm for vaccine administration is at the subject's discretion. Aseptic

technique will be used for the withdrawal and administration of each dose of study product using a sterile needle appropriate in length for each participant. Detailed information regarding administration of study vaccine will be provided in the protocol-specific MOP.

4.4 Pre-determined Modification of Study Intervention/Investigational Product for an Individual Subject

There will be no dose modifications. If a subject's second study vaccination is deferred, it should be rescheduled to occur within the acceptable protocol-specified window for that visit. No exceptions to the protocol-specified window will be made.

4.5 Accountability Procedures for the Study Intervention/Investigational Product(s)

After receipt of study products, the site principal investigator is responsible for study product distribution and disposition, and has ultimate responsibility for study product accountability. The site principal investigator may delegate to the participating VTEU site's research pharmacist responsibility for study product accountability. The participating VTEU site's research pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, storage conditions, and final disposition of the study product(s). The pre-filled HEV-239 single use syringe and the placebo syringe will be disposed of per site processes **once the vaccine is administered.**

HEV-239 and placebo normal saline vials will be stored and shipped from the DMID contract Clinical Material Services (CMS) to the Clinical Sites. Once received, HEV-239 and placebo normal saline vials will be stored in and dispensed by the Investigational Pharmacy. Unused product will be handled in accordance with the MOP.

The Food and Drug Administration (FDA) requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product disposition is maintained and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the subject number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records must be available for inspection by the DMID monitoring contractors, and is subject to inspection by a regulatory agency (e.g., FDA) at any time. An assigned Study Monitor will review the pharmacy records.

Unused investigational product HEV239 syringes stored at [2°C to 8°C] and sterile normal saline at 20°C to 25°C (68°F to 77°F) will be stored in the Investigational Pharmacy until clinical trial accountability is completed, monitored and released for disposition when written authorization from the study Sponsor (CPM/DMID) received. At study termination, all unused investigational product will be handled in accordance with the MOP following complete drug accountability and monitoring.

5 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL

Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses.

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

5.1 Eligibility Criteria

Screening for eligible subjects must be performed within 28 days before Day 1. The investigator should ensure that all study enrollment criteria have been met at the end of the screening period. If a subject's status changes (including laboratory results or the receipt of additional medical records) after screening but before the prime vaccination such that the subject no longer meets all eligibility criteria, then the subject should be excluded from participation in the study.

Enrollment will be based solely on the eligibility criteria outlined below and will be open to both sexes and all races/ethnicities. Sex and minority breakdown cannot be predicted for this study but should follow the general population distribution. Pregnant women and women who are breastfeeding will not be eligible for inclusion.

5.1.1 Subject Inclusion Criteria

Prospective subjects must meet all of the following inclusion criteria to be considered eligible for enrollment:

1. Subject must provide written informed consent
2. Subject must be able to comprehend and willing to comply with all study visits and procedures (up to 13 months from enrollment).
3. Subject must be a man or a non-pregnant woman¹ aged 18-45 years (inclusive).

¹Females of childbearing potential must have a negative serum human chorionic gonadotropin (β -HCG) pregnancy test at screening and negative urine β -HCG pregnancy test within 24 hours prior to (each) vaccination.

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4. Subject must be in good general health as determined by medical history, vital signs,² body mass index (BMI),³ physical examination, and clinical judgment of the investigator.
- ²Oral temp <38.0°C/100.4°F; pulse 51 to 100 bpm; systolic blood pressure 90 to 140 mm Hg, and diastolic blood pressure 55 to 90 mmHg.
- ³BMI ≥18.5 and <35 kg/m².
5. Subject's screening laboratory values^{4,5} must be within site normal limits⁶ within 28 days of enrollment.
- ⁴Screening labs will include:
- White blood cell (WBC) count
 - Hemoglobin (HgB)
 - Platelets
 - Absolute neutrophil count (ANC)
 - Absolute eosinophil count (AEC)
 - Creatinine
 - Glucose (random, must be <140)
 - Alanine Aminotransferase (ALT)
 - HIV 1/2 antibody/antigen test, Hepatitis B surface antigen (HBsAg), and Hepatitis C virus (HCV) antibody
- ⁵Minor abnormalities are considered acceptable if not clinically significant (e.g., MCV). Repeating the screening tests once is permitted for out-of-range values provided there is an alternative explanation for the out-of-range value. The alternative explanation for the out-of-range value should be documented in the subject's source documents.
- ⁶See [Appendix B](#) for site normal values. Creatinine, glucose, and ALT values lower than the normal range may be acceptable if the PI or a designated licensed clinician determines that these laboratory findings are not clinically significant. The HIV 1/2 antibody/antigen test, Hepatitis B surface antigen (HBsAg), and Hepatitis C virus (HCV) antibody must be non-reactive.
6. Subject's HEV-specific IgG and IgM are negative by ELISA at screening.
7. Subject agrees to not to participate in another clinical trial during the study period.

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8. Subject agrees not to donate blood from screening through Day 270.
 9. Female subjects must be of non-childbearing potential⁷ OR must use an acceptable method of contraception⁸ from 28 days before prime vaccination until at least 3 months after the last vaccination.

⁷Surgically sterile via tubal ligation, bilateral oophorectomy, hysterectomy or postmenopausal for ≥ 1 year.

⁸Abstinence (defined as refraining from heterosexual intercourse), monogamous relationship with vasectomized partner, barrier methods such as male or female condoms with spermicide or diaphragms with spermicide, intrauterine devices, and licensed hormonal methods (such as birth control pills, skin patches, Implanon®, Nexplanon®, DepoProvera®, or NuvaRing®).
 10. Male subjects must be surgically sterile via vasectomy OR must use an acceptable method of contraception⁹ from prime vaccination until at least 3 months after the last boost vaccination.

⁹Abstinence (defined as refraining from heterosexual intercourse), or condoms with spermicide.
 11. Subjects must have consistent access to the internet to perform electronic data entry.

5.1.2 Subject Exclusion Criteria

Prospective subjects must not meet any of the following exclusion criteria to be considered eligible for enrollment:

1. Has a previous HEV infection or chronic liver disease.
2. Has received any experimental agent¹⁰ within 30 days prior to first vaccination, or the expected recipient of any experimental agent during this trial-reporting period.

¹⁰Including vaccines, drugs, biologics, devices, and/or blood products
3. Female subject is pregnant (or has a positive pregnancy test prior to vaccination) or breast feeding, or planning to become pregnant within 3 months after the last boost vaccination.

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4. Fever ($\geq 38.0^{\circ}\text{C}/100.4^{\circ}\text{F}$) or other acute illness within 3 days prior to first vaccination.
 5. Infection requiring systemic antibiotics or antiviral treatment within the 7 days prior to first vaccination.
 6. Has a positive urine drug screen for amphetamines¹¹, cocaine, opiates, or phencyclidine.

¹¹Prescription amphetamines are not exclusionary.

7. Chronic, clinically significant medical or psychiatric conditions that,¹² in the opinion of the investigator, may pose additional risk to the subject if she/he participates in the study.

¹²Permissible conditions include but are not limited to mild, well-controlled asthma, well-controlled depression, well-controlled anxiety, seasonal allergies, and well-controlled hypertension.

8. Receipt of immunosuppressive drugs¹³⁻¹⁵ or biologic agents within the 30 days prior to enrollment.

¹³This includes use of oral or parental prednisone. This also includes allergy desensitization injections from 14 days prior to each vaccination through 14 days after each vaccination. The use of topical steroids for mild uncomplicated dermatitis permissible after therapy is completed. Over-the-counter (OTC) corticosteroid nasal sprays for allergic rhinitis are permissible. The use of low or moderate dose inhaled steroids is permissible. Doses are 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.

¹⁴Receipt of systemic, prescription medications for the treatment of chronic medical conditions or variations of normal physiologic functions may be permissible if, in the opinion of the investigator, they are used for conditions that are not clinically significant and would not impact the safety of the subject or the safety and immunogenicity outcomes of the protocol.

¹⁵Use of systemic, over-the-counter medications and PRN systemic, prescription medication may be allowed if, in the opinion of the

investigator, they pose no additional risk to subject safety or assessment of immunogenicity/reactogenicity.

9. Has known neoplastic disease¹⁶ anticancer therapy, or radiation therapy within 3 years prior to first study vaccination.
¹⁶Excluding non-melanoma skin cancer, such as squamous cell skin cancer or basal cell skin cancer, cured by surgical excision.
10. Has a history of any hematologic malignancy at any time.
11. Has a known or suspected congenital or acquired disease that impairs the immune system, including functional asplenia or immunosuppression as a result of underlying illness or treatment.
12. Has prior organ and/or stem cell transplant.
13. Has a history of abuse of alcohol or drugs that, in the opinion of the investigator, may interfere with the subject's ability to comply with the protocol.
14. Has behavioral or cognitive impairment or psychiatric conditions that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.
15. Has received blood products or immunoglobulin within six months prior to vaccination.
16. Travel to Asia, the Middle East, Africa, or Central America or to an area with an active Hepatitis E outbreak¹⁷ within the last 90 days or intention to travel to such areas during the study.
¹⁷See study-specific Manual of Operating Procedures (MOP) for a listing of outbreaks within the last 3 years.
17. Receipt of any inactivated vaccine from 2 weeks prior to each vaccination through 2 weeks after each vaccination.
18. Receipt of any live vaccine from 4 weeks prior to each vaccination through 4 weeks after each vaccination.

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19. Known hypersensitivity or allergy to aluminum, any component of the vaccine, or other serious adverse reactions to vaccines or vaccine products.
 20. Subject who, in the opinion of the investigator, is unlikely to adhere to the requirements of the study.
 21. Any condition that, in the opinion of the investigator, might interfere with assessing the study objectives.

