Mucosal immune responses against *Neisseria gonorrhoeae* following meningococcal immunization in healthy young adults

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human subjects research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

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 Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- 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

SIGNATURE PAGE

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.

Site Investigator Signature:

Signed: _____ Date: _____

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The Hope Clinic of the Emory Vaccine Center

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LIST OF ABBREVIATIONS

4CMenB	Bexsero® or multi-component meningococcal serogroup B vaccine
ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event/Adverse Experience
aPTT	Activated Partial Thromboplastin Time
BLA	Biologics License Applications
BMI	Body Mass Index
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
COVID-19	Coronavirus disease 2019
CRF	Case Report Form
CRO	Contract Research Organization
CROMS	Clinical Research Operations and Management Support
CSR	Clinical Study Report
CT	*Chlamydia trachomatis*
DCC	Data Coordinating Center
DGI	Disseminated Gonococcal Infection
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS

eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FHbp	Factor H Binding Protein
FWA	Federal Wide Assurance
GC	*Neisseria gonorrhoeae*
GCP	Good Clinical Practice
GMFR	Geometric Mean Fold Rise
GMT	Geometric Mean Titer
HEENT	Head/Ear/Eyes/Nose/Throat
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ICS	Intracellular Cytokine Staining
IDCRC	Infectious Diseases Clinical Research Consortium
IDE	Investigational Device Exemption
IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee

IgA	Immunoglobin A
IgG	Immunoglobin G
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IUD	Intrauterine Device
JAMA	Journal of the American Medical Association
LLC	Limited Liability Corporation
MAAEs	Medically-Attended Adverse Events
MBC	Memory B Cell
MedDRA®	Medical Dictionary for Regulatory Activities
MeNZB	New Zealand Meningococcal Group B Vaccine
mL	Milliliter
mm	Millimeter
MM	Medical Monitor
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NAAT	Nucleic Acid Amplification Test
NadA	Neisserial Adhesion A
NDA	New Drug Application
NEJM	New England Journal of Medicine
NHBA	Neisserial Heparin Binding Antigen

NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NSAID	Non-Steroidal Anti-Inflammatory Drug
OER	Office of Extramural Research
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
OMV	Outer Membrane Vesicle
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate-Buffered Saline
PHI	Protected Health Information
PI	Principal Investigator
PID	Pelvic Inflammatory Disease
PK	Pharmacokinetics
PLT	Platelet Count
PT	Prothrombin Time
QA	Quality Assurance
QC	Quality Control
RPR	Rapid Plasma Reagin
SAE	Serious Adverse Event/Serious Adverse Experience
SAP	Statistical Analysis Plan
SBA	Serum Bactericidal Antibody
SDCC	Statistical and Data Coordinating Center
SMA	Secondary Medical Assessor

SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
SUSAR	Suspected Unexpected Serious Adverse Event
US	United States
USP	United States Pharmacopeial Convention
VTEU	Vaccine and Treatment Evaluation Unit
WBC	White Blood Count
WFI	Water For Injection
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Mucosal immune responses against *Neisseria gonorrhoeae* following meningococcal immunization in healthy young adults
Design of the Study:	This is a mechanistic clinical trial to assess the systemic and mucosal immunogenicity of the multicomponent meningococcal serogroup B vaccine (4CMenB or Bexsero®) (group 1, approximately 40 subjects) against *N. gonorrhoeae* (GC), using a placebo vaccine (normal saline) as a comparator (group 2, approximately 10 subjects).
Study Phase:	2
Study Population:	This trial will enroll approximately 50 (maximum of 60 subjects) healthy male and non-pregnant female subjects aged 18 to 49 years, and will be conducted in the US
Number of Sites:	1 (Emory University VTEU)
Description of Study Product or Intervention:	The active product will be Bexsero® (4CMenB), a FDA-approved multi-component meningococcal serogroup B vaccine that contains outer membrane vesicles (OMVs) containing PorA serosubtype P1.4, as well as three recombinant protein antigens (neisserial adhesion A [NadA], factor H binding protein [FHbp] fusion protein, and neisserial heparin binding antigen [NHBA] fusion protein). 4CMenB is presented as a single-dose prefilled syringe that is administered intramuscularly on a 2 dose (0.5-Milliliter (mL) each) schedule, with the second dose to be given 1 month apart from the first. The comparator product will be a normal saline placebo.
Study Objectives:	Primary: • To characterize the rectal mucosal Immunoglobin G (IgG) antibody response to GC elicited by the 4CMenB

vaccine as compared with the placebo vaccine in healthy adult subjects.

Secondary:

- To characterize the serum IgG antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To assess the safety and reactogenicity of 4CMenB in healthy adult subjects.
- Exploratory:
- To characterize the vaginal mucosal IgG antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the oropharyngeal mucosal IgG antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the serum Immunoglobin A (IgA) antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the mucosal IgA antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the systemic B cell responses to GC antigens elicited by 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the systemic T cell responses to GC antigens elicited by 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.

- To characterize the mucosal T cell responses to GC antigens elicited by 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the serum bactericidal antibody responses to GC elicited by 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To evaluate the impact of 4CMenB vaccine-induced antibodies on gonococcal adhesion to human cervical cells.
- To characterize immune responses to N. meningitidis elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects for use as controls.

Duration of Individual Subject Participation:	The duration of each subject's participation is approximately 8 months, from recruitment through the last study visit.
Estimated Time to Last Subject/Last Study Day:	Approximately 14 months. The enrollment period will be approximately 6 months and subjects will be followed through 6 months after their first vaccination.

Table 1: Treatment Arm Goals*

	Rectal Mucosal Biopsy Cohort (N=20)		No Rectal Mucosal Biopsy Cohort (N=30)		Total
Vaccine	Male	Non-pregnant Female	Male	Non-Pregnant Female	
4CMenB	8	8	12	12	40
Placebo	2	2	3	3	10

*As noted in the study design, this trial will enroll approximately 50 (maximum of 60 subjects) healthy male and non-pregnant female subjects aged 18 to 49 years, randomized in a ratio of 4 to 1 4CMenB to placebo

1 KEY ROLES

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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Gonorrhea is a sexually transmitted infection (STI) caused by the gram-negative diplococcus GC. It causes urethritis and cervicitis in sexually active men and women respectively, and in women can lead to upper genital tract disease (i.e., pelvic inflammatory disease (PID)), which is a risk factor for ectopic pregnancy and infertility. Extragenital infections of other mucosal sites (e.g., rectum and pharynx) are also possible, as are invasive infections (i.e., disseminated gonococcal infection, or DGI) such as gonococcal arthritis/dermatitis syndrome, septic arthritis, and endocarditis. Finally, perinatally acquired infection – which typically occurs among newborns of asymptomatic women – can cause sight-threatening conjunctivitis (ophthalmia neonatorum).

In 2012 the World Health Organization estimated approximately 78 million new cases of gonorrhea globally.[1] In the United States, gonorrhea is the second most commonly reported notifiable disease, with more than 550,000 cases reported in 2017. Rates of gonococcal disease have been steadily increasing since 2009 irrespective of age, sex, ethnic group, or geographic region.[2]

Though gonococcal infections are curable with antibiotic therapy, antimicrobial resistance has become a global public health problem. The prevalence of resistance to multiple antibiotic classes has increased gradually since the 1940s[2], and GC isolates with reduced susceptibility to current first-line therapy – ceftriaxone and azithromycin – have emerged.[3] Given the large and growing burden of disease, and the threat of untreatable gonococcal infections due to antimicrobial resistance, a safe and effective vaccine is urgently needed. Unfortunately, previous efforts to develop a gonorrhea vaccine have been largely unsuccessful.

Recently, a retrospective case-control study in New Zealand demonstrated 31% (95% confidence interval (CI), 21-39) effectiveness of an OMV meningococcal group B vaccine (MeNZB) against incident gonorrhea.[4] The same group of investigators also reported 24% (95% CI, 1-42) vaccine effectiveness against hospitalization caused by gonorrhea.[5] Previous ecological studies had suggested a possible protective effect of OMV vaccines against gonorrhea in Norway[6] and Cuba.[7] Meningococcal OMV vaccines have been used successfully to control epidemic strains of *N. meningitidis* serogroup B.[8] These vaccines elicit serum bactericidal antibody (SBA) responses directed primarily against meningococcal strain-specific porin protein, PorA.[9] The precise mechanism of cross-protection conferred by meningococcal OMV vaccines against GC remains unknown.

A four component meningococcal serogroup B vaccine (Bexsero® or 4CMenB) was recently licensed for use in the United States and was given a category B recommendation by the Advisory Committee on Immunization Practices (ACIP) for use in adolescents and young adults up to 25 years of age.[10] Bexsero includes OMVs containing PorA serosubtype P1.4, as well as three recombinant protein antigens (neisserial adhesion A [NadA], factor H binding protein [FHbp] fusion protein, and neisserial heparin binding antigen [NHBA] fusion protein).[11] An analysis of gonococcal incidence following a 4CMenB vaccination campaign in one region of Quebec, Canada found a non-significant reduction in gonorrhea cases in the post-vaccination period[12], supporting the findings from New Zealand, and suggesting that 4CMenB vaccine could protect against gonorrhea.

A recent study found a high level of amino acid sequence identity between the 4CMenB OMV antigens and those in the MeNZB OMV in the vaccine studied in New Zealand, and with OMV homologues in a laboratory strain of GC. The same study found that rabbits vaccinated either with MeNZB or the OMV component of 4CMenB raised antibodies against GC, and that 4CMenB vaccination in humans elicited serum antibodies against GC.[13] In contrast, another study found vaccination with a native OMV vaccine raised SBA responses against GC in mice, but 4CMenB vaccination did not elicit similar SBA responses in humans.[14]

Given the potential public health impact of an effective vaccine against gonorrhea, a better understanding of the immunologic responses to GC following 4CMenB vaccination is needed. Specifically, although two studies have characterized antibody responses against GC following 4CMenB in serum, gonorrhea is a mucosal disease. While there are studies looking at the ability of meningococcal serogroup B vaccines to modulate human nasopharyngeal mucosal immunity to *N. meningitidis*, there are no data on the impact of 4CMenB on immunologic responses at the human mucosal surfaces where GC is typically encountered (urethra, rectum, cervix, and pharynx).

2.2 Scientific Rationale

2.2.1 Purpose of Study

This is a mechanistic clinical trial to assess the systemic and mucosal immunogenicity of the multicomponent meningococcal serogroup B vaccine (4CMenB or Bexsero®) against GC, using a placebo vaccine (normal saline) as a comparator, in healthy young adults.