5.2 Requirements Prior to Administration of the Second and Third Vaccinations

The following assessments should be performed prior to the administration of the second and third vaccinations to determine if the subject remains eligible:

Subjects will not receive the subsequent vaccine doses (first or second boost) if any of the pre-specified individual halting criteria are met (see below and [Section 8.6.2](#)).

- For a woman of childbearing potential, an acceptable form of birth control must be verified. A urine pregnancy test must be performed and the subject must not be pregnant before administering the vaccine.
- Subjects who do not meet inclusion criteria 2 – 4 and 7 – 11, and exclusion criteria 7 – 21 will not be given the second or third vaccination.
- Subjects who have a fever (defined as an oral temperature $\geq 38.0^{\circ}\text{C}$) or a moderate or severe acute illness at the time of or in the three days prior to boost vaccination administration may have the boost vaccination deferred until they recover as long as this remains within the study window (see [Section 6.3](#)).
- Subjects who do not receive the second dose of vaccine for any reason will not receive the third dose of vaccine.

5.3 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination

5.3.1 Withdrawal from the Study or Discontinuation of the Study Product

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

An investigator may also withdraw a subject from receiving the study product for any reason. Follow-up safety evaluations and blood draws will be conducted, if the subject agrees. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the electronic case report forms (eCRFs).

The reasons, might include, but are not limited to the following:

- Subject no longer meets eligibility criteria
- Subject meets individual halting criteria (reference to [Section 8.6.2](#))
- Subject becomes noncompliant
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject lost to follow-up
- Subject becomes pregnant, if applicable
- Determined by a physician's discretion to require additional therapy not indicated in the protocol to ensure subject's health and well-being (or treatment failure, if applicable)

The investigator should be explicit regarding study follow-up (e.g. safety follow-up) that might be carried out despite the fact the subject will not receive further study product. If the subject consents, every attempt will be made to follow all AEs through resolution. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent or the investigator may seek subsequent informed consent using an IRB/IEC-approved consent form with the revised procedures.

The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study.

5.3.2 Subject Replacement

Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the informed consent form (ICF), randomization and receipt of study vaccine will not be replaced. However, if a subject withdraws after signing the ICF, but before randomization and/or receipt of study vaccine, they may be replaced.

5.3.3 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, the IRB, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. If the DMID agrees, then other study procedures (e.g., safety follow-up, blood sampling for safety and immunogenicity) may be continued through the remaining study visits but no further vaccinations will occur. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6 STUDY PROCEDURES

6.1 Screening Visit: Visit 00 (Day -28 to -1, All Groups)

Up through 28 days before baseline (the day of the subject's prime vaccination, Day 1), screening assessments as indicated in [Sections 5.1.1](#) and [5.1.2](#) will occur. Screening may be split into multiple days or visits.

The ICF will be signed before any study-specific procedures at the start of the screening period (see [Section 9.2](#)). For women of childbearing potential, it should be confirmed that adequate birth control measures were used from at least 28 days before the prime vaccination with a negative serum β -human chorionic gonadotropin (β -hCG) pregnancy test available before Day 1 and a negative urine β -hCG pregnancy test immediately (within 24 hours) prior to each study vaccination. All women, except for those of non-childbearing potential, will be asked to use adequate birth control from signing of the ICF onwards until at least 3 months after the last boost vaccination.

The investigator will provide detailed information on the study to the subject and will obtain written informed consent prior to each subject's study participation. The procedures indicated in [Section 7](#) will only be performed after the subject's written informed consent has been obtained.

The following assessments are performed during the Screening Visit to determine eligibility requirements as specified in the inclusion and exclusion criteria ([Section 5.1.1](#) and [5.1.2](#)). The written informed consent must be signed prior to conducting any of the assessments listed below:

- Obtain medical history (see [Section 7.1.1](#))
- Review prestudy medications and therapies up to 30 days prior to start of screening and record on the eCRF.
- Measure vital signs (HR, BP, and oral body temperature). This will also include height and weight for determination of BMI.
- Perform abbreviated physical examination. This will be done by a licensed clinician listed on FDA Form 1572.
- Review of birth control history with female subjects.
- Counsel subjects to use adequate birth control methods to avoid pregnancy

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- Obtain blood and urine for baseline screening laboratories (see [Section 2.3.1](#) for additional details).
 - Review inclusion and exclusion criteria

Laboratory values will be determined according to the [Appendix B Toxicity Table](#).

The overall eligibility of the subject to participate in the study will be assessed once all screening values and results of any other required evaluations are available. Retesting of values (e.g., baseline screening laboratories) that lead to exclusion is allowed **once** using an unscheduled visit during screening to assess eligibility, provided there is an alternative explanation for the out of range value. Study subjects who qualify for inclusion will be contacted and scheduled for enrollment and prime vaccination within the window for enrollment.

6.2 Enrollment/Baseline: Visit 01, Day 1, Vaccination Clinic Visit (All Groups)

The investigator should ensure that all enrollment criteria have been met during screening. If a subject's status (including any unscheduled laboratory results or the receipt of additional medical records) after screening but before the prime vaccination changes such that the subject no longer meets all enrollment criteria, then the subject should be excluded from participation in the study.

If the initial laboratory screening occurred more than 28 days before baseline (Day 1) but the subject was unable to be vaccinated within the 28-day window (e.g., due to meeting [Exclusion Criteria](#) in [Section 5.1.2](#) or for other reasons), the subject must have safety laboratories repeated (see protocol-specific MOP for details about laboratories).

Prior to Vaccination

- Review inclusion and exclusion criteria to ensure the subject remains eligible for enrollment
- Review medical history for any interval changes
- Review prestudy and concomitant medications
- Obtain vital signs (HR, BP, and oral temperature)
- Subjects who have a diastolic blood pressure value >90 mmHg or a systolic blood pressure value >140 mmHg prior to the prime vaccination can receive the vaccination if the elevated blood pressure is considered to be not clinically significant and the subject had acceptable blood pressure at screening.

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- Perform symptom-directed PE (if needed).
 - Perform urine pregnancy test for women of childbearing potential within 24 hours prior to immunization. Test result must be known and negative prior to vaccination.
 - Review of birth control history with female subjects
 - Counsel subjects to use adequate birth control methods to avoid pregnancy
 - Subjects will be enrolled in Advantage eClinical® and randomly assigned to a Study Group (see [Section 10.3.1 Randomization Procedures](#) for details).
 - Obtain serum for immunogenicity assays.
 - Collect PBMCs and plasma for future assays.
 - Study vaccine will be prepared on-site by an unblinded site research pharmacist (see [Section 4.3](#)).
 - Pre-administration reactogenicity assessments will be performed prior to the first study vaccination to establish baseline. A subject with mild pre-administration reactogenicity which is transient, resolving, or clinically insignificant may receive vaccination at the investigator's discretion.

Vaccination

- The unblinded study vaccine administrator will vaccinate the subject according to vaccination assignment in the subject's preferred arm.
- Following receipt of study product, subjects may have the injection site covered with an adhesive bandage (e.g. BAND-AID®) as needed.

Following Vaccination

- Observe subjects for a minimum of 30 minutes following immunization.
- Provide subjects with the e-Memory aid instructions, ruler and thermometer and instruct the subject on the observance, grading and recording of unsolicited reactions and solicited injection site reactions, systemic reactions and daily maximum temperatures through Day 8, beginning with the day of vaccination (Day 1). Subjects will be instructed on how to complete the e-Memory aid. Reactions that persist beyond Day 8 will continue to be recorded

until the subject is symptom-free. As a back-up method in the event of internet connectivity issues, subjects will also be provided a paper memory aid.

- Instruct subjects as to when to contact the investigator about unsolicited AEs.
- Review hypersensitivity symptoms with the subject and counsel to contact study personnel or seek medical attention immediately.
- Post-administration reactogenicity assessments will be performed, vital signs will be obtained, and the vaccination site assessed at a minimum of 30 minutes following immunization.
- Evaluate vaccination site.
- Solicited and Unsolicited AEs, and SAEs will be collected and documented on the eCRFs, together with the information on any concomitant medications.

6.3 Planned Study Visits

6.3.1 Follow-up

6.3.1.1 Visit 02, Day 4 +/- 1 Day; Telephone Call

- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.
- Review concomitant medications

6.3.1.2 Visit 03, Day 8 +/- 1 Day; Clinic Visit

- Obtain vital signs (HR, BP, and oral temperature)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site
- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.

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- If symptoms persist, clinic staff will attempt to follow up with the subject until the symptoms resolve. Maximum severity post Day 8 and stop date of the symptoms will be recorded by clinic staff on the Solicited Events eCRF.
 - Counsel subjects to use adequate birth control methods to avoid pregnancy
 - Review concomitant medications.
 - Obtain blood for safety laboratories ([Section 2.3.1](#)).
 - Obtain serum for immunogenicity assays
 - Collect PBMCs and plasma for future assays.

6.3.1.3 Visit 04, Day 15 +/- 2 Days; Clinic Visit

- Obtain vital signs (HR, BP, and oral temperature)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site (if needed)
- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.
- Counsel subjects to use adequate birth control methods to avoid pregnancy
- Review concomitant medications.
- Obtain blood for safety laboratories (if needed due to abnormalities at prior visit, [Section 2.3.1](#))
- Obtain serum for immunogenicity assays
- Collect PBMCs and plasma for future assays.

6.3.1.4 Visit 05, Day 29 +/- 2 Days; Clinic Visit

Prior to Vaccination

- Review medical history for any interval changes

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- Review concomitant medications
 - Obtain vital signs (HR, BP, and oral temperature)
 - Subjects who have a diastolic blood pressure value >90 mmHg or a systolic blood pressure value >140 mmHg prior to the prime vaccination can receive the vaccination if the elevated blood pressure is considered to be not clinically significant and the subject had acceptable blood pressure at screening.
 - Perform symptom-directed PE (if needed)
 - Perform urine pregnancy test for women of childbearing potential within 24 hours prior to immunization. Test result must be known and negative prior to vaccination.
 - Review of birth control history with female subjects.
 - Counsel subjects to use adequate birth control methods to avoid pregnancy.
 - Obtain serum for immunogenicity assays.
 - Collect PBMCs and plasma for future assays.
 - Review inclusion and exclusion criteria to ensure the subject remains eligible for boost vaccination.
 - Study vaccine will be prepared on-site by an unblinded site research pharmacist (see [Section 4.3](#)).
 - Pre-administration reactogenicity assessments will be performed prior to vaccination to establish baseline. A subject with mild pre-administration reactogenicity which is transient, resolving, or clinically insignificant may receive vaccination at the investigator's discretion.

Vaccination

- The unblinded study vaccine administrator will vaccinate the subject according to vaccination assignment in the subject's preferred arm.
- Following receipt of study product, subjects may have the injection site covered with an adhesive bandage (e.g. BAND-AID®) as needed.

Following Vaccination

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- Observe subjects for a minimum of 30 minutes following immunization.
 - Provide subjects with the e-Memory aid instructions, ruler and thermometer and instruct the subject on the observance, grading and recording of unsolicited reactions and solicited injection site reactions, systemic reactions and daily maximum temperatures through Day 8, beginning with the day of vaccination (Day 1). Subjects will be instructed on how to complete the e-Memory aid. Reactions that persist beyond Day 8 will continue to be recorded until the subject is symptom-free. As a back-up method in the event of internet connectivity issues, subjects will also be provided a paper memory aid.
 - Instruct subjects as to when to contact the investigator about unsolicited AEs.
 - Review hypersensitivity symptoms with the subject and counsel to contact study personnel or seek medical attention immediately.
 - Post-administration reactogenicity assessments will be performed, vital signs will be obtained, and the vaccination site assessed at a minimum of 30 minutes following immunization.
 - Evaluate vaccination site.
 - Solicited and Unsolicited AEs and SAEs will be collected and documented on the eCRFs, together with the information on any concomitant medications.

6.3.1.5 Visit 06, Day 32 (Day 4 after First Boost; +/- 1 Day); Telephone Call

- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.
- Review concomitant medications

6.3.1.6 Visit 07, Day 36 (Day 8 after First Boost; +/- 1 Day); Clinic Visit

- Obtain vital signs (HR, BP, and oral temperature)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site

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- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
 - Review of Unsolicited AEs and SAEs.
 - If symptoms persist, clinic staff will attempt to follow up with the subject until the symptoms resolve. Maximum severity post boost Day 8 and stop date of the symptoms will be recorded by clinic staff on the Solicited Events eCRF.
 - Counsel subjects to use adequate birth control methods to avoid pregnancy
 - Review concomitant medications.
 - Obtain blood for safety laboratories ([Section 2.3.1](#)).
 - Obtain serum for immunogenicity assays
 - Collect PBMCs and plasma for future assays.

6.3.1.7 Visit 08, Day 43 (Day 15 after First Boost; +/- 2 Days); Clinic Visit

- Obtain vital signs (HR, BP, and oral temperature)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site (if needed)
- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.
- Counsel subjects to use adequate birth control methods to avoid pregnancy
- Review concomitant medications.
- Obtain blood for safety laboratories (if needed due to abnormalities at prior visit, [Section 2.3.1](#))
- Obtain serum for immunogenicity assays
- Collect PBMCs and plasma for future assays.

6.3.1.8 Visit 09, Day 57 (Day 29 after First Boost; +/- 2 Days); Clinic Visit

- Perform symptom-directed PE (if needed)
- Review of Unsolicited AEs and SAEs.
- Counsel subjects to use adequate birth control methods to avoid pregnancy
- Review concomitant medications.
- Obtain serum for immunogenicity assays
- Collect PBMCs and plasma for future assays.

6.3.1.9 Visit 10, Day 120 (Day 92 after First Boost; +/- 14 Days); Telephone Call

- Review of SAEs.
- Review concomitant medications if associated with an SAE.
- Counsel subjects to use adequate birth control methods to avoid pregnancy

6.3.1.10 Visit 11, Day 180 +/- 14 Days from Prime Vaccination; Clinic Visit

Prior to Vaccination

- Review medical history for any interval changes
- Review concomitant medications
- Obtain vital signs (HR, BP, and oral temperature)
- Subjects who have a diastolic blood pressure value >90 mmHg or a systolic blood pressure value >140 mmHg prior to the prime vaccination can receive the vaccination if the elevated blood pressure is considered to be not clinically significant and the subject had acceptable blood pressure at screening.
- Perform symptom-directed PE (if needed).
- Perform urine pregnancy test for women of childbearing potential within 24 hours prior to immunization. Test result must be known and negative prior to vaccination.
- Review of birth control history with female subjects.

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- Counsel subjects to use adequate birth control methods to avoid pregnancy.
 - Obtain blood for safety laboratories ([Section 2.3.1](#)).
 - Obtain serum for immunogenicity assays.
 - Collect PBMCs and plasma for future assays
 - Review inclusion and exclusion criteria to ensure the subject remains eligible for boost vaccination
 - Study vaccine will be prepared on-site by an unblinded site research pharmacist (see [Section 4.3](#)).
 - Pre-administration reactogenicity assessments will be performed prior to vaccination to establish baseline. A subject with mild pre-administration reactogenicity which is transient, resolving, or clinically insignificant may receive vaccination at the investigator's discretion.

Vaccination

- The unblinded study vaccine administrator will vaccinate the subject according to vaccination assignment in the subject's preferred arm.
- Following receipt of study product, subjects may have the injection site covered with an adhesive bandage (e.g. BAND-AID®) as needed.

Following Vaccination

- Observe subjects for a minimum of 30 minutes following immunization.
- Provide subjects with the e-Memory aid instructions, ruler and thermometer and instruct the subject on the observance, grading and recording of unsolicited reactions and solicited injection site reactions, systemic reactions and daily maximum temperatures through Day 8, beginning with the day of vaccination (Day 1). Subjects will be instructed on how to complete the e-Memory aid. Reactions that persist beyond Day 8 will continue to be recorded until the subject is symptom-free. As a back-up method in the event of internet connectivity issues, subjects will also be provided a paper memory aid.
- Instruct subjects as to when to contact the investigator about unsolicited AEs.

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- Review hypersensitivity symptoms with the subject and counsel to contact study personnel or seek medical attention immediately.
 - Post-administration reactogenicity assessments will be performed, vital signs will be obtained, and the vaccination site assessed at a minimum of 30 minutes following immunization.
 - Evaluate vaccination site.
 - Solicited and Unsolicited AEs and SAEs will be collected and documented on the eCRFs, together with the information on any concomitant medications.

6.3.1.11 Visit 12, Day 184 (Day 4 after Second Boost; +/- 1 Day); Telephone Call

- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.
- Review concomitant medications

6.3.1.12 Visit 13, Day 187 (Day 8 after Second Boost; +/- 1 Day); Clinic Visit

- Obtain vital signs (HR, BP, and oral temperature)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site
- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.
- If symptoms persist, clinic staff will attempt to follow up with the subject until the symptoms resolve. Maximum severity post boost Day 8 and stop date of the symptoms will be recorded by clinic staff on the Solicited Events eCRF.
- Counsel subjects to use adequate birth control methods to avoid pregnancy
- Review concomitant medications.

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- Obtain blood for safety laboratories ([Section 2.3.1](#)).
 - Obtain serum for immunogenicity assays
 - Collect PBMCs and plasma for future assays.

6.3.1.13 Visit 14, Day 194 (Day 15 after Second Boost; +/- 2 Days); Clinic Visit

- Obtain vital signs (HR, BP, and oral temperature)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site (if needed)
- Interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data.
- Review of Unsolicited AEs and SAEs.
- Counsel subjects to use adequate birth control methods to avoid pregnancy
- Review concomitant medications.
- Obtain blood for safety laboratories (if needed due to abnormalities at prior visit, [Section 2.3.1](#)).
- Obtain serum for immunogenicity assays
- Collect PBMCs and plasma for future assays.

6.3.1.14 Visit 15, Day 208 (Day 29 after Second Boost; +/- 2 Days); Clinic Visit

- Perform symptom-directed PE (if needed)
- Review of Unsolicited AEs and SAEs.
- Counsel subjects to use adequate birth control methods to avoid pregnancy
- Review concomitant medications.
- Obtain serum for immunogenicity assays
- Collect PBMCs and plasma for future assays.

6.3.1.15 Visit 16, Day 270 (Day 90 after Second Boost; +/- 14 Days); Telephone Call

- Review of SAEs.
- Review concomitant medications if associated with an SAE.

6.3.2 Final Study Visit: Visit 17, Day 360 +/- 28 days (Day 180 after Second Boost); Clinic Visit

- Perform symptom-directed PE (if needed)
- Review of SAEs.
- Review concomitant medications if associated with an SAE.
- Obtain serum for immunogenicity assays
- Collect PBMCs and plasma for future assays.