4CMenB is licensed for use in those aged 10-25 years and is administered intramuscularly as a two dose-series, with the second dose given at least one month after the first dose.

2.2.2 Study Population

The study population will include approximately 50 males and non-pregnant females, 18 to 49 years of age, inclusive, who are in good health and meet all eligibility criteria.

Subjects will be recruited from the community at large and enrolled at 1 VTEU site (Hope Clinic, Emory VTEU).

This age range was selected to represent the population with the highest incidence of gonorrhea[2] therefore children are excluded from this study. There are no plans currently to include children in future studies of mucosal immune responses to GC after meningococcal immunization.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential risks of participating in this trial are those related to having venous blood drawn, the intramuscular injections, possible reactions to the 4CMenB or placebo vaccines, undergoing oropharyngeal, vaginal and rectal mucosal sampling, and breach of confidentiality.

Blood draw and Intramuscular (IM) injection

Drawing blood may cause transient discomfort and fainting and lead to or exacerbate iron deficiency in those who are not anemic. Fainting is usually transient and managed by having the subject lie down and elevating 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.

Bexsero® (4CMenB)

Bexsero® was licensed by the FDA for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroup B in 2005 and is approved for use in individuals aged 10 through 25 years.

A comprehensive description of the risks of adverse events (AEs) are described in the package insert.[11] The most common (frequency ≥10%) solicited adverse reactions observed in clinical trials were pain at the injection site (≥83%), myalgia (≥48%), erythema (≥45%), fatigue (≥35%), headache (≥33%), induration (≥28%), nausea (≥18%), and arthralgia (≥13%).

From post marketing experience, lymphadenopathy been reported after receiving Bexsero. Since this was voluntarily reported from a population of uncertain size, the incidence rate cannot be estimated.

There are limited safety data for 4CMenB in adults over age 25 years. Although the vaccine is licensed for use in individuals aged 10 through 25 years, the ACIP has noted that there are no theoretical differences in safety for persons aged >25 years compared with those aged 10–25 years, thus the ACIP supports the routine use of MenB vaccines in persons aged ≥10 years who are at increased risk for serogroup B meningococcal disease.[15]

An analysis of two mass vaccination campaigns that used 4CMenB in response to two university outbreaks of MenB included safety data from 16,974 individuals who received at least one dose of 4CMenB with a median age of 20 years (range 16-65 years).[16] In this analysis, 8.4% of vaccinated individuals reported any AE, with the most common AEs being pain in the injected arm (10%), fever (9%), headache (5%), nausea (4%), and fatigue (4%). A total of 54 individuals (0.3%) experienced a Serious Adverse Event (SAE), only three of which were suspected to be related to the 4CMenB. In one phase 1 study of healthy adults aged 18-40 years[17] and three phase 2 studies of laboratory workers (age range 18-65 years)[18-20] receiving 4CMenB vaccination, the vaccine was well-tolerated and there were no SAEs.

Oropharyngeal mucosal secretion sampling

Oropharyngeal secretion sampling may cause transient discomfort. Participants will be asked to stop some activities before these samples are collected, including oral sex, or use of certain oral or inhaled immunomodulatory agents – such as corticosteroids – which may be inconvenient.

Vaginal mucosal secretion sampling

Vaginal mucosal secretion sampling may cause anxiety, transient discomfort, and embarrassment. Participants will be asked to stop some activities before these samples are collected, including vaginal sex, or use of anything in or around their vagina – such as tampons, spermicide, lubricants, or medications (e.g., topical yeast treatments) – which may be inconvenient.

Rectal mucosal secretion sampling

Rectal mucosal secretion sampling may cause anxiety, transient discomfort, and embarrassment. Participants will be asked to stop some activities before these samples are collected, including receptive anal intercourse, or insertion of anything into their anus – such as cleaning products, lubricant, enemas, or douches (including water) – which may be inconvenient.

Rectal mucosal biopsy

Risks associated with lower gastrointestinal endoscopy include colitis from chemicals for endoscope sterilization, bowel perforation, bleeding, diverticulitis, and infection. All procedures will be performed by Dr. Kelley or a nurse practitioner trained by Dr. Kelley. Non-physician medical providers have performed endoscopic procedures for diagnostic and therapeutic procedures for years. Many of these require mastery of flexible sigmoidoscopes, detailed anatomy of the full colon, and familiarity with sedation procedures.[21, 22] Procedures utilizing flexible instruments that access a deeper area of the colon and may or may not require sedation are more complicated and risky than the procedure detailed in this protocol which utilizes a rigid sigmoidoscope and only accesses the sigmoid colon a maximum of 15 cm from the anal verge. Therefore, it is appropriate for a trained, licensed mid-level provider to perform the procedure. Dr. Kelley's team has performed >300 similar procedures for other IRB approved protocols with zero complications.

All procedures will utilize disposable rigid sigmoidoscopes, forceps, and guides to reduce risk of infection and obviate the need for instrument sterilization between participants. To minimize risks, rigid proctoscopy, rather than flexible sigmoidoscopy or full colonoscopy, will be used in this study and the number of biopsies taken will be limited to 12. Colonoscopy has been shown to be associated with a still low, but significantly greater risk of complications than rectosigmoidoscopy.[23] The frequency of serious complications after flexible sigmoidoscopy is extremely low and complications from rigid sigmoidoscopy are presumably even lower, but unknown. In two large studies including a combined 144,832 clinically indicated procedures, the incidence of serious complications ranged from 0.06 to 0.8% utilizing flexible sigmoidoscopy.[23, 24] Obtaining biopsies may be associated with an increased risk of complications. The best available data on the risk of multiple biopsies comes from studies of dysplasia surveillance among patients with long-standing inflammatory bowel disease, in whom large numbers of "blind" biopsies are obtained throughout the colon for early detection of malignant transformation. In two such studies including a combined 3,011 procedures and a median of eight and 17 biopsies, respectively, there was only one serious complication, for an incidence of approximately 0.33%.[25, 26] In a study of subjects undergoing endoscopic procedures exclusively for research purposes, including 64 flexible sigmoidoscopies with a mean of 25 biopsies obtained from the rectosigmoid, there were no major complications. Thirteen subjects experienced minor symptoms (self-limited bleeding and pain), which were not related to the number of biopsies.[27]

A summary of procedures across several Microbicide Trial Network (an NIH-funded, international network designed to develop topical agents for Human Immunodeficiency Virus (HIV) prevention) was published in 2017.[28] This manuscript reported on the safety of 1,004

sigmoidscopy procedures with >15,000 biopsy collection from 278 research participants. Many participants underwent multiple procedures (median 3 procedures). There were no SAEs, and an AE related to sigmoidoscopy was reported in 1.6% of procedures. Eight of the 16 related AEs reported were abdominal pain, flatulence, bleeding, diarrhea, and bloating. Fourteen of the 16 related AEs were grade 1 and 2/16 were grade 2; median time to resolution was 1 day. The authors concluded that repeated intestinal mucosal biopsies for research purposes are safe. Thus, based on the available data, the risk of serious complications from the proposed study procedures, even with up to 10 biopsy specimens, is expected to be very low (<1:5000).

There is theoretical risk of increased acquisition of HIV or other infection if a study participant is exposed soon after the rectal biopsy procedure (i.e. while the mucosal surface is damaged). Therefore, study subjects will be counseled not to engage in anal intercourse for 1 week after the rectal biopsy procedure.

Biologic samples will be coded with a unique identifier prior to processing and storage for immunologic assays. Therefore, lab personnel will be unable to link specimens with participants. Only the PI and designated co-investigators/study personnel will be able to access information to identify specimens of individual participants.

Breach of Confidentiality

Subjects will be asked to provide protected 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 a subject's PHI. All records will be kept in a locked file cabinet or maintained in a locked room at the site. 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 this PHI. Any publications from this trial will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the site for quality assurance (QA) and data analysis include groups such as NIAID (or its designee) and FDA.

A description of this 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. At most, this web site will include a summary of the results.

There may be other unknown risks, discomforts, or side effects.

2.3.2 Potential Benefits

For subjects assigned to the Bexsero® group of the study, this meningococcal vaccine is known to elicit protective antibodies against *N. meningitidis* serogroup B in adults aged 10-25 years of age. There may also be benefits to society through the improvement of our understanding of mucosal immune responses to GC following 4CMenB vaccination.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

This is a single-center phase 2 double blinded mechanistic clinical trial to assess the systemic and mucosal immunogenicity of the multicomponent meningococcal serogroup B vaccine (4CMenB or Bexsero®) against GC, using a placebo vaccine (normal saline) as a comparator.

This trial will enroll approximately 50 healthy male and non-pregnant female subjects aged 18 to 49 years, inclusive, who meet all eligibility criteria. Subjects will be randomized 4:1 to one of two treatment groups. Group 1 (approximate N=40) will receive two doses of 4CMenB on Day 1 and Day 29. Group 2 (approximate N=10) will receive two placebo injections on Day 1 and Day 29. *The goal will be to ensure adequate representation of subjects by sex in both groups (Table 1)*

Vaccine preparation and administration will be unblinded. Only pharmacy personnel, the Emmes unblinded team and an unblinded vaccinator will have access to the study codes, and all other study personnel will be blinded. Subjects will be followed through 6 months after their first vaccination (Day 181).

All subjects will undergo sampling of mucosal secretions for testing for antibodies against GC. Male subjects will undergo oropharyngeal and rectal mucosal sampling, and female subjects will undergo oropharyngeal, vaginal and rectal mucosal sampling. A subset of subjects in each treatment group (N=16 in Group 1, N=4 in Group 2) will undergo rectal mucosal biopsy at two time points (baseline and following the second vaccination) for assessment of tissue GC specific cellular responses. *The goal will be to ensure adequate representation of subjects by sex in both groups in the rectal mucosal biopsy cohort (Table 1).*

This trial is expected to take approximately 14 months to complete, from initiation through availability of a final report on the primary outcomes of mucosal antibody responses and the secondary outcomes of serum antibody responses and safety outcomes related to mucosal sampling procedures and 4CMenB vaccination. For additional details on study procedures and evaluations and study schedule by study visits/days, see Sections 6, 7, and 8 and well as Appendices Appendix A and Appendix B.

Schematic of Study Design: See Table 1.

3.2 Study Objectives

3.2.1 Primary

- To characterize the rectal mucosal IgG antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine (normal saline) in healthy adult subjects.

3.2.2 Secondary

- To characterize the serum IgG antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To assess the safety and reactogenicity of 4CMenB in healthy adult subjects.