6.3.3 Early Termination Visit

The following activities may be performed at the early termination visit for subjects who withdraw, or are withdrawn or terminated from this study:

- Obtain vital signs (BP, HR, and oral temperature) (if needed)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site if within Day 15 after each vaccination
- Review Unsolicited AEs if within Day 29 after each vaccination
- Review of SAEs.
- If occurring through Day 8 after any vaccine dose, interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data. If visit occurs between Day 8 and Day 29, if needed the subject will be interviewed and ongoing solicited AEs will be reviewed.
- If symptoms persist post Day 8, clinic staff will attempt to follow up with the subject daily until the symptoms resolve. Maximum severity post Day 8 and stop date of the symptoms will be recorded by clinic staff on the Solicited Events eCRF.
- Review concomitant medications.
- Obtain blood for safety laboratories (if needed)
- Obtain serum for immunogenicity assays (if needed)

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- Collect PBMCs and plasma for future assays (if needed).
 - Review of birth control history with female subjects.
 - Counsel subjects to use adequate birth control methods to avoid pregnancy if before 3 months after the last study vaccination
 - A urine pregnancy test for women of childbearing potential may be performed.

6.4 Unscheduled Study Visits

Unscheduled visits may occur at any time during this study. Any of the following activities may be performed:

- Obtain vital signs (BP, HR, and oral temperature) (if needed)
- Perform symptom-directed PE (if needed)
- Evaluate vaccination site if within Day 15 after each vaccination
- Review Unsolicited AEs if within Day 29 after each vaccination
- Review of SAEs.
- If occurring through Day 8 after any vaccine dose, interview the subject and review the e-Memory aid data. Address any incomplete, missing, or inconsistent data. If visit occurs between Day 8 and Day 29, if needed the subject will be interviewed and ongoing solicited AEs will be reviewed.
- If symptoms persist post Day 8, clinic staff will attempt to follow up with the subject daily until the symptoms resolve. Maximum severity post Day 8 and stop date of the symptoms will be recorded by clinic staff on the Solicited Events eCRF.
- Review concomitant medications.
- Obtain blood for safety laboratories (if needed)
- Obtain serum for immunogenicity assays (if needed)
- Collect PBMCs and plasma for future assays (if needed).
- Review of birth control history with female subjects (if needed).
- Counsel subjects to use adequate birth control methods to avoid pregnancy if before 3 months after the last study vaccination.
- A urine pregnancy test for women of childbearing potential may be performed.

6.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

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 principal investigator and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the DCC protocol deviation reporting procedures.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

7.1.1 Research Procedures: Medical History

Complete medical history will be obtained by interview of the subjects at the Screening Visit. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A focused vaccination history will be obtained. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. History of receipt of medications which modify the host immune response (e.g., cancer chemotherapeutic agents, parenteral corticosteroids), any experimental therapeutic agent, and blood product or immunoglobulin receipt will be solicited. A history of use of contraception will be solicited. Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required. Pre-vaccination reactogenicity symptoms will be solicited and recorded.

7.1.2 Research Procedures: Physical Examination

At the screening visit, an abbreviated physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator. An abbreviated PE is distinguished from a complete PE as all assessments are not required (e.g., pelvic, rectal). The abbreviated PE will include assessments of the following organs and organ systems: skin, HEENT, neck, lungs, heart, liver, spleen, abdomen, extremities, lymph nodes (axillary and cervical), and nervous system. On Day 1 and at follow-up visits after the first study vaccination a symptom-directed PE may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator, if indicated based on subject's interim medical history. This symptom-directed PE will be performed as indicated by the investigator based on any clinically relevant issues, clinically relevant symptoms and medical history. The symptom-directed PE may be repeated if deemed necessary by the investigator. Any abnormal finding that represents an increase in toxicity grade post study vaccination must be recorded on the AE page of the eCRF. All events should be followed to resolution, or until reaching a clinically stable endpoint.

7.1.3 Research Procedures: Vital Signs

Vital signs (oral temperature, pulse, and BP) will be collected at the Screening Visit, and prior to and at a minimum of 30 minutes after each study vaccination (Day 1, Day 29, and Day 180). Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline. Vital signs will also be assessed at Days 8, 15, 36, 43, 187, and 194 in all subjects. Blood pressure and heart rate measurements should be preceded by at least 5 minutes of rest in a quiet setting without distractions (e.g., television, cell phones). Confirmatory vital sign measurements can be repeated once. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. The repeated measurement may be used to determine eligibility per the judgment of the investigator. Height and weight will be collected at the Screening Visit for the calculation of BMI. See the protocol specific MOP for additional details.

7.1.4 Research Procedures: Post Vaccination

Subjects will be observed in the clinic for at least 30 minutes after each study vaccination (Days 1, 29, and 180). Vital signs will be obtained at a minimum of 30 minutes after each study vaccination. The study vaccination site will be examined, post-reactogenicity symptoms will be recorded, and any AE/SAEs will be assessed and recorded on the appropriate eCRF prior to discharge from the clinic. The study vaccination site will also be examined at follow-up visit Day 8 after each study vaccination. The study vaccination site will be examined at follow-up visit Day 15 after each study vaccination as well, if needed.

All subjects will enter reactogenicity symptoms via an e-Memory aid to the SDCC beginning on the evening of the day of vaccination (Day 1, Day 29, and Day 180) through Day 8 after each study vaccination. Subject e-Memory aids will be reviewed with the subject for AEs on Days 4 (by telephone), Day 8, and if needed on Day 15 after each study vaccination. Reactogenicity assessments will include an assessment of solicited AEs including an assessment of injection site reactions of pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and tenderness as well as systemic reactions including fever, feverishness (chills/ shivering/ sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, nausea, and vomiting.

7.1.5 Assessment of Concomitant Medications/Treatments other than Study Product

Medications history (concomitant medications) will include a review of all current medications and medications taken within 30 days prior to signing the informed consent form through Day 57, or Day 29 for those who only receive one dose of study vaccine, or early termination (if prior to Day 29 after any study vaccine dose), whichever occurs first. Concomitant medications will also include all current medications and medications taken within 30 days prior to Vaccine Dose 3 (Day 180) through Day 208. Concomitant therapies outside these times should only be recorded if administered in conjunction with SAEs. Prescription and over-the-counter drugs and vaccines will be included as well as vitamins, herbals, and supplements. Assessment of eligibility will also include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see [Section 5.1.1](#) and [5.1.2](#)).

Medications that might interfere with the evaluation of the investigational product should not be used unless necessary. Medications in this category include, but are not limited to, glucocorticoids (i.e., oral, parenteral and high-dose inhaled steroids), and immunosuppressive or cytotoxic drugs. Analgesics and antipyretics should be avoided for the 24 hours following study injection, unless necessary.

7.1.6 Assessment of Subject Compliance with the Study Visit Schedule

Study product will be administered to subjects by an unblinded study vaccine administrator via IM injection at all dosing times and as described in [Section 4.3](#). Thus, subject compliance is not anticipated to be an issue. Deviations from the dose schedule may only occur as described in [Section 4.4](#).

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

7.2.1.1 Baseline Screening Laboratories

Serum pregnancy tests will be performed locally by site laboratory at the screening visit and urine pregnancy test will be performed prior to each study vaccination on all women of childbearing potential. Results must be known and negative prior to randomization on Day 1 to be eligible for participation in this trial. Baseline laboratories will be obtained as defined in [Section 2.3.1](#).

7.2.1.2 Subsequent Testing Including Safety Laboratories

Urine pregnancy tests will be performed by locally by site laboratory within 24 hours prior to each study vaccination (Days 1, 29, and 180) on all women of childbearing potential. Results must be known and negative prior to administration of each study vaccination to be eligible for participation in the study and receipt of each dose of study vaccine, respectively. Safety laboratories will be obtained as defined in [Section 2.3.1](#). Subjects will have follow-up safety laboratories obtained on Days 8, 36, 180, and 187 (about 13 mL).

Laboratory toxicities will be determined according to [Appendix B](#). Laboratory reports 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, document this review, and record any abnormal laboratory value that represents an increase in toxicity grade post study vaccination on the AE page of the eCRF. The laboratory reports must be filed with the source documents. All events should be followed to resolution, or until reaching a clinically stable endpoint.

The volume of venous blood to be collected for clinical safety laboratory evaluations is presented in [Appendix A](#).

7.2.2 Research Assays

The volume of venous blood to be collected for immunogenicity assays and future research is presented in [Appendix A](#).

Immunogenicity

Assays to determine serum levels of hepatitis E antibodies (IgG and IgM) will be performed at Emory's VTEU laboratory. Venous blood samples (approximately 10 mL) for antibody assays will be collected from each subject at screening and sent directly to Emory's VTEU laboratory for baseline testing. Seronegative samples will be defined as per the Wantai HEV-IgM and IgG ELISA package inserts (A/C.O. <0.9).

Venous blood samples (approximately 10 mL) will be collected immediately prior to each study vaccination (Days 1, 29, and 180) and Days 8, 15, after each study vaccination (Days 8, 15, 36, 43, 187, 194), Day 29 after each boost vaccination (Days 57 and 208), and Day 360. Subjects who withdraw early will have hepatitis E antibody assays run on available sera. Samples for immunogenicity assessments will be shipped from the DMID CMS to Emory's VTEU laboratory for vaccine immunogenicity assessments beginning with the completion of the Day 360 visit.

Seroconversion will be defined as a change from a seronegative result to a seropositive result as defined by the Wantai HEV-IgM and IgG ELISA package inserts (A/C.O. >1.1). As defined by

prior studies,²⁴ the antibody concentration in samples negative for hepatitis E virus IgG was set at 0.0385 Wu/mL which was half the lower limit of quantification. A 4-fold seroconversion will be defined as a HEV IgG ≥ 0.154 in a subject that was HEV seronegative at Day 1.

Future Use:

Residual samples are those that are left over after the study has been completed and are stored for possible use in future research studies. Future use samples are extra tube(s) of blood collected and stored for possible use in future research studies. Retention of residual and future use samples and the potential for use in future research studies will be a condition of study participation. Subjects who sign the informed consent form for the study are consenting to allow the collection, storage and use of any residual samples (serum or cells derived from venous blood samples) or future use samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. Future use research studies may include, but are not limited to non-traditional immune assay development, assessing innate immune factors, and the ability of HEV vaccine-induced antibodies to cross-react with other viruses.

Residual and future use samples will be encoded with a barcode label and unique tracking number to protect subject identity. Samples will be stored indefinitely at a DMID designated central storage facility. Residual samples may be shared for purposes other than per protocol analysis with investigators at participating VTEU sites and with investigators at other institutions once the clinical study report has been finalized. Future use research samples may be requested from DMID and shipped from the DMID CMS at any time.

Residual and future use samples will not be sold or used directly for production of any commercial product. Genetic tests may be performed on samples. There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will NOT be kept in their health records.

Subjects may withdraw consent for study participation at any time by notifying the study doctors or nurses in writing. However, any data from sample(s) collected prior to the withdrawn consent will not be removed including genomic data. There will be no further use of residual samples or collection and use of future use samples after consent has been withdrawn.

Venous blood will be collected for PBMCs and plasma (16-64 mL) for future use on Days 1, 8, 15, 29, 36, 43, 57, 180, 187, 194, 208, and 360.

7.2.2.1 Laboratory Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the central (clinical) laboratory manual and protocol-specific MOP as appropriate.

7.2.2.2 Laboratory Specimen Shipping

Specimen shipment will occur at intervals during the course of this trial following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the central (clinical) laboratory manual and protocol-specific MOP as appropriate.

Specimens for safety laboratory evaluation will be shipped from the Emory VTEU to the clinical laboratory.

Screening: Specimens for initial immunogenicity laboratory evaluations at screening will be sent to the Emory VTEU laboratory in an unblinded manner. Results will be conveyed back to the Emory VTEU clinical team.

Enrolled Subjects: Specimens for immunogenicity laboratory evaluations beginning Day 1 and continuing throughout the study will be shipped from the Emory VTEU to the central repository and then provided by the DMID CMS to Emory VTEU laboratory in a blinded manner.

Further instructions for specimen shipment are included in the central (clinical) laboratory manual and protocol-specific MOP, as appropriate.

8 ASSESSMENT OF SAFETY

Regulatory requirements including the FDA regulations, ICH Guidelines for GCP, and European Union (EU) Clinical Trials Directive set forth safety monitoring and reporting responsibilities of sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

Responsibilities

Investigators participating in this clinical trial are responsible for and will:

- Evaluate subject safety including assessment of AEs for seriousness, severity, and causality;
- Follow up AEs resolution through study follow up period;
- Notify the sponsor (DMID) of SAEs immediately;
- Follow up SAEs until resolution, even if this extends beyond the follow up period (resolution of an AE/SAE is defined as the return to baseline or stabilization of the condition with the expectation that it will remain chronic) provide detailed written reports, including necessary documentation requested by the sponsor or Institutional Review Board (IRB)/Independent Ethics Committee (IEC), promptly following immediate initial reports, and;
- Inform the IRB/IEC of AEs as required by applicable regulatory requirements.

8.1 Assessing and Recording Safety Parameters

8.1.1 Adverse Events (AEs)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

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. The occurrence of an AE may come to the attention of study personnel during study

visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs, including solicited local (injection site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and eCRF. 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 Form FDA 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. AEs will be followed through resolution.

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.

8.1.1.1 Adverse Events Grading

All AEs (laboratory and clinical symptoms) will be graded for severity and assessed for relationship to study product (see definitions). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

Severity of Event:

AEs will be assessed by the investigator using a protocol-defined grading system (toxicity table included as an appendix). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify 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.

Relationship to Study Product: The assessment of the AE's relationship to study product will be done by the licensed study physician indicated on the Form FDA 1572 and the assessment will be part of the documentation process. Whether the AE is related or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. The relationship to study product will be assessed for AEs using the terms related or not related:

- **Related** – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study product caused the event.

8.1.2 Reactogenicity (Solicited Local and Systemic Events)

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. The subject will be instructed on noting the presence and severity of symptoms through D8 after administration of study vaccine: The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Table 3: Local (Injection Site) Reactogenicity Grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site (spontaneous discomfort)	No pain medication or it requires use of a non-narcotic pain reliever ≤24hours OR does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours OR interferes with activity	Any use of narcotic pain reliever OR prevents daily activity
Tenderness – hurts only when injection site is	Discomfort only to touch	Discomfort with movement	Significant discomfort at rest

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
touched or the arm is moved			
Pruritus (Itching)	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Ecchymosis (Bruising)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Induration (Hardness)/Swelling*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

* Will also be measured in mm but size will not be used as halting criteria.

Erythema (redness), ecchymosis, and induration (hardness)/swelling as analyzed by measurement will be graded as follows:

Table 4: Local (Injection Site) Reactogenicity Measurements

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Ecchymosis (Bruising)*	25mm – 50mm	51 mm – 100mm	>100 mm
Erythema (Redness)*	25 mm – 50 mm	51 mm – 100 mm	>100 mm
Induration (Hardness)/Swelling*	25 mm – 50 mm	51 mm – 100 mm OR interferes with activity	>100 mm OR prevents daily activity

*Will not be used as halting criteria.

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

Table 5: Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness (Chills/Shivering/Sweating)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fatigue (Tiredness)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise (General Unwell Feeling)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia (Body Aches/Muscular Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (Joint Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Repeated use of non-narcotic pain reliever >24 hours OR some interference with activity	Significant; any use of narcotic pain reliever OR prevents daily activity OR requires triptans
Nausea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity or requires IV hydration

* Not at injection site.

Oral temperature[#] will be graded as follows:

Table 6: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral [†]	38.0°C – 38.4°C 100.4°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

[#] Oral temperature assessed on Day 1 prior to the first study vaccination will be considered as baseline.

* A fever can be considered not related to the study product if an alternative etiology can be documented.

† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

Any symptoms still present at the D8 visit after each vaccination will continue to be followed by subject until symptom resolution. Subjects will also be asked to call the site if they experience any severe symptoms other than what's listed on the e-Memory aid, if they have any severe symptoms that prevents daily activity or any other symptoms and health complaints, even if they are not listed on the e-Memory aid. Sites will review the concomitant medications with the subject at subsequent clinic visits. The subject's input into the electronic data capture system will be reviewed with the subject at subsequent clinic visits.

Subjects will be instructed on how to record daily temperature using a thermometer provided for home use. Subjects should record the oral temperature in the evening post vaccination, and then daily through D8 after each vaccination. Temperature should be measured at approximately the same time each day. If more than one measurement is made on any given day, the highest temperature will be recorded. This information will be reviewed by study staff during follow-up subject contacts.

The site PI/study staff will also assess the vaccination site and measure edema and erythema at the visits post vaccination. Vital sign measurements will be performed at the time points indicated in the [Section 6](#). Blood pressure and heart rate measurements should be preceded by at least 5 minutes of rest and in a quiet setting without distractions (e.g., television, cell phones). Confirmatory vital sign measurements can be performed if inconsistent with a prior measurement. A symptom-directed examination will be performed as indicated by the investigator based on any clinically relevant issues, clinically relevant symptoms and medical history. The symptom-directed PE may be repeated if deemed necessary by the investigator. PEs will be performed by a study clinician licensed to make medical diagnoses and listed on the FDA Form 1572 as the site principal investigator or sub-investigator.

8.1.3 Unsolicited Adverse Events

8.1.4 Serious Adverse Events (SAEs)

An AE or suspected adverse reaction is considered a serious adverse event (SAE) if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event¹,
- inpatient hospitalization or prolongation of existing hospitalization,

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- 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 hospitalizations 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, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

¹Life-threatening adverse event. An AE is considered “life-threatening” if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

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 Form FDA 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 IND studies, a physician listed on the Form FDA 1572 as the site Principal Investigator or Sub-Investigator).
- Reviewed and evaluated by DMID, an Independent Safety Monitor (ISM), the SMC (periodic review unless related), and the IRB/IEC.

8.2 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Serious adverse events (see [Section 8.1.4](#)) occurring from the time of the first study vaccination through the last study visit (Day 360).
2. Solicited Adverse Events (see [Section 8.1.2](#)) – reactogenicity events occurring on the day of each study vaccination through Day 8 after each study vaccination:

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- a) Injection site reactions including pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and tenderness.
 - b) Systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, nausea, and vomiting.
3. Clinical safety laboratory AEs (see [Appendix B](#)) at Day 8 after each study vaccination. Parameters to be evaluated include WBC, ANC, AEC, Hgb, PLT, Cr, and ALT ([Section 2.3.1](#)).
 4. Unsolicited Adverse Events (see [Section 8.1.3](#)) – non-serious adverse events occurring from the time of the first study vaccination until Day 57 then from Day 180 through Day 208. Unsolicited AEs will be collected at all visits from the time of the first study vaccination until D57, then from Day 180 through 208. Any safety-related changes that occur post-vaccination during these timeframes and that represent an increase in grade must be recorded on the eCRF. Thereafter, recording will be limited to SAEs.