3.2.3 Exploratory

- To characterize the vaginal mucosal IgG antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the oropharyngeal mucosal IgG antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the serum IgA antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the mucosal IgA antibody response to GC elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the systemic B cell responses to GC antigens elicited by 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the systemic T cell responses to GC antigens elicited by 4CMenB vaccine as compared with placebo vaccine in healthy adult subjects.
- To characterize the mucosal T cell responses to GC antigens elicited by 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To characterize the serum bactericidal antibody responses to GC elicited by 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects.
- To evaluate the impact of 4CMenB vaccine-induced antibodies on gonococcal adhesion to human cervical cells.

- To characterize immune responses to *N. meningitidis* elicited by the 4CMenB vaccine as compared with the placebo vaccine in healthy adult subjects for use as controls.

3.3 Study Endpoints or Outcome Measures

3.3.1 Primary

- Rectal mucosal IgG concentrations (geometric mean titers, GMT) against GC OMV antigens at Day 1, 29, 43, 57, and 181 in each treatment group

3.3.2 Secondary

- Serum IgG concentrations (GMT) against GC OMV antigens at Day 1, 29, 43, 57, and 181 in each treatment group
- Frequency and severity of any AE related to 4CMenB immunization through the end of the study.
- Frequency of SAEs through the end of the study.

3.3.3 Exploratory

- Rectal mucosal IgG concentrations (GMT) against GC antigens before and after vaccination in each treatment group
- Serum IgG concentrations (GMT) against GC antigens before and after vaccination in each treatment group
- Vaginal mucosal IgG concentrations geometric mean titer (GMT) against GC antigens before and after vaccination in female subjects in each treatment group
- Oropharyngeal mucosal IgG concentrations (GMT) against GC antigens before and after vaccination in each treatment groupSerum IgA concentrations (GMT) against GC antigens before and after vaccination in each treatment group
- Rectal mucosal IgA concentrations (GMT) against GC antigens before and after vaccination in each treatment group
- Vaginal mucosal IgA concentrations (GMT) against GC antigens before and after vaccination in female subjects in each treatment group
- Oropharyngeal mucosal IgA concentrations (GMT) against GC antigens before and after vaccination in each treatment group

- Frequencies and function of memory B cells specific for antigens from GC before and after vaccination by ELISpot in each treatment group.
- Frequencies and function of peripheral blood T cells specific for antigens from GC before and after vaccination by ELISpot in each treatment group.
- Frequencies, phenotype, and function of peripheral blood CD4+ T cells specific for antigens from GC based on cytokine profile before and after vaccination by intracellular cytokine staining in each treatment group.
- Frequencies, phenotype, and function of peripheral blood CD8+ T cells specific for antigens from GC based on cytokine profile before and after vaccination by intracellular cytokine staining in each treatment group.
- Frequencies, phenotype, and function of rectal mucosal CD4+ T cells specific for antigens from GC based on cytokine profile before and after vaccination by intracellular cytokine staining in each treatment group.
- Frequencies, phenotype, and function of rectal mucosal CD8+ T cells specific for antigens from GC based on cytokine profile before and after vaccination by intracellular cytokine staining in each treatment group.
- Serum bactericidal antibody titers against GC before and after vaccination in each treatment group.
- Percent inhibition of gonococcal adhesion to human cervical cell line (ME180) by mucosal antibodies at Day 43 in each treatment group.
- Serum IgG concentrations (GMT) against *N. meningitidis* antigens before and after vaccination in each treatment group
- Frequencies and function of memory B cells specific for antigens from *N. meningitidis* before and after vaccination by ELISpot in each treatment group.
- Frequencies and function of peripheral blood T cells specific for antigens from *N. meningitidis* before and after vaccination by ELISpot in each treatment group.
- Frequencies, phenotype, and function of peripheral blood CD4+ T cells specific for antigens from *N. meningitidis* based on cytokine profile before and after vaccination by intracellular cytokine staining in each treatment group.

- Frequencies, phenotype, and function of peripheral blood CD8+ T cells specific for antigens from *N. meningitidis* based on cytokine profile before and after vaccination by intracellular cytokine staining in each treatment group.

4 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

4.1 Study Product Description

Bexsero®[11]: Active Product

Each 0.5-mL dose of Bexsero® (4CMenB) is formulated to contain 50 micrograms each of recombinant proteins NadA, NHBA, and fHbp, 25 micrograms of OMV, 1.5 mg aluminum hydroxide (0.519 mg of Al3+), 3.125 mg sodium chloride, 0.776 mg histidine, and 10 mg sucrose at pH 6.4 – 6.7. The NadA component is a fragment of the full-length protein derived from *N. meningitidis* strain 2996 (peptide 8 variant 2/3). The NHBA component is a recombinant fusion protein comprised of NHBA (peptide 2) and accessory protein 953 derived from *N. meningitidis* strains NZ98/254 and 2996, respectively. The fHbp component is a recombinant fusion protein comprised of fHbp (variant 1.1) and the accessory protein 936 derived from *N. meningitidis* strains MC58 and 2996, respectively. These 3 recombinant proteins are individually produced in *Escherichia coli* and purified through a series of column chromatography steps. The OMV antigenic component is produced by fermentation of *N. meningitidis* strain NZ98/254 (expressing outer membrane protein PorA serosubtype P1.4), followed by inactivation of the bacteria by deoxycholate, which also mediates vesicle formation. The antigens are adsorbed onto aluminum hydroxide. Each dose contains less than 0.01 micrograms kanamycin (by calculation)

Placebo

The placebo is 0.9% Sodium Chloride, USP injection.

4.1.1 Formulation, Packaging, and Labeling

Bexsero®: Active Product

Bexsero® (4CMenB) is a sterile, white, opalescent, suspension for intramuscular injection. 4CMenB is supplied as a 0.5-mL suspension in a glass prefilled syringe (packaged without needles). The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex.

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

Placebo

Placebo will be supplied as 0.9% Sodium Chloride Injection, USP which is a colorless, sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection (WFI). Each mL contains supplied chloride 9 mg. It contains no bacteriostatic, antimicrobial agent, or added buffer and is supplied only in single-dose containers. The placebo, 0.9% Sodium Chloride, contains no preservatives. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3 [4.5 to 7.0]).

4.1.2 Product Storage and Stability

Bexsero®: Active Product

Bexsero® (4CMenB) must be stored between 2°C to 8°C (36°F to 46°F). Do not freeze. Do not use if the vaccine has been frozen. Protect from light.

Placebo

0.9% Sodium Chloride, USP injection must be stored at 20ºC to 25ºC (68°F to 77ºF) [See USP Controlled Room Temperature; excursions between 15°C and 30°C (59°F and 86°F) are permitted]. See protocol-specific manual of procedures (MOP) for further instructions.

4.2 Acquisition/Distribution

Bexsero®: Active Product

4CMenB will be provided by DMID Clinical Materials Services (CMS, Fisher BioServices).

Placebo

Normal saline will be used as the placebo and will be provided by DMID CMS (Fisher BioServices).

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

Bexsero® (4CMenB): Active Product

Bexsero® (4CMenB) will be administered as a 2 dose series (0.5-mL each) with the second dose given 1 month after the first dose (Day 1 and Day 29) in participants randomized to Group 1.

Shake the syringe immediately before use to form a homogeneous suspension. Do not use the vaccine if it cannot be resuspended. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if particulate matter or discoloration is found.

Bexsero® will be administered as a 0.5-mL intramuscular injection into the deltoid muscle of the upper arm. The vaccine dose will be administered within 30 minutes following removal from the refrigerator.

Placebo

The placebo will be administered as a 2-dose series (0.5-mL each) with the second dose given 1 month after the first dose (Day 1 and Day 29) in participants randomized to Group 2.

Gently invert the normal saline vial 5 to 7 times. Using aseptic technique, puncture the septum top of the saline vial with a 1-inch, 23- or 25-gauge disposable, sterile needle attached to a 1-mL disposable, sterile syringe. Withdraw 0.5 mL from the saline vial. The prepared saline dose in the syringe will be allowed to store at room temperature and administered within 30 minutes. The dose will be administered IM over the deltoid region of the preferred arm.

Syringes for both Bexsero and placebo will be overlayed with a blinding tape and flagged (wrapped) with a prescription label. Labeling specifics will be referenced in the MOP. Labeled syringes will be provided to the unblinded vaccine administrator. The unblinded vaccine administrator will be credentialed to administer vaccines but will not be involved in study-related assessments, subject contact, or data collection following vaccination.

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

Study vaccines will be stored and shipped from the DMID contract CMS to the Clinical Sites. Once received, vaccines will be stored in and dispensed by the Investigational Pharmacy. DMID will determine the disposition of unused product.

The 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 syringes will be stored at 2ºC-8ºC in the Investigational Pharmacy until clinical trial accountability is completed. At study termination, all unused investigational product will be disposed in accordance with the MOP following complete drug accountability and monitoring.

5 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL

Subjects will be recruited through: posting of IRB-approved flyers on the Emory University campus; use of social media, list serves (such as CDC, Emory University, Emory Vaccine Center, and Vaccine Dinner Club), and clinical trial recruitment websites; a HIPAA-compliant clinical trials database to identify subjects of previous studies at the Hope Clinic who have agreed to future contact; presentations by Hope Clinic faculty at various University and community venues; and volunteer word-of-mouth.

If subjects are agreeable and interested, they will be screened initially over the phone for general eligibility criteria and their PHI will be saved in a locked cabinet in a secure office of the study coordinator/recruiter. When appropriate, an appointment at the Hope Clinic is then scheduled for an in-person clinic visit. Research staff will then obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

This study will enroll both male and non-pregnant female subjects. The goal will be to ensure approximately adequate representation of subjects by sex in both treatment groups (Table 1).

To facilitate this, enrollment will be stratified by both sex and treatment arm. Enrollment of subjects by sex and "cohort" (either "biopsy" or "no biopsy") will be based on the numbers listed in Table 1, and all subjects will be randomized 4:1 to either 4CMenB or placebo.

Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator (PI) or sub-investigator. 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

5.1.1 Subject Inclusion Criteria

Subjects must meet all the following inclusion criteria to be eligible for this trial:

1. Must be aged 18-49 years old (inclusive) at the time of vaccination.
2. Must be able to provide written informed consent.
3. Must have a body mass index (BMI) ≥18.5 and <35.0 kg/m^2

4. Must be in good health based on physical examination, vital signs*, medical history, safety labs** (as applicable to the rectal biopsy and no biopsy cohorts) and the investigator's clinical judgment.

**Vital signs must be within the normal ranges in Appendix C If a subject has elevated systolic or diastolic blood pressure, subject may rest for 10 minutes in a quiet room and the blood pressure may be retaken.*

***Safety labs must be within the normal ranges in Appendix C, and the normal ranges will be those used by the reference clinical lab.*

5. For female subjects only: If a female participant is of childbearing potential*, she must use contraception** from 30 days before study product administration through the end of study participation.