All Grade 1, 2, and 3 changes except for Grade 1 and 2 solicited adverse events must be assessed for relatedness to the study vaccination. The assessment of causality must be determined by a study clinician licensed to make medical diagnoses and listed on the FDA Form 1572 as the site principal investigator or sub-investigator.

8.3 Reporting Procedures

Solicited injection site and systemic reactogenicity events will be documented and reported from the time of each study vaccination through D8 after each study vaccination.

Unsolicited non-serious AEs will be documented and reported at all visits from signing of the ICF onwards until D57, then from Day 180 through Day 208.

SAEs will be collected from the time of the first study vaccination through the last study visit (Day 360).

8.3.1 Reporting Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)

6500 Rock Spring Dr. Suite 650

Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the DCC system. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

8.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site Principal Investigator or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected unexpected serious adverse reaction (SUSAR). DMID will report an AE as a suspected unexpected adverse event only if there is evidence to suggest a causal relationship between the study intervention and the AE. DMID will submit an IND safety report to the FDA and will notify all participating site Principal

Investigators (i.e., all Principal Investigators to whom the sponsor is providing drug under its IND(s) or under any Principal Investigator's IND(s) of potential serious risks from clinical studies or any other source, as soon as possible. DMID 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, DMID 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.

All SAEs designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

8.3.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via Advantage eClinical® on the Pregnancy Report eCRF. No further study vaccinations will be administered to pregnant subjects, but 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.

8.4 Type and Duration of Follow-up of Subjects after Adverse Events

Solicited AEs are reactogenicity events occurring on the day of each study vaccination through Day 8 after each study vaccination. Unsolicited AEs are non-serious adverse events occurring from the time of the first study vaccination until Day 57, then from Day 180 through Day 208. AEs will be assessed, and followed from initial recognition of the AE through end of the protocol defined follow-up period. AEs will be followed to adequate resolution. Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

SAEs will be collected from the time of the first study vaccination through the last study visit (Day 360). SAEs will be followed to adequate resolution or stabilization even if this extends beyond the study-reporting period.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate eCRF.

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

Samples will be collected for hematology, serum chemistry and urinalysis. The study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator must review the laboratory results, document this review, and record attribution to study product and clinical significance for all abnormal laboratory values on the Local Laboratory eCRF. Abnormal laboratory results meeting the protocol-defined toxicity grading scale are considered adverse events; however, they ARE NOT reported as unsolicited AEs on the Adverse Event eCRF. The laboratory reports must be filed with the source documents.

8.6 Halting Rules

8.6.1 Study Halting Criteria

Further enrollment and study vaccinations 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 vaccination.
- Two or more subjects who have received at least one dose of study vaccine to date experience the same (at MedDRA PT level) severe (grade 3) study vaccine-related unsolicited adverse event.
- Two or more subjects who have received at least one dose of study vaccine to date experience a severe (grade 3) study vaccine-related laboratory adverse event in the same laboratory parameter.
- Two or more subjects who have received at least one dose of study vaccine to date experience the same severe (grade 3) solicited injection site adverse event that persists for 3 or more days

and does not resolve or decrease to a lower grade (The size [measured in mm] of ecchymosis, erythema and induration/swelling will not be used as a halting criterion).

- Two or more subjects who have received at least one dose of study vaccine to date experience the same severe (grade 3) study vaccine-related solicited systemic adverse event that persists for 3 or more days and does not resolve or decrease to a lower grade (Subjective systemic reaction corroborated by study personnel).
- Any subject experiences a study vaccine-related laryngospasm, bronchospasm, or anaphylaxis within 1 day of study vaccination.
- Any subject experiences a study vaccine-related allergic reaction (e.g., generalized urticaria) within 3 days of study vaccination.
- Any subject experiences a study vaccine-related SAE from the time of the first study vaccination through the subject's last study visit.

Grading scales for solicited local (injection site) and systemic (subjective and quantitative) reactions are included in [Section 8.1.2](#).

If any of the halting rules above are met following any subject receipt of any vaccination, the study will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the SMC to proceed. DMID retains the authority to suspend additional enrollment and study interventions/ administration of study product during the entire study, as applicable.

8.6.2 Individual Halting Criteria

Individual subjects will not be administered boost vaccination doses (2nd or 3rd vaccination) if he or she experiences ANY of the following:

- Anaphylaxis within 1 day after administration of a study vaccine.
- Allergic reaction (e.g., generalized urticaria) within 3 days after administration of a study vaccine.
- A serious adverse event (SAE) that is considered to be study vaccine-related.
- A severe (grade 3) laboratory adverse event that is considered to be study vaccine-related and persists for 3 or more days.
- A severe (grade 3) unsolicited adverse event that is considered to be study vaccine-related and does not resolve or decrease to a lower grade.

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- A severe (grade 3) solicited systemic adverse event that is considered to be study vaccine-related and persists for 3 or more days and does not resolve or decrease to a lower grade (Subjective systemic reaction corroborated by study personnel).
 - A severe (grade 3) solicited injection site adverse event that persists for 3 or more days and does not resolve or decrease to a lower grade. (The size [measured in mm] of ecchymosis, erythema, and induration/swelling will not be used as a halting criterion).

Vaccinations for an individual subject may be suspended for safety concerns other than those described above, at the discretion of the investigator if he/she feels the subject's safety may be threatened. The investigator may ask for an *ad hoc* SMC meeting to be held for any single event or combination of multiple events which, in his/her professional opinion, could jeopardize the safety of the subjects or the reliability of the data.

8.7 Safety Oversight (ISM, SMC)

8.7.1 Independent Safety Monitor (ISM)

For certain clinical trials, DMID will require an Independent Safety Monitor (ISM) to be assigned for each study site and the requirement for an ISM will be specified in the protocol. An ISM is a physician with relevant expertise whose primary responsibility is to provide to DMID an independent safety assessment in a timely fashion. The ISM will review SAEs in real time and other AEs as needed and provide an independent assessment to DMID.

For this clinical trial an ISM is required. Participation is for the duration of the DMID study and is a voluntary position that does not receive payment. The ISM must meet the requirements of the NIAID conflict of interest policy. The ISM:

- Is in close proximity to the study site and has the authority and ability to readily access study participant records in real time.
- May be a member of the participating institution's staff but preferably be from a different organizational group within the institution.
- Should not be in a direct supervisory relationship with the investigator.
- Should have no direct involvement in the conduct of the study.

The ISM will:

- Sign a COI certification at the time they are asked to participate and provide updates to this information as needed.

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- Receive reports of Serious Adverse Events (SAEs) from the site investigator and will be notified by email when DMID is notified of the SAE.
 - Evaluate the SAE and report their clinical assessment to DMID, through DMID-CROMS SOCS in a timely manner using the attached report form and email the report to DMID-CROMS SOCS.
 - Communicate with the investigator at the participating site as needed.
 - Review additional safety related events at the request of DMID.
 - Provide additional information to DMID and/or the SMC by teleconference as requested.

8.7.2 Safety Monitoring Committee (SMC)

This clinical study will utilize a SMC, which is an independent group of experts that advises the DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to the DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in a SMC charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study.

As defined in the charter, the SMC will review data at specified times during the course of the study for subject and overall study progress, and will conduct ad hoc reviews as appropriate when a halting rule is met or for immediate concerns regarding observations during this study.

The SMC will review study progress and subject clinical, safety, and reactogenicity data at the following time points:

- The SMC will be provided with top line safety data through Day 57 for all participants.
- Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded safety and efficacy data for this trial. The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID.
- *Ad hoc* when a halting rule is met, for immediate concerns regarding observations during this trial, or as needed.

9 HUMAN SUBJECTS PROTECTION

9.1 Institutional Review Board/Independent Ethics Committee

Each site principal investigator will obtain IRB approval for this protocol to be conducted at his/her research site(s), and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-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/IEC before they are implemented. IRB/IEC 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 and the IRB/IEC. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

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.

9.2 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 clinical trial, verbally and with a written consent form. The explanation will be organized, and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study, 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/products. This will include aspects of the trial that are experimental, the probability for random assignment to treatment groups, 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 procedures that may be available and the important potential benefits and risks of these available alternative 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 investigator) 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 monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical 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 informed consent form, 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 specimens will be used for additional research, even if identifiers are removed.

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

Informed consent forms will be IRB-approved and subjects will be asked to read and review the consent form. Subjects must sign the informed consent form prior to starting any study procedures being done specifically for this trial.

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

Study personnel may employ 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, site 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.

New information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

9.3 Consent for Future Use of Stored Specimens and Data

Residual samples are those that are left over after the study has been completed and are stored for possible use in future research studies. Future use samples are extra tube(s) of blood collected and stored for possible use in future research studies. Retention of residual and future use samples and the potential for use in future research studies will be a condition of study participation. Subjects who sign the informed consent form for the study are consenting to allow the collection, storage and use of any residual samples (serum or cells derived from venous blood samples) or future use samples for possible use in future research studies. Subjects may withdraw consent for study participation at any time by notifying the study doctors or nurses in writing. Data from sample(s) collected prior to the withdrawing consent will not be removed. There will be no further use of residual samples or collection and use of future use samples after consent has been withdrawn.

9.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all healthy adults who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, sex, or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations.

Children will be excluded from this Phase I trial since safety and immunogenicity data for HEV 239 in US healthy adult populations are currently lacking.

9.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. This confidentiality extends to research and future use test results, in addition to the clinical information relating to participating subjects. No information concerning the study or the data generated from the study will be released to any third party without prior written approval of the DMID. Subject confidentiality will be maintained when study results are published or discussed in conferences. The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study 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 specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

9.6 Certificate of Confidentiality

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

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, and for the purposes of other research that is in compliance with applicable Federal regulations governing the protection of human subjects in research.

9.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, or by the participating site for any injury suffered due to participation in this trial.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

The primary goal of this study is to assess the safety and immunogenicity of HEV 239 administered in three successive intramuscular injections. This study will also collect blood for future use. This study, like other Phase I studies, is exploratory rather than confirmatory, and thus is not designed to formally test any hypotheses.

10.2 Sample Size Considerations

A total of 25 subjects, aged 18 to 45 years (inclusive), will be enrolled and randomized to HEV 239 vaccine (20 subjects) versus placebo (5 subjects) in a blinded manner. Each subject will receive three study injections (0, 1, and 6 months). The sample size for this study was selected to obtain preliminary estimates of HEV 239 vaccine safety and immunogenicity in a time sensitive manner.

Given an N of 20 vaccinees, there is an ~88% probability of detecting an adverse event occurring in 10% of the population and a ~99% probability of detecting an adverse event occurring in 20%. Placebo subjects are included in order to reduce observer bias and are not intended to be a comparison group. Probabilities of observing at least one adverse event for a given true event rate are presented in the table below.

Table 7: Probability (%) of Observing at Least One Adverse Event

Adverse Event Frequency	N = 20
0.01% Rare	0.20
0.1% Uncommon	1.98
1% Common	18.21
10% Very Common	87.84
20% Very Common	98.85

10.3 Treatment Assignment Procedures

10.3.1 Randomization Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at each participating VTEU 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 SDCC Advantage eClinical® (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subject will be enrolled. Subjects will be assigned randomly to receive three IM doses of either placebo or HEV 239 vaccine. The randomization scheme for this study is listed below and also presented in [Figure 1](#).

- HEV 239 vaccine group (N=20) will receive a three-dose regimen of HEV 239 administered IM on Days 1, 29, and 180.
- Placebo group (N=5) will receive three-doses of administered placebo IM on Days 1, 29, and 180.

Enrollment of subjects will be done online using the enrollment module of Advantage eClinical®. The randomization code will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. Advantage eClinical® will assign each subject to a group 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.

10.3.2 Masking Procedures

This is a double-blind study. Subjects, investigators, study personnel performing any study-related assessments following study vaccine administration, and laboratory personnel performing antibody assays will be blinded to whether the subject received placebo or HEV 239.

The randomization scheme will be generated by the SDCC and provided to unblinded study personnel including pharmacists performing study vaccination preparations and unblinded study vaccine administrator.

The unblinded study vaccine administrator is a study personnel member credentialed to administer vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration. He or she will only be involved with vaccine administration.

10.4 Planned Interim Analyses

No formal interim analysis is planned.

10.5 Final Analysis Plan

A formal SAP will be developed and finalized prior to any analysis, or database lock.

10.5.1 Analysis Populations

The Safety Analysis population includes all subjects who received at least one study vaccination.

The modified intent-to-treat (mITT) population includes all subjects who received at least one study vaccination and contributed both pre- and at least one post-study vaccination blood samples for immunogenicity testing for which valid results were reported. Subjects will be analyzed according to the study arm to which they were randomized.

The per protocol (PP) population includes all subjects in the mITT subset with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline.
- Data from all visits subsequent to major protocol deviations, such as:
 - Second or third study vaccination not received,
 - Receipt of non-study licensed live vaccine within 30 days prior to or after each study vaccination,
 - Receipt of non-study licensed inactivated vaccine within 14 days prior to or after each study vaccination,
 - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days prior to or after each study vaccination.
- Data from any visit that occurs substantially out of window.
- In the case of miss-randomization, subjects will be analyzed according to the study product actually received.

10.5.2 Safety Data

Safety data will be summarized for the Safety Analysis Population. Subjects receiving a vaccination according to a study arm other than the study arm to which they were randomized, will be analyzed according to the vaccination they actually received. Solicited AEs will be summarized by severity for each day after each study vaccination for Days 1-8 after each study vaccination. The number, percentage (observed rate), and exact two-sided 95% CI for subjects reporting each solicited AE within 8 days following vaccination will be summarized. In addition, maximum severity for each solicited AE will be determined for each subject and reported by study arm and the resulting number and percentage of subjects will be summarized by severity grade (none, mild, moderate, severe). Summaries of solicited AEs will be presented separately by study arm for each study vaccination as well as overall study vaccinations.

All AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). The final clinical study report will include a summary of all SAEs as well as a summary of all SAEs by relatedness to study product. The number of SAEs considered related to study vaccination are likely to be small in this trial and will be reported by detailed listings showing the event description, MedDRA® preferred term and SOC, relevant dates (study vaccinations and AEs), severity, relatedness, and outcome for each event. Non-serious unsolicited vaccine-related AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA® preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the proportion of subjects and exact 95% confidence intervals of AEs by study arm for each study vaccination as well as overall study vaccinations and by MedDRA® categories will be calculated.

The number and percentage (observed rate) of subjects that withdrew or discontinued their vaccination series will be tabulated for each study arm.

10.5.3 Immunogenicity Data

Immunogenicity data summaries and analysis will be presented for the mITT and PP populations.

Immune responses for antibodies against HEV-specific IgM and IgG will be summarized at each time point by study arm. GMCs and corresponding 95% confidence intervals will be reported. The number and percentage of subjects with seroconversion (defined as crossing the threshold from negative to positive) and 4-fold rise from baseline will be reported for each post-vaccination time point.

Graphical presentations of immune response may include reverse cumulative distribution (RCD) curves, and longitudinal presentation of GMCs.

10.5.4 Missing Values and Outliers

All attempts will be made to collect all data per protocol. As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

11 ELECTRONIC CASE REPORT FORMS AND ACCESS TO SOURCE DATA

The participating VTEU site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents (paper and electronic), which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, 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 the 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 trial.

The study uses direct data entry for the participating clinic site and a web-based e-Memory aid for subjects. All eCRFs serve as the source documents. Subjects will be trained to use a database to complete a web based “e-Memory aid” and are expected to enter information in the e-Memory aid each day. Subjects using the e-Memory aid will also be provided a paper memory aid for their use in the event they are unable to access the web-based system. The subjects will be asked to enter the information from the paper memory aid into the e-Memory aid once they are able to access the web-based system.

Subjects will record temperature, local and systemic symptoms and any new medications used following vaccination or changes to previously reported medications daily for 8 days after any vaccination.

Subjects will be instructed to contact the clinic staff immediately if they experience severe symptoms at any time during the study, for prompt follow-up in real time. The study clinic will be alerted in real time of any potential solicited events of Grade 3 severity entered in the e-Memory Aid. An email alert will be sent to the clinic site and the Emmes study team. Within one business day of site awareness, the site must attempt to follow up with the subject on the severe solicited event and send an email to Emmes confirming attempted follow up with the subject. Instructions for completing the e-Memory aid are provided in the MOP and in a

separate e-Memory aid instructions document that will be provided to the subjects at each vaccination. The site staff must review the e-Memory aid information and interview the subject at the next scheduled visit. The subject-entered data will be available for review by the clinician during the clinical interview.

The site staff will be the data originators for the clinically reviewed data in Advantage eClinical® that will be used for the study endpoints. A list of all authorized site staff data originators will be included on the Study Personnel/Signature Responsibility List.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating 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 source data/data collection forms, 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 documentations are maintained on site.

The SDCC 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 participating site for clarification and resolution.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

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

Electronic case report forms will be created by the SDCC to record and maintain data for each subject enrolled in this study.

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the eCRF.

13.2 Data Management Responsibilities

All electronic case report forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events must be recorded on the appropriate electronic case report form, assessed for severity and relationship, and reviewed by the site principal investigator or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

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

13.3 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), reactogenicity and immunogenicity data will be entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the SDCC. 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 be entered directly into electronic case report forms by the study personnel.

13.4 Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, and immunogenicity data).

13.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study drug disposition records will be retained for 2 years after a marketing application is approved for the study product 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 study product, 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 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 principal investigator when these documents no longer need to be retained. The participating VTEU sites must contact DMID for authorization prior to the destruction of any study records.

14 CLINICAL MONITORING

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

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

15 PUBLICATION POLICY

Following completion of the study, the lead Principal Investigator is expected to publish the results of this research in a scientific journal. 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 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 results 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 trial the responsible party is DMID, which will register the 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

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17 APPENDICES

Appendix A. Schedule of Events

Study Visit	Screening	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	
Study day from 1 st vaccine	Screening (≤28 days) ^{a,b}	Day 1 ^c	Day 4** +/-1	Day 8 +/-1	Day 15 +/-2	Day 29 +/-2	Day 32**	Day 36	Day 43	Day 57	Day 120**	Day 180 +/- 14	Day 184 **	Day 187	Day 194	Day 208	Day 270**	Day 360 +/-28	Early Termination/ Unscheduled
Study day from 2 nd vaccine						Day 1	Day 4** +/-1	Day 8 +/- 1	Day 15 +/-2	Day 29 +/-2	Day 92** +/- 14								
Study day from 3 rd vaccine												Day 1	Day 4** +/-1	Day 8 +/- 1	Day 15 +/-2	Day 29 +/-2	Day 90** +/-14	Day 180 +/- 14	
Obtain Informed Consent	X ^b																		
Medical History*	X	X				X						X							
Serum pregnancy test ^e	X																		
Urine pregnancy test ^e		X ^h				X ^h						X ^h							{X}
Review of birth control history with female subjects	X	X ^e				X ^e						X ^e							X
Counsel on pregnancy avoidance ^e	X	X		X	X	X		X	X	X	X	X		X	X	X			{X}
Physical examination ⁱ	X	{X} ^h		{X}	{X}	{X} ^h		{X}	{X}	{X}		{X} ^h		{X}	{X}	{X}		{X}	{X}
Vital signs ^j	X	X		X	X	X		X	X			X		X	X				{X}
Review of Eligibility Criteria	X	X ^{c,h}				X ^h						X ^h							