**A woman is considered of childbearing potential unless post-menopausal (defined as history of ≥1 year of spontaneous amenorrhea), or permanently surgically sterilized (bilateral oophorectomy, salpingectomy, hysterectomy).*

***Acceptable methods of contraception include: abstinence or no sex with a male, monogamous relationship with a man who had a vasectomy at least 6 months before the 1st study vaccine, prescription oral contraceptives, intrauterine device (IUD), birth control implants or injections, contraceptive patch, vaginal ring, condoms and diaphragms*/cervical *cap with spermicide ("double barrier" method)*

6. Must be available and willing to participate for the duration of this trial.
7. Willing to provide mucosal samples: vaginal secretions for women and oropharyngeal and rectal secretions for men and women.
8. For the rectal biopsy cohort only, willing to provide rectal biopsies.

5.1.2 Subject Exclusion Criteria

Subjects meeting any of the following exclusion criteria at baseline will be excluded from study participation:

1. Has ever been diagnosed with meningococcal infection or gonococcal infection at any anatomic site.
2. Has ever received any serogroup B meningococcal vaccine.

3. Any positive test result for STI (including GC/*Chlamydia trachomatis* (CT), Rapid Plasma Reagin (RPR) and HIV) at screening*.

**Female subjects will also be tested for Trichomonas at screening.*

4. Any history of Chlamydia trachomatis or syphilis infection at any body site in the preceding 12 months.
5. Has known allergy or history of anaphylaxis or other serious adverse reaction to a vaccine or vaccine products.
6. Has severe allergy or anaphylaxis to latex.
7. Has an acute illness or temperature ≥38.0°C on Day 1*.

**Subjects with fever or acute illness on the day of vaccination may be re-assessed and enrolled if healthy or only minor residual symptoms remain within 3 days.*

8. Has a history of a bleeding disorder, or is taking chronic anti-coagulant (e.g. warfarin, direct thrombin inhibitors, heparin products, etc.), anti-platelet, or Non-Steroidal Anti-Inflammatory Drug (NSAID) therapy.
9. Has history of autoimmune disease, or clinically significant cardiac, pulmonary, gastrointestinal, hepatic, rheumatologic, or renal disease by history or physical examination.
10. Has history of active malignancy other than squamous cell or basal cell skin cancer, unless there has been surgical excision that is considered to have achieved cure*.

**Subjects with a history of skin cancer must not be vaccinated at the previous tumor site.*

11. Has known or suspected congenital or acquired immunodeficiency, or recent history or current use of immunosuppressive therapy*.

**Anti-cancer chemotherapy or radiation therapy within the preceding 3 years, or long-term (≥2 weeks within the previous 3 months) systemic corticosteroid therapy (at a dosage of ≥0.5 mg/kg/day). Intranasal or topical prednisone (or equivalent) are allowed.*

12. Is post-organ and/or stem cell transplant, whether or not on chronic immunosuppressive therapy.
13. Had major surgery (per the investigator's judgment) within 4 weeks before study entry or planned major surgery during this trial.

14. Has history of diabetes* mellitus type 1 or type 2, including cases controlled with diet alone*.

**History of isolated gestational diabetes is not an exclusion criterion.*

15. Received live attenuated vaccines from 30 days before first vaccination until 30 days after second vaccination.

16. Received killed or inactivated vaccines* from 14 days before first vaccination until 14 days after second vaccination.

**For inactivated influenza vaccine, from 7 days before either vaccination until 7 days after either vaccination*

17. Received mRNA, viral vector, or any other technology platform COVID-19 vaccine within 14 days prior to first dose of the study product.*

**COVID-19 vaccination should take priority over administration of the study product.*

18. Received experimental therapeutic agents within 12 months before first vaccination or plans to receive any experimental therapeutic agents during this trial except for EUA COVID-19 therapy.*

**Only exclusionary if, in the opinion of the investigator, they would interfere with safety or endpoint assessment.*

19. Is currently participating or plans to participate in another clinical study which would involve receipt of an investigational product or undergoing a procedure*.

**Only exclusionary if, in the opinion of the investigator, they would interfere with safety or endpoint assessment.*

20. Received blood products or immunoglobulin in the 3 months before study entry or planned use during this trial.

21. Has major psychiatric illness in the past 12 months that in the opinion of the investigator would preclude participation.

22. Has current alcohol use or current or past abuse of recreational or narcotic drugs by history as judged by the investigator to potentially interfere with study adherence.

23. In the opinion of the investigator cannot communicate reliably, is unlikely to adhere to the requirements of this trial, or has any condition which would limit the ability to complete this trial.
24. Is pregnant or breast feeding.

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

5.2.1 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 discontinue a subject from receiving the study product for any reason. Follow-up safety and immunogenicity evaluations 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 case report forms (CRFs).

The reasons for subject withdrawal or discontinuation of study product, 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.

5.2.2 Subject Replacement

Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and administration of the study product may be replaced at the investigator's discretion. Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before administration of the study product may be replaced at the investigator's discretion.

5.2.3 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, 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. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6 STUDY PROCEDURES

6.1 Screening

6.1.1 Visit 00A, Day -56 to -1 for subjects in the no biopsy cohort / -56 to -28 for subjects in the biopsy cohort, 1st Screen, Clinic Visit

Potential subjects will be screened for eligibility up to 56 days before the first study vaccination. The following activities will be performed at the 1st screening visit.

- Begin the informed consent process. Provide subjects with a description of this trial (purpose and study procedures) and ask them to read the ICF. Provide an opportunity to the potential subject to ask questions. If she/he wishes to proceed with participation, the ICF should be signed before performing any screening procedures
- Review eligibility criteria.
- Interview subjects to collect demographics, medical history, sexual history, vaccination history, and pre-study medication use. Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required.
- Review subjects' concomitant medications taken within 30 days before signing the ICF.
- Obtain vital signs, including oral temperature, pulse, and blood pressure to assure eligibility.
- Measure height and weight and calculate BMI.
- Perform a physical examination including the following organs and organ systems: general appearance, head/eyes/ears/nose/throat (HEENT), neck, lungs, heart, abdomen, extremities, musculoskeletal, lymph nodes, skin, and nervous system. Genital and rectal exams will be performed at the investigator's discretion. All physical exams will be done by a clinician licensed to make medical diagnoses and listed on Form FDA 1572 as site PI or sub-investigator
- Collect venous blood for RPR and HIV-1/2 antibody testing.
- During screening for the rectal biopsy cohort only, collect venous blood for complete blood count (White Blood Count (WBC), hemoglobin, Platelet Count (PLT)), prothrombin time (PT) and activated partial thromboplastin time (aPTT)

- Collect urine for pregnancy test from all female subjects of childbearing potential.
- Collect pharyngeal and rectal swabs for nucleic acid amplification testing (NAAT) for GC and CT
- Collect urine for NAAT for GC/CT from male subjects
- Collect a self-collected vaginal swab for NAAT for GC/CT/Trichomonas from female subjects
- The overall eligibility of the subject to participate in this trial will be assessed once all screening test values and results of any other required evaluations are available. Subjects who qualify for inclusion will be contacted and scheduled for baseline rectal mucosal biopsy or first vaccination within 28 days.
- Subjects who qualify for inclusion in the rectal biopsy cohort will be instructed to abstain from receptive anal intercourse or insertion of anything into their anus, for at least 2 days prior to the baseline rectal mucosal biopsy visit
- Subjects who qualify for inclusion will be instructed to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the enrollment/first vaccination visit

6.1.2 Visit 00B, Day -7 to -1 for subjects in the no biopsy cohort / -34 to -28 for subjects in the biopsy cohort, 2nd Screen, Clinic Visit (optional)

This screening visit will only occur to repeat a screening assessment whose initial result is thought to be temporary). The following activities will be performed at the 2nd screening visit if it occurs.

- Review eligibility criteria, including results of available clinical screening lab evaluations.
- Review medical history and any updates obtained by interview of subjects since the screening visit (Visit 00A) to assure continued eligibility.
- Review interim sexual history to identify any new sexual partners since the last screening visit
- Review all concomitant medications recorded on the appropriate Electronic Case Report Form (eCRF).

- Obtain vital signs including oral temperature, blood pressure, and pulse, if needed.
- Measure height and weight and calculate BMI, if needed.
- Perform a targeted physical examination, if needed.
- Collect blood and urine for safety labs, if needed
- Subjects who qualify for inclusion in the rectal biopsy cohort will be instructed to abstain from receptive anal intercourse or insertion of anything into their anus, for at least 2 days prior to the baseline rectal mucosal biopsy visit

6.1.3 Visit 00C, Day -28 to -14, Baseline Rectal Mucosal Biopsy, Clinic Visit

This visit will only take place for subjects being enrolled in the rectal biopsy cohort. The following activities will be performed at Visit 00C.

- Ensure participant is safe to proceed with biopsy procedure, including results of all clinical screening lab evaluations*.

  *If the initial safety labs are >28 days old, these tests will need to be repeated and results obtained before rectal mucosal biopsy (see Section 7.2).
- Review medical history and any updates obtained by interview of subjects since the last screening visit (Visit 00A or, if performed, Visit 00B) to assure the participant is safe to proceed with biopsy procedure.
- Review interim sexual history to identify any new sexual partners since the last screening visit
- Review all concomitant medications and recent (within 28 days) vaccinations with subjects before the first vaccination for accuracy and completeness. Any new medications taken since the last screening visit (Visit 00A or, if performed, Visit 00B) will be recorded on the appropriate eCRF and reviewed to assure the participant is safe to proceed with biopsy procedure.
- Assess all AE/SAEs from the biopsy procedure and record them on the appropriate Electronic Case Report Form (eCRF).

- Obtain vital signs including oral temperature, blood pressure, and pulse to assure the participant is safe to proceed with biopsy procedure. Vital signs obtained on the day of biopsy will not be considered "screening", nor used for determining eligibility.
- Perform a targeted physical examination as needed before the biopsy procedure, if indicated based on review of complete medical history and updates obtained by interview of subjects since the last screening visit (Visit 00A or, if performed, Visit 00B).
- Perform rigid sigmoidoscopy and collect rectal mucosal biopsy samples for immunologic assays as described in the Schedule of Events (Appendix A) (for subjects in the mucosal biopsy subgroup).
- Subjects will be counseled not to engage in anal intercourse for 1 week after the rectal biopsy procedure.
- Subjects will be reminded to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the enrollment/first vaccination visit.

6.1.4 Visit 00P, Day -27 to -12, Baseline Rectal Mucosal Biopsy, Telephone Visit

This visit will take place 24-48 hours after the baseline rectal biopsy procedure only for subjects enrolled in the rectal biopsy cohort. The following activities will be performed at Visit 00P.

- Assess all AE/SAEs from the biopsy procedure and record them on the appropriate eCRF.
- Subjects will be reminded not to engage in anal intercourse for 1 week after the rectal biopsy procedure.
- Subjects will be reminded to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the enrollment/first vaccination visit.

6.2 Enrollment

6.2.1 Visit 01, Day 1, Randomization and First Vaccination, Clinic Visit

The following activities will be performed at the first vaccination visit.

- Reconfirm the subject's willingness to participate before performing any study procedures, including their first vaccination.
- Review eligibility criteria, including results of HIV, RPR, and STI screening lab evaluations, with subjects before the first vaccination to assure continued eligibility.
- Review interim sexual history to identify any new sexual partners since the last screening visit. Subjects that report new sexual exposures should undergo repeat STI screening and enrollment should be deferred until results are available and assure continued eligibility.
- Review medical history and any updates obtained by interview of subjects since the last screening visit (Visit 00A or, if performed, Visits 00B and/or 00C) to assure continued eligibility.
- Review all concomitant medications and recent (within 28 days) vaccinations with subjects before the first vaccination for accuracy and completeness. Any new medications taken since the last screening visit (Visit 00A or, if performed, Visits 00B and/or 00C) will be recorded on the appropriate eCRF and assessed for continued eligibility.
- Review COVID-19 vaccine history to ensure the subject is at least 14 days out from any COVID-19 vaccine before receiving study vaccine.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse before the first vaccination. Perform a targeted physical examination as needed before the first vaccination, if indicated based on review of complete medical history and updates obtained by interview of subjects since the last screening visit (Visit 00A or, if performed, Visits 00B and/or 00C).
- Perform a urine pregnancy test within 24 hours before first vaccination on all female subjects of childbearing potential. Results must be negative and known before first vaccination.
- Collect blood samples for baseline immunologic assays as described in the Schedule of Events (Appendix A)
- Collect rectal and oropharyngeal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)
- From female subjects, collect vaginal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)

- Subjects will be enrolled in Advantage eClinical and randomly assigned before the first vaccination.
- Perform pre-administration reactogenicity assessments before the first vaccination to establish baseline. Subjects will then receive a single dose of vaccine via IM injection into the deltoid muscle of the preferred arm. The site of injection (right or left arm) and time of administration will be recorded on the appropriate eCRF.
- Observe subjects in the clinic for ≥15 minutes after vaccination to monitor for any acute reactions.
- Evaluate the vaccination site and assess for reactogenicity and AE/SAEs for ≥15 minutes after vaccination and before discharge from clinic and record on the appropriate CRF.
- Subjects will enter their reactogenicity information in an electronic memory aid (with a paper back up). Subjects will be provided a thermometer and ruler and will record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will have their electronic memory aid reviewed with them at their next study visit.

6.3 Planned Study Visits

6.3.1 Visit 02, Day 8 (±1 day), Clinic Visit

The following activities will be performed at Visit 02.

- Obtain interim medical history by interview of subjects and note any changes since the previous clinic visit.
- Record all concomitant medications on the appropriate eCRF.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse, if indicated based on review of complete medical history.
- A targeted physical examination will be performed, if needed, by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on review of complete medical history.
- Assess for reactogenicity phenomena through subject interview and review memory aid.

- Examine vaccination site for local reactions.
- Collect blood samples for immunologic assays as described in the Schedule of Events (Appendix A).

6.3.2 Visit 03, Day 15 (±1 day), Clinic Visit

The following activities will be performed at Visit 03.

- Obtain interim medical history by interview of subjects and note any changes since the previous clinic visit.
- Record all concomitant medications on the appropriate eCRF.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse, if indicated based on review of complete medical history.
- A targeted physical examination will be performed, if needed, by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on review of complete medical history.
- Collect blood samples for immunologic assays as described in the Schedule of Events (Appendix A)
- Subjects will be instructed to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the next study visit

6.3.3 Visit 04, Day 29 (±1 day), Second Vaccination, Clinic Visit

The following activities will be performed at Visit 04.

- Obtain interim medical history by interview of subjects and note any changes since the previous clinic visit.
- Review interim sexual history to identify any new sexual partners since the last screening visit
- Record all concomitant medications on the appropriate eCRF.

- Review COVID-19 vaccine history to ensure the subject is at least 14 days out from any COVID-19 vaccine before receiving study vaccine.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- A targeted physical examination will be performed, if needed, by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on review of complete medical history.
- Collect venous blood for RPR testing
- Collect pharyngeal and rectal swabs for nucleic acid amplification testing (NAAT) for GC and CT
- Collect urine for NAAT for GC/CT from male subjects
- Collect a self-collected vaginal swab for NAAT for GC/CT from female subjects
- Collect blood samples for immunologic assays as described in the Schedule of Events (Appendix A)
- Collect rectal and oropharyngeal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)
- From female subjects, collect vaginal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)
- Perform a urine pregnancy test within 24 hours before second vaccination on all female subjects of childbearing potential. Results must be negative and known before second vaccination.
- Perform pre-administration reactogenicity assessments before the second vaccination to establish baseline. Subjects will then receive a single dose of vaccine via IM injection into the deltoid muscle of the preferred arm. The site of injection (right or left arm) and time of administration will be recorded on the appropriate eCRF.
- Observe subjects in the clinic for ≥15 minutes after vaccination to monitor for any acute reactions.
- Evaluate the vaccination site and assess for reactogenicity and AE/SAEs for ≥15 minutes after vaccination and before discharge from clinic and record on the appropriate CRF.

- Subjects will enter their reactogenicity information in an electronic memory aid (with paper back up). Subjects will be provided a thermometer and ruler (if needed) and will record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will have their electronic memory aid reviewed with them at their next study visit.

6.3.4 Visit 05, Day 36 (±1 day), Clinic Visit

The following activities will be performed at Visit 05.

- Obtain interim medical history by interview of subjects and note any changes since the previous clinic visit.
- Record all concomitant medications on the appropriate eCRF.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse, if indicated based on review of complete medical history.
- A targeted physical examination will be performed, if needed, by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on review of complete medical history.
- Assess for reactogenicity phenomena through subject interview and review memory aid
- Examine vaccination site for local reactions
- Collect blood samples for immunologic assays as described in the Schedule of Events (0)
- Subjects will be instructed to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the next study visit

6.3.5 Visit 06, Day 43 (±3 days), Rectal Mucosal Biopsy, Clinic Visit

The following activities will be performed at Visit 06.

- Obtain interim medical history by interview of subjects and note any changes since the previous clinic visit.

- Obtain interim sexual history to identify any new sexual partners since the start of the study and the second vaccination visit
- Record all concomitant medications on the appropriate eCRF.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- A targeted physical examination will be performed, if needed, by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on review of complete medical history.
- If indicated based on the subject's interim sexual history: Collect pharyngeal and rectal swabs for NAAT for GC and CT, collect urine for NAAT for GC/CT from male subjects, and collect a self-collected vaginal swab for NAAT for GC/CT from female subjects
- Collect blood samples for immunologic assays as described in the Schedule of Events (Appendix A)
- Collect rectal and oropharyngeal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)
- From female subjects, collect vaginal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)
- Perform rigid sigmoidoscopy and collect rectal mucosal biopsy samples for immunologic assays as described in the Schedule of Events (Appendix A) (for subjects in the mucosal biopsy subgroup)
- Subjects will be counseled not to engage in anal intercourse for 1 week after the rectal biopsy procedure.
- Subjects will be instructed to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the next study visit

6.3.6 Visit 06P, Day 44 (+1 day), Telephone Visit

This visit will take place 24-48 hours after the baseline rectal biopsy procedure only for subjects enrolled in the rectal biopsy cohort. The following activities will be performed at Visit 06P.

- Assess all AE/SAEs from the biopsy procedure and record them on the appropriate eCRF.
- Subjects will be reminded not to engage in anal intercourse for 1 week after the rectal biopsy procedure.
- Subjects will be reminded to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the next study visit.

6.3.7 Visit 07, Day 57 (±1 day), Clinic Visit

The following activities will be performed at Visit 07.

- Obtain interim medical history by interview of subjects and note any changes since the previous clinic visit.
- Review interim sexual history to identify any new sexual partners since the last screening visit
- Record all concomitant medications on the appropriate eCRF.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse, if indicated based on review of complete medical history.
- A targeted physical examination will be performed, if needed, by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on review of complete medical history.
- Collect blood samples for immunologic assays as described in the Schedule of Events (Appendix A)
- Collect rectal and oropharyngeal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)
- From female subjects, collect vaginal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)

- Subjects will be instructed to abstain from receptive anal and/or vaginal intercourse, insertion of anything into their vagina and/or anus, or oral sex for at least 2 days prior to the next study visit

6.3.8 Visit 08, Final Study Visit, Day 181 (±7 days), Clinic Visit

The following activities will be performed at Visit 08.

- Obtain interim medical history by interview of subjects and note any changes since the previous clinic visit.
- Obtain interim sexual history to identify any new sexual partners since the last study visit
- Record all concomitant medications on the appropriate eCRF.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse, if indicated based on review of complete medical history.
- A targeted physical examination will be performed, if needed, by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on review of complete medical history.
- Collect venous blood for RPR and HIV-1/2 antibody testing
- Collect pharyngeal and rectal swabs for NAAT for GC and CT
- Collect urine for NAAT for GC/CT from male subjects
- Collect a self-collected vaginal swab for NAAT for GC/CT from female subjects
- Collect blood samples for immunologic assays as described in the Schedule of Events (Appendix A)
- Collect rectal and oropharyngeal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)
- From female subjects, collect vaginal mucosal secretion samples for immunologic assays as described in the Schedule of Events (Appendix A)

6.3.9 Early Termination Visit

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

- Obtain interim medical history by interview of subjects and note any changes since the last study visit.
- Obtain interim sexual history to identify any new sexual partners since the last study visit
- Record all concomitant medications on the appropriate eCRF.
- Assess all AE/SAEs and record them on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse, if needed.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Examine vaccination site for local reactions if visit occurs ≤7 days after last injection.
- Assess for reactogenicity phenomena through subject interview and review memory aid if visit occurs ≤7 days after last injection.
- Perform a urine pregnancy test on all female subjects of childbearing potential (if indicated)
- If subject allows, obtain blood samples for immunologic assays appropriate to that visit if visit occurs within window of a regular study visit as described in the Schedule of Events (Appendix A).
- If subject allows, collect rectal and oropharyngeal mucosal secretion samples for immunologic assays appropriate to that visit if visit occurs within window of a regular study visit as described in the Schedule of Events (Appendix A).
- If subject allows, from female subjects, collect vaginal mucosal secretion samples for immunologic assays appropriate to that visit if visit occurs within window of a regular study visit as described in the Schedule of Events (Appendix A).