Study Visit	Screening	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	Early Termination/ Unscheduled
Study day from 1 st vaccine	Screening (≤28 days) ^{a,b}	Day 1 ^c	Day 4** +/-1	Day 8 +/-1	Day 15 +/-2	Day 29 +/-2	Day 32** +/-1	Day 36	Day 43	Day 57	Day 120** +/- 14	Day 180 +/- 14	Day 184 **	Day 187	Day 194	Day 208	Day 270** +/-28	Day 360 +/-28	
Study day from 2 nd vaccine						Day 1	Day 4** +/-1	Day 8 +/- 1	Day 15 +/-2	Day 29 +/-2	Day 92** +/- 14								
Study day from 3 rd vaccine												Day 1	Day 4** +/-1	Day 8 +/- 1	Day 15 +/-2	Day 29 +/-2	Day 90** +/-14	Day 180 +/- 14	
Pre-Administration Reactogenicity Assessments		X ^h				X ^h						X ^h							
Randomization		X ^h																	
Study vaccine administration		▲				▼						▼							
Distribution of memory aid		X ^k				X ^k						X ^k							
Review of subject diary/solicited events by staff			X	X	X		X	X	X				X	X	X				{X}
AE/SAE Assessment ^m		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	{X}
Evaluate vaccination site		X		X	{X}	X		X	{X}			X		X	{X}				{X}
Post-Administration Reactogenicity Assessments		X				X						X							
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X ^l	X	X	X	X	X	X ^l	X ^l	X ^l
Hematology, chemistry ^f (mL)	13 ⁿ			13	{13}			13	{13}			13 ^h		13	{13}				{13}
Hepatitis B surface antigen, hepatitis C antibody, HIV types 1 and 2 antigen/antibody	10																		
Urine drug screen	X																		
Hepatitis E IgM and IgG antibodies	10																		
Serum: Immunogenicity (mL)		10 ^h		10	10	10 ^h		10	10	10		10 ^h		10	10	10		10	{10}

Study Visit	Screening	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	
Study day from 1 st vaccine	Screening (≤28 days) ^{a,b}	Day 1 ^c	Day 4** +/-1	Day 8 +/-1	Day 15 +/-2	Day 29 +/-2	Day 32**	Day 36	Day 43	Day 57	Day 120**	Day 180 +/- 14	Day 184 **	Day 187	Day 194	Day 208	Day 270**	Day 360 +/-28	Early Termination/ Unscheduled
Study day from 2 nd vaccine						Day 1	Day 4** +/-1	Day 8 +/- 1	Day 15 +/-2	Day 29 +/-2	Day 92** +/- 14								
Study day from 3 rd vaccine												Day 1	Day 4** +/-1	Day 8 +/- 1	Day 15 +/-2	Day 29 +/-2	Day 90** +/-14	Day 180 +/- 14	
PBMC and Plasma Collection for Future Use		48 ^h		16	36	48 ^h		16	36	32		48 ^h		16	36	32		32	{≤48}
Total Blood Volume per visit (mL) ^a	33	58		39	{59}	58		39	{59}	42		71		39	{59}	42		42	{71}
Cumulative Blood Volume ^a	33	91		130	{189}	247		286	{345}	387		458		497	{556}	598		640	

▲Prime dose: *HEV 239 or Placebo*; ▼Boost dose: *HEV 239 or Placebo*;

NOTE 1: In case of early withdrawal due to an adverse event, the investigator will collect all information relevant to the AE and safety of the subject, and will follow the subject to resolution, or until reaching a clinically stable endpoint. If feasible, blood will be drawn for immunologic assays. Subjects who wish to withdraw consent will be offered an optional visit for safety follow-up (before the formal withdrawal of consent). The subject has the right to refuse.

- Screening may be split into multiple days or visits. This will include collection of baseline demographics and subject contact information.
- Signing of the informed consent form (ICF) needs to be done before the first study-related activity.
- The investigators should ensure that all study enrollment criteria have been met at the end of the screening period. If a subject's status changes (including laboratory results or the receipt of additional medical records) after screening but before Day 1 such that the subject no longer meets all eligibility criteria, then the subject should be excluded from participation in the study.
- Pre-study medications and therapies will be reviewed and recorded for the 30 days prior to the start of screening.
- Subjects will be counselled to use adequate birth control methods to avoid pregnancy.
- Baseline laboratories include complete blood count with differential (which will include white blood cells, absolute neutrophil count (ANC), absolute eosinophil count (AEC), hemoglobin, and platelets), creatinine, glucose, Alanine aminotransferase (ALT), hepatitis B surface antigen, hepatitis C antibody, HIV

types 1 and 2 antigen/antibody, hepatitis E IgM and IgG antibodies, and urine drug screen ([Section 2.3.1](#)). Retesting of values that lead to exclusion is allowed once using an unscheduled visit during the screening period, provided there is an alternative explanation for the out of range value. If the initial laboratory screening occurred more than 28 days before baseline (Day 1) but the subject was unable to be vaccinated within the 28-day window (e.g., due to meeting [Exclusion Criteria 5.1.2](#)), the subject must have safety laboratories repeated (see protocol-specific MOP for details). Safety laboratories will be obtained on Days 8, 36, 180, and 187 will include complete blood count with differential (which will include white blood cells, ANC, AEC, hemoglobin, and platelets), Cr, and ALT ([Section 2.3.1](#)).

h) Prior to study vaccine administration.

i) An abbreviated physical examination will be carried out at screening. At other visits, symptom-directed examination will be performed as indicated by the investigator.

j) Includes heart rate, blood pressure and oral body temperature after at least 5 minutes rest. To be assessed prior to study vaccine administration and at a minimum of 30 minutes after study vaccine administration. At screening this will also include height and body weight for determination of body mass index (BMI).

k) When the memory aid is distributed to subjects, they will also be instructed as to when to contact the investigator about unsolicited AEs.

Hypersensitivity symptoms will also be reviewed with the subject and they will be counselled that if any of these were occur they must contact study personnel or seek medical attention immediately.

l) Concomitant therapies between D57 and D150 and from D208 – D360 should only be recorded if administered in conjunction with SAEs.

m) Solicited AEs: Inclusive of solicited injection site reactions and systemic reactogenicity events performed on the day of each study vaccination through at least D8 after each study vaccination (see [Sections 8.1.2](#)). Unsolicited AEs: Collection of unsolicited AEs from time of first vaccination through D57; then from Day 180 – 208. SAEs: SAEs will be collected from the time of the first study vaccination through the last study visit (Day 360).

n) Volumes are the maximum volumes expected to be drawn. Samples may be fasted or non-fasted. The total volume at an unscheduled or early termination visit will not exceed the individual time point volume.

{ } If indicated based upon timing and/or symptoms.

* See [Section 7.1.1](#) regarding details of medical history to be collected.

** Telephone Visit

Appendix B.

Table 8: TOXICITY TABLE

Laboratory Adverse Events				
Blood, Serum, or Plasma	Normal Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Creatinine – mg/dL (Female)	0.5 – 1.10	≥1.11 – 1.79	≥1.80-2.09	≥2.10
Creatinine – mg/dL (Male)	0.6 – 1.35	≥1.36 – 1.79	≥1.80-2.09	≥2.10
ALT U/L (Female)	6 – 29	30 - 105	106-175	>175
ALT U/L (Male)	9 – 46	47 - 105	106-175	>175
Hemoglobin (Female) - g/dL	11.7 – 15.5	11.0-11.6	9.5-10.9	<9.5
Hemoglobin (Male) - g/dL	13.2 – 17.1	12.0 – 13.1	10.0 – 11.9	<10.0
WBC Increase (Thousand/uL)	3.8 – 10.8	10.9 – 15.0	15.1 – 20.0	> 20.0
WBC Decrease (Thousand/uL)	3.8 – 10.8	2.5-3.7	1.5-2.4	<1.5
Absolute Neutrophil Count Decrease (cells/uL)	1500 – 7800	1490 – 1499	1000-1489	<1000
Absolute Eosinophil Count Increase (cells/uL)	15 – 500	501-749	750-1500	>1500
Platelets Decreased (Thousand/uL)	140 – 400	120-139	100-119	<100

Clinical Adverse Events			
Category	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Allergic reaction	Pruritus without rash*	Localized urticarial OR requires oral therapy	Generalized urticaria; angioedema OR anaphylaxis OR requires epinephrine
Illness or clinical adverse event	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.	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	Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

* Not at injection site.

Table 9: VITAL SIGNS REFERENCE RANGES

VITAL SIGNS	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever (°C) *	38.0 – 38.4	38.5 – 38.9	≥39.0
Tachycardia - beats per minute	101 – 115	116 – 130	>130 or ventricular dysrhythmias
Bradycardia - beats per minute	45 - 50 bpm	40 – 44 bpm	<40 bpm
Hypertension (systolic)- mm Hg**	141-150	151-160	>160
Hypertension (diastolic) - mm Hg**	91-95	96-100	>100
Hypotension (systolic) - mm Hg**	85-89	80-84	< 80
Hypotension (diastolic) – mm Hg**	50 – 54	45 – 49	<45

* Oral temperature; no recent hot or cold beverages or smoking.

** Assuming subject is awake, and resting for AEs; 3 measurements on the same arm with concordant results.