6.4 Unscheduled Study Visits

Unscheduled visits may occur at any time during this trial. Labs may be drawn at PI discretion. Any of the following activities may be performed:

- Obtain interim medical history by interview of subjects and note any changes since the last study visit.
- Record all concomitant medications on the appropriate eCRF.
- Obtain vital signs including oral temperature, blood pressure, and pulse, if indicated.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Examine vaccination site for local reactions if visit occurs ≤7 days after study vaccination
- Assess for reactogenicity phenomena through subject interview if visit occurs ≤7 days after last injection.
- Obtain blood for safety if needed.
- Perform a urine pregnancy test on all female subjects of childbearing potential (if indicated).
- Assess all AE/SAEs and record them on the appropriate eCRF.

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 investigator, or the study site staff. As a result of deviations, corrective actions should be developed by the site and implemented promptly. It is the responsibility of the site PI and other study personnel to use continuous vigilance to identify and report protocol deviations. All individual protocol deviations will be addressed in subject study records. All protocol deviations, either individual, product, or site-specific will be collected and the record stored at the study site. Protocol deviations must be sent to the local IRB/IEC per its guidelines and will be reported to the sponsor. The site PI and other study personnel are responsible for knowing and adhering to their IRB/IEC requirements.

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

- Complete medical history will be obtained by interviewing the subjects at the first study 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 history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At subsequent follow-up visits, an interim medical history will be obtained by interview of the subjects noting any changes since the previous clinic visit.
- Concomitant medications will be collected as described in Section 7.1.2.
- At the first screening visit (Visit 00A), a physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator. For all subsequent visits, a targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on Form FDA 1572 as the site PI or sub-investigator, if indicated based on subject's interim medical history.
- Vital signs (oral temperature, pulse, and blood pressure) will be collected at each study visit if indicated. Vital signs assessed on the screening visits (V00A and V00B) will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes before taking oral temperature. In the event of an abnormal heart rate or blood pressure due to physiological variation or activity, the subject may rest for 10 minutes in a quiet room, and then blood pressure and/or heart rate may be re-measured. The repeated measurement may be used to determine eligibility per the judgement of the investigator.
- Height and weight will be collected on the screening visit to determine BMI.
- Pre-administration reactogenicity assessments will be performed before each vaccination. A subject with mild (Grade 1) pre-administration reactogenicity which is transient, resolving, or clinically insignificant may be vaccinated at the investigator's discretion.

- Subjects will be observed in the clinic for ≥15 minutes after each vaccination. The vaccination site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be assessed and recorded on the appropriate eCRF before discharge from the clinic.
- All subjects will complete an electronic memory aid (with paper back up) on the day of each vaccination through seven days following each vaccination. Subject memory aids will be reviewed with the subjects for AEs (solicited injection site and systemic reactions, and unsolicited AEs). In addition, after each vaccination, whenever the subject is seen in the clinic, solicited and unsolicited reactogenicity and AEs will be reviewed.
- Reactogenicity assessments will include an assessment of solicited AEs occurring from the time of each vaccination through seven days following each vaccination, which include injection site reactions (pruritus, erythema, ecchymosis, induration/swelling, pain, and tenderness) and systemic reactions (fever, chills/shivering/sweating, fatigue, malaise, myalgia and arthralgia (exclusive of the injection site), headache, and nausea).

7.1.1 Research Procedures

Oropharyngeal mucosal secretion sampling

Trained, delegated study staff will be performing all oropharyngeal secretion (saliva) sampling procedures. Saliva will be collected and stored in the appropriate container. Instructions for saliva collection, handling, and storage are included in the MOP.

Vaginal mucosal secretion sampling

A trained, delegated clinician will be performing all vaginal mucosal sampling procedures with the assistance of a study coordinator utilizing a lubricated speculum and light source. Vaginal mucosal secretion samples will be collected and stored in the appropriate container. Instructions for vaginal secretion sample collection, handling, and storage are included in the MOP.

Rectal mucosal secretion sampling and biopsy

A trained, delegated clinician will be performing all rectal mucosal sampling procedures with the assistance of the study coordinator.

All subjects will have rectal mucosal secretions sampled utilizing a disposable anoscope and a light source. A lubricated anoscope will be inserted into the anus and 2-3 swabs or wicks will be used to collect secretions from the bowel wall for antibody measurements.

For subjects in the rectal mucosal biopsy cohort, a rigid sigmoidoscope, light source, and jumbo biopsy forceps will be utilized. Following rectal mucosal secretion sampling, without the administration of any previous enemas or other preparation, a rigid sigmoidoscope will be inserted and 8-12 adequate ~1.0 millimeter (mm) thick biopsy specimens will be taken from normal-appearing rectal mucosa approximately 10 cm above the external anal aperture using flexible sigmoidoscopic forceps mounted on a semi-flexible rod. All biopsy specimens will be coded with a unique numeric identifier such that the laboratories that receive the specimens will be unable to link them back to the study participants. The biopsy specimens will be placed in phosphate-buffered saline (PBS) and transported to a lab where the specimens will be processed for flow cytometry.

Twenty-four to forty-eight hours after the procedure, study personnel will call the participants who donated rectal biopsy samples and inquire about symptoms, complications, or adverse events related to study procedures. Participants who report symptoms suggestive of any significant complications will receive advice on seeking care and will be given referrals to appropriate healthcare professionals as needed. This follow-up may be completed over the phone or through electronic communication.

7.1.2 Assessment of Concomitant Medications/Treatments other than Study Product

Administration of any medications, therapies, or vaccines will be documented on the appropriate eCRF. Concomitant medications will include all current medications and non-study vaccinations taken within 30 days before signing the ICF through approximately 28 days after the last study vaccination, and for new-onset chronic medical conditions through the final study visit for each subject. Subjects who do not receive all vaccinations will have concomitant medications collected through approximately 28 days after the last vaccination, or early termination, whichever occurs first. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements.

Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Medications that might interfere with the evaluation of the study vaccines should not be used during the trial unless absolutely necessary. Medications in this category include the prohibited medications per the subject exclusion criteria (see Section 5.1.2). In addition, the site PI or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity. Use of medications as prophylaxis before study vaccination is prohibited.

To the sponsor's knowledge, there are no known drug-vaccine interactions with the study vaccines and subjects are not being asked to discontinue current medications not listed in the exclusion criteria. In the event medical conditions dictate use of medications, subjects are encouraged to obtain adequate care, comply with the course of therapy as prescribed by their physician, and inform the Investigator as soon as practicable. Details of all medications taken during this trial (date, brand or generic name) will be recorded.

7.2 Laboratory Evaluations

Laboratory assays and blood volumes are detailed in Appendix B.

7.2.1 Clinical Laboratory Evaluations

Screening

To be eligible for participation in this trial and receipt of the first vaccine, the subject's clinical screening lab evaluations (Visit 00A) must be confirmed to meet the subject inclusion criteria (see Section 5.1). All subjects will be screened for GC and CT infection at all relevant mucosal sites with NAAT. Female subjects will also be screened for *Trichomonas vaginalis* vaginal infection by NAAT. NAAT will be performed as follows:

- Rectal and pharyngeal swabs, and urine specimens will be obtained from male subjects
- Rectal and pharyngeal swabs, and self-collected vaginal swabs will be obtained from female subjects

Subjects will be screened for RPR and HIV-1/2 antibody. If a positive result on any of these screening tests occurs, the subject will be referred for appropriate follow-up and results reported as required by state law. These screening tests must be negative for the subject to be eligible to participate.

Urine pregnancy tests will be performed by the site laboratory at the screening visit (Visit 00A) and within 24 hours before initial vaccination (Day 1 (Visit 01)) on all female subjects of childbearing potential. Results must be negative and known before randomization on Day 1 (Visit 01) and before receipt of vaccination.

In the subset of participants who will undergo rectal mucosal biopsy, additional laboratory tests at the screening visit (Visit 00A) will include complete blood count (WBC, hemoglobin, PLT), PT and aPTT.

Follow-Up

Urine pregnancy tests will be performed by the site laboratory within 24 hours before second vaccination (Day 29 (Visit 04)) on all female subjects of childbearing potential. Results must be negative and known before receipt of vaccination.

Subjects will be re-screened for GC and CT infection at all relevant mucosal sites with NAAT prior to the second vaccination at Day 29 (Visit 04) and at the final study visit (Day 181 (Visit 08)) as follows:

- Rectal and pharyngeal swabs, and urine specimens will be obtained from male subjects
- Rectal and pharyngeal swabs, and self-collected vaginal swabs will be obtained from female subjects

Subjects will also be re-screened for RPR at Day 29 (Visit 04) and Day 181 (Visit 08). If a positive result on any of these screening tests occurs, the subject will be referred for appropriate follow-up and results reported as required by state law.

7.2.2 Research Assays

7.2.2.1 Antibody assays

Antibody assays will be performed at the study site (Stephens laboratory) and will include:

- Measurement of IgG concentrations against GC antigens at mucosal sites (rectum, vagina, oropharynx) and in serum as described in the Schedule of Events (Appendix A) by enzyme-linked immunosorbent assay (ELISA)
- Measurement of IgA concentrations against GC antigens at mucosal sites (rectum, vagina, oropharynx) and in serum as described in the Schedule of Events (Appendix A) by ELISA
- Measurement of serum bactericidal antibody titers as described in the Schedule of Events (Appendix A)
- Measurement of IgG concentrations against *N. meningitidis* antigens in serum as described in the Schedule of Events (Appendix A) by ELISA

7.2.2.2 Cell-mediated immunity assays

Cell-mediated immunity assays will be performed at the study site (Hope Clinic) and will include:

- Frequencies and function of memory B cells (MBC) specific for antigens from GC and *N. meningitidis* as described in the Schedule of Events (Appendix A) by ELISpot
- Frequencies and function of peripheral blood T cells specific for antigens from GC and *N. meningitidis* as described in the Schedule of Events (Appendix A) by ELISpot
- Frequencies, phenotype, and function of peripheral blood CD4+ and CD8+ T cells specific for antigens from GC and *N. meningitidis* as described in the Schedule of Events (Appendix A) by intracellular cytokine staining (ICS)
- Frequencies, phenotype, and function of rectal mucosal CD4+ and CD8+ T cells specific for antigens from GC as described in the Schedule of Events (Appendix A) by ICS

7.2.2.3 Bacterial adhesion assay

Serum for bacterial adhesion assays will be collected as described in the Schedule of Events (Appendix A). For samples scheduled to be collected during vaccination visits, samples will be collected before vaccination.

The bacterial adhesion assay will be performed at the study site (Stephens laboratory). Instructions for specimen preparation, handling, storage, and labeling are included in the MOP.

7.2.2.4 Laboratory Specimen Preparation, Handling, and Storage

Serum for antibody assays will be collected as described in the Schedule of Events (Appendix A). For samples scheduled to be collected during vaccination visits, samples will be collected before vaccination.

Peripheral blood mononuclear cell (PBMC) samples for MBC and T-cell assays will be collected as described in the Schedule of Events (Appendix A). For samples scheduled to be collected during vaccination visits, samples will be collected before vaccination. Plasma from the PBMC separations will be saved as described in the MOP.

Instructions for specimen preparation, handling, storage, and labeling are included in the MOP.

7.2.2.5 Laboratory Specimen Shipping

Specimen shipment will occur at intervals during 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 MOP.

8 ASSESSMENT OF SAFETY

8.1 Assessing and Recording Safety Parameters

Safety will be assessed by the frequency and severity of:

- Any AE related to 4CMenB immunization through the end of the study.
- Any AE related to rectal mucosal sampling procedures through the end of the study.
- Any SAEs through the end of the study.

8.1.1 Adverse Events

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. In addition, AEs specific to the rectal mucosal biopsy cohort will be collected prior to study drug administration and are defined as any unfavorable and unintended sign, symptom, or disease temporally associated with the rectal mucosal biopsy procedure. 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 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.

8.1.1.1 Adverse Events Grading

All AEs (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 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

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. The Toxicity Grading Scale (Appendix C) will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions.

8.1.3 Serious Adverse Events

An AE or suspected adverse reaction is considered a SAE if, in the view of either the site PI or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event[1],
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require 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.

[1] Life-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 or procedure 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 PI or Sub-Investigator.
- Recorded on the appropriate SAE eCRF.

- Followed through resolution by a licensed study physician (for investigational new drug application (IND) studies, a physician listed on the Form FDA 1572 as the site PI or Sub-Investigator).
- Reviewed and evaluated by DMID, an Independent Safety Monitor (ISM) (as deemed necessary), or Safety Monitoring Committee (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:

- Any AE related to 4CMenB immunization through the end of the study.
- Any AE related to rectal mucosal sampling procedures through the end of the study.
- Any SAEs through the end of the study.

8.2.1 Solicited Events

Solicited events are AEs that are common and known to occur following administration of study product. The Toxicity Grading Scale (Appendix C) will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions. All subjects will complete a subject memory aid on the day of each vaccination through 7 days after each vaccination (i.e. Day 8 and Day 36 visits). Subject memory aids will be reviewed with the subjects for AEs (solicited injection site and systemic reactions, and unsolicited AEs). In addition, after each vaccination, whenever the subject is seen in the clinic or a phone call occurs (i.e., at all opportunities), solicited and unsolicited reactogenicity and AEs will be reviewed. Reactogenicity assessments will include an assessment of solicited AEs occurring from the time of each vaccination through seven days following each vaccination, which include injection site reactions (pruritus, erythema, ecchymosis, induration/swelling, pain, and tenderness) and systemic reactions (fever, chills/shivering/sweating, fatigue, malaise, myalgia and arthralgia (exclusive of the injection site), headache, and nausea).

Solicited injection site and systemic reactogenicity events will be documented and reported from Day 1 through Day 8 and Day 29 through Day 36,

8.2.2 Unsolicited Events

Unsolicited events are any other AEs that occur following rectal biopsy procedures or administration of study product. Unsolicited AEs will be collected and assessed through the end of the protocol-defined follow up period. Unsolicited AEs are followed through resolution or stabilization even if this extends beyond the reporting period.

8.2.3 Medically-Attended Adverse Events (MAAEs)

For each AE experienced, the subject will be asked if he/she had received medical attention, defined as hospitalization, an ER visit, or an otherwise unscheduled visit to or from medical personnel for any reason. AEs characterized by such unscheduled medical care will be designated as MAAEs.

8.3 Reporting Procedures

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 or frequency of any pre-existing medical condition worsens, it should be recorded as an AE.

If an event meets both the criteria of a study endpoint and an AE, the event will be reported either as a study endpoint or as an AE (not both).

For the rectal biopsy cohort, SAEs will be documented and reported from the time of the baseline rectal mucosal biopsy through approximately 6 months after the first study vaccination. For the non-rectal biopsy cohort, SAEs will be documented and reported from the first study vaccination through approximately 6 months after the first study vaccination.

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. [redacted]

Bethesda, MD 20817, USA

SAE Hot Line: [redacted]

SAE FAX Number: [redacted]

SAE Email Address: [redacted]

In addition to the SAE form, select SAE data fields must also be entered into the data coordinating center (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 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 PI 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 PI or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected unexpected serious adverse event (SUSAR) as an IND safety report to the FDA and will notify all participating site PIs (i.e., all PIs to whom the sponsor is providing drug under its IND(s) or under any PI'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 that are not SUSARs will be reported to the FDA at least annually in a summary format.

8.3.3 Reporting of Pregnancy

Upon awareness, pregnancies occurring in subjects will be recorded on the Pregnancy Report eCRF. Subjects who become pregnant will be discontinued from further study procedures, but will be followed and recorded for safety until pregnancy outcome as per the protocol.

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

AEs will be assessed, and followed from initial recognition of the AE through end of the protocol defined follow-up period.

SAEs will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined follow-up period.

Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

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

The site PI or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this trial, regardless of the relationship to the vaccine. AE/SAEs or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately.

All subjects will be screened for GC and CT infection at all relevant mucosal sites with NAAT at screening (Visit 00A) and prior to rectal mucosal sampling procedures at Day 29 (Visit 04) and at the final study visit (Day 181 (Visit 08)). Female subjects will also be screened for *Trichomonas vaginalis* infection with NAAT at screening (Visit 00A). Subjects will also be screened for RPR at screening (Visit 00A), Day 29 (Visit 04) and Day 181 (Visit 08), as well as HIV-1/2 antibody (Visit 00A and 08). If a positive result on any of these screening tests occurs, the subject will be referred for appropriate follow-up and results reported as required by state law.

Safety labs will only be required at screening in the rectal biopsy cohort. Safety labs may be repeated during the study at the investigator's discretion as part of the assessment of abnormal clinical findings.

8.6 Halting Rules

8.6.1 Study Halting Criteria

The study will be halted for SMC review/recommendations if any of the following are reported:

- Any subject experiences an SAE after administration of study product that is considered related to study product.
- Any subject experiences an SAE after a study procedure that is considered related to the study procedure.
- An overall pattern of symptomatic, clinical, or laboratory events that the DMID Medical Monitor (MM) or SMC consider associated with the study product and that may appear minor in terms of individual events, but that may collectively represent a serious potential concern for safety.

If any of the halting rules are met, the study will not continue without a review of the safety data by and recommendation from the SMC before proceeding.

SMC retains the authority to suspend additional enrollment and administration of the study product during the entire study, as applicable.

8.6.2 Individual Halting Criteria

A subject will be discontinued from second vaccination if:

- Subject develops a new medical condition or medication change for which continued participation, in the opinion of the investigator, would pose a risk to the subject
- Subject is no longer willing and/or able to adhere to study restrictions outlined in the Inclusion and Exclusion Criteria
- Subject has a fever (defined as an oral temperature ≥38.0 °C) or an acute illness at the time of boost vaccination administration. The next study vaccination can be deferred until

the fever or illness resolves or only minor residual symptoms remain, that in the opinion of the investigator, will not interfere with the ability to assess safety parameters as required by the protocol, provided that the boost is given within the protocol-defined study window.

- Subject has received COVID-19 vaccine <14 days prior to scheduled dose #2. The next study vaccination can be deferred until at least 14 days elapse from the time of any COVID-19 vaccination, if in the opinion of the investigator, will not interfere with study assessments as required by the protocol, provided that the boost is given within the protocol-defined study window.
- Permission to administer vaccination outside the protocol-defined study window must be obtained from DMID Medical Officer.

Note: In case the boost vaccination is postponed, the timing of the safety/immunogenicity visits post-boost will be planned relative to actual vaccination day.

8.7 Safety Oversight

8.7.1 Independent Safety Monitor (ISM)

For this clinical trial an ISM is <u>not</u> required. However, **upon DMID Medical Monitor request,** the PI will identify a physician with relevant expertise, to act as a Secondary Medical Assessor (SMA). The SMA will examine a subject and/or medical records and provide a medical assessment (or second medical opinion) to the DMID of the safety event in question. The PI will send to the DMID MM, a summary of the event and include the PI and SMA assessments.

Note: In the case that DMID has requested this type of evaluation multiple times, DMID may request the site(s) identify an ISM to assist DMID with safety oversight.

8.7.2 Safety Monitoring Committee (SMC)

This clinical study will utilize an 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. A simple majority will be considered a quorum for voting purposes. The SMC will consist of members with appropriate 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 convene for the following meetings: Organizational meeting, scheduled data review meetings, ad hoc meetings for a specific safety concern or issue, final meeting.

9 HUMAN SUBJECTS PROTECTION

9.1 Institutional Review Board/Independent Ethics Committee

Each site PI 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.

The institution engaged in this research will hold a current FWA issued by the Office of 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), and the expected duration of the subject's participation in the trial.

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.

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

Once signed, a copy of the ICF 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 PI 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 ICFs that they sign.

Human Genetic Testing

No human genetic testing will be performed in this study.

9.3 Consent for Future Use of Stored Specimens and Data

Residual samples/specimens and data are those that are left over after protocol-specified testing and this study has been completed. Subjects will be asked for permission to keep any data and any remaining (residual) specimens (serum and PBMCs) derived from venous blood samples, swabs and other mucosal samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. These residual specimens will be stored coded indefinitely at a DMID-contracted storage facility. Data or specimens may be shared with investigators at the participating site and with other investigators at U.S. or international institutions. The recipients of data or specimens will be informed that these specimens have a NIH certificate of confidentiality. The information provided to a recipient will not contain direct identifiable information. Use of the specimens will require a new protocol and review and approval of that protocol and the specimens for use by an IRB.

There are no benefits to subjects in the collection, storage and subsequent future use of their data/samples/specimens. These data/samples/specimens will not be sold or used directly for production of any commercial product. The data/sample/specimen will be encoded (labeled) only with a barcode and a unique tracking number that connects to a code key at the study site. Restricted access to the code key is maintained by the principal investigator to protect subject confidentiality. Reports from future research studies performed using subjects' data/samples/specimens will NOT be kept in their health records.

Subjects may be given the option to decide if they want their **residual** specimens with their data to be used for future research or have these specimens destroyed at the end of this trial. The subject's decision can be changed at any time by notifying the study doctors or nurses in writing. However, if the subject originally consents to the future use of **residual** specimens with their data and subsequently changes his/her decision, any data already collected from a previously collected specimen may still be used for future research.

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

This trial will include all healthy adults who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background. Only subjects, aged 18 to 49 years inclusive, will be included. This age range was selected to represent the population with the highest incidence of gonorrhea therefore children are excluded from this study. Special populations, e.g., non-English speakers, illiterate or non-writing individuals, and vulnerable populations, for which no benefit of trial participation has been identified, will not be enrolled in this trial.

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. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the subject. 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 FDA.

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

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

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, *or* for the purposes of other research that 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. Procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party. 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 PI 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 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 systemic and mucosal immunogenicity of two doses of 4CMenB compared to a placebo comparator. A secondary goal is to assess the safety and reactogenicity of 4CMenB. Though the study is not designed to formally test any hypotheses, p-values may be reported that compare the immunogenicity and/or safety data between treatment groups. The null hypotheses for these tests will be that there is no difference between treatment groups and the alternative hypotheses will be two-sided.

10.2 Sample Size Considerations

A total of approximately 50 (maximum of 60), subjects will be enrolled and randomized to one of two study arms based on a 4:1 allocation ratio to the 4CMenB arm and placebo arm, respectively.. The 4:1 allocation ratio was not selected based on any formal statistical criteria but instead was selected to maximize the number of subjects administered 4CMenB, and thus maximize the amount of immunogenicity data collected on subjects administered 4CMenB. Of the approximate 50 subjects, approximately 20 subjects will be enrolled into the Rectal Mucosal Biopsy Cohort. As with the overall sample size, this number was not selected based on any statistical criteria but was selected weighing the feasibility of enrolling subjects who would consent to the biopsy procedure against ensuring ample data was collected via rectal mucosal biopsy as there are no data on the impact of 4CMenB on immunologic responses at the human mucosal surfaces where GC is typically encountered.

The study is not designed to formally test any hypotheses associated with the immunogenicity data. Using the ELISA GMT estimates reported in Table 2 of Semchenko et al.,[13] estimated GMT 95% confidence intervals were calculated for the 4CMenB group using the post-vaccination estimates and for the placebo group using the pre-vaccination estimates. Table 2 provides the estimated GMTs and GMT ratios between groups. To account for a small percentage of the approximate 50 randomized subjects not being eligible for the primary analysis (e.g., due to not having immunogenicity data available), a range of sample sizes from 36 to 50 was explored.

Table 2: 95% Confidence Intervals for GMTs and GMT Ratios across a range of Sample Sizes and Antigens

N (Vaccine / Placebo)	Antigen[a]	Treatment Group	GMT Estimate	GMT 95% CI	GMT Ratio	GMT Ratio 95% CI
30 / 6	Ng OMV	Vaccine	42224	35234, 50601	1.23	0.77, 1.97
		Placebo	34297	16638, 70697		
	Ng whole cell	Vaccine	78793	61639, 100721	1.62	0.86, 3.05
		Placebo	48503	19329, 121713		
	Nm whole cell	Vaccine	388023	262806, 572900	4.00	1.53, 10.47
		Placebo	97006	29287, 321311		
	Ng rNHBA	Vaccine	1176267	876783, 1578046	34.3	16.98, 69.26
		Placebo	34297	16638, 70697		
35 / 8	Ng OMV	Vaccine	42224	35748, 49873	1.23	0.81, 1.87
		Placebo	34297	19275, 61026		
	Ng whole cell	Vaccine	78793	62863, 98760	1.62	0.93, 2.83
		Placebo	48503	23305, 100944		
	Nm whole cell	Vaccine	388023	271134, 555305	4.00	1.73, 9.26
		Placebo	97006	37363, 251856		
	Ng rNHBA	Vaccine	1176267	897654, 1541356	34.3	18.63, 63.14
		Placebo	34297	19275, 61026		
40 / 10	Ng OMV	Vaccine	42224	36161, 49303	1.23	0.85, 1.79
		Placebo	34297	20947, 56156		
	Ng whole cell	Vaccine	78793	63850, 97233	1.62	0.98, 2.68
		Placebo	48503	25906, 90811		
	Nm whole cell	Vaccine	388023	277922, 541742	4.00	1.88, 8.51
		Placebo	97006	42880, 219456		
	Ng rNHBA	Vaccine	1176267	914549, 1512881	34.3	19.85, 59.27
		Placebo	34297	20947, 56156		

a: From Semchenko (2018), Table 2. Vaccine group estimates are derived from the post-vaccination estimates and placebo group estimates are derived from the pre-vaccination estimates.

10.3 Treatment Assignment Procedures

10.3.1 Randomization Procedures

Within each biopsy cohort (Rectal Mucosal Biopsy Cohort and No Rectal Mucosal Biopsy Cohort), subjects will be randomized 4:1 to 4CMenB or placebo. Randomization will be stratified by sex. Approximately twenty (20) subjects, approximately 10 males and 10 females, will be enrolled into the Rectal Mucosal Biopsy Cohort and approximately 30 subjects, approximately 15 males and 15 females, will be enrolled into the No Rectal Mucosal Biopsy Cohort.

The list of randomized treatment assignments will be included in the enrollment module of Emmes' Internet Data Entry System (IDES). Advantage eClinical® will assign each enrolled participant to a treatment arm after demographic and eligibility data have been entered into the system. A designated individual at each participating site will be provided with a coded 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 a participating site temporarily loses access to the Internet or the online enrollment system is unavailable.

10.3.2 Masking Procedures

This is a double-blinded clinical trial. Participants, site investigators, and study personnel who perform study assessments following study product administration, data entry personnel at the sites, and laboratory personnel performing study assays will be blinded to treatment assignment. Syringes will be labeled with an overlay/blinding tape containing the subject ID, and the treatment number from the treatment key, and expiration time for the syringe, and provided to the unblinded vaccine administrator. The unblinded vaccine administrator will be credentialed to administer vaccines but will not be involved in study-related assessments, subject contact, or data collection following vaccination (data entry related to the vaccination is acceptable).

The randomization scheme will be generated by the Statistical and Data Coordinating Center (SDCC) and provided to unblinded study personnel (i.e., research pharmacists performing study product preparations and unblinded study product administrators) at the participating sites. The unblinded study product administrator is a study personnel member credentialed to administer study product and may also participate in dose preparation but will not be involved in study-related assessments or have participant contact for data collection following study product administration to the participant.

The SMC may receive data in aggregate and presented by study arm. The SMC may be unblinded to individual study treatment assignments, as needed, to adequately assess safety issues. The SMC will review grouped and unblinded data in the closed session only.

10.4 Planned Interim Analyses

10.4.1 Interim Safety Review

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.

10.4.2 Interim Immunogenicity Review

There will be an interim immunogenicity analysis, which may be used to optimize and prioritize research laboratory assays for exploratory outcomes. Results of the interim analyses will not be used to make any decisions concerning the conduct of this trial.

The interim immunogenicity analysis will include all available antibody data for the first 20 subjects who complete Visit 6, which includes data addressing the primary and secondary objectives, as well as several exploratory objectives. The interim report will be prepared by the unblinded statistician at the SDCC after all relevant immunogenicity data through Visit 6 are received. Results will be shared only with relevant study personnel (i.e. PI, laboratory investigator(s), and DMID) and only in aggregate by treatment arm. Study personnel will remain blinded to individual subject treatment assignments.

The interim immunogenicity analyses will include summaries of descriptive and aggregate antibody data against antigens from *Neisseria gonorrhoeae* and *Neisseria meningitidis* stratified by antigen, specimen type (serum and mucosal sites), pre- versus post-treatment timepoints, and treatment arm (4CMenB or placebo). No hypothesis tests or modeling will be included in the interim immunogenicity analysis. This interim analysis of the data is not intended to impact the conduct of this trial. No statistical adjustments are planned.

10.5 Final Analysis Plan

A separate Statistical Analysis Plan (SAP) will be written that will contain the details of all planned analyses. This section outlines the major components of the analyses.

10.5.1 Analysis Populations

10.5.1.1 Immunogenicity Population

For the primary outcome analysis, the Immunogenicity Population at a specified follow-up visit will include all eligible subjects who have received all doses of study product prior to that visit and have immunogenicity data available for that visit. Subjects will be classified according to the study product received. To account for subjects having data available only for a subset of assays, assay-specific populations will be defined.

Per-protocol populations may also be defined that further restrict the immunogenicity analysis populations to exclude subjects with major protocol deviations or events that may impact study product effectiveness or the ascertainment or interpretation of assay results. These will be described in the SAP. For supplementary and exploratory analyses, additional analysis populations may be defined; details will be provided in the SAP.

10.5.1.2 Safety Population

The Safety Population will include all subjects who received at least one dose of study product. Subjects will be classified according to the treatment received.

10.5.2 Baseline Characteristics

Baseline and demographic characteristics will be summarized overall and by study arm. For both continuous and categorical variables, appropriate summary statistics will be applied. For continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation, median, minimum, and maximum. For categorical variables, descriptive statistics will include counts and percentages per category.

10.5.3 Immunogenicity Analysis Plan

For the analyses of the primary and secondary immunogenicity outcomes, the GMT and geometric mean fold rise (GMFR) for ELISA titers will be calculated for Days 1, 29, 43, 57, and 181 in each study arm. Point estimates and their 95% confidence intervals will be reported for each time point. The distribution of titers will also be graphically summarized using reverse cumulative frequency distributions. Anatomical sites will be summarized separately.

Summaries of the ratio of serum to mucosal IgG concentration will be generated at each time point. Geometric means and confidence intervals will be reported and scatter plots of serum vs. mucosal IgG concentrations will be generated. Summaries of pairwise concentration ratios, comparing anatomical sites, will also be generated at each time point. Summaries will include geometric means and confidence intervals as well as graphical summaries.

All geometric mean calculations will use $\log_{10}$ transformed data, and the anti-log of the resulting point estimates will be reported for means and confidence intervals. Missing immunogenicity data will not be imputed and no search for outliers will be performed. The logarithmic transformation is used to improve the distributional properties of the immunogenicity data to reduce the impact of potential outliers.

Additional supplementary and/or sensitivity analyses of the primary and secondary outcomes as well as analysis of the exploratory immunogenicity outcomes will be described in the separate SAP.

10.5.4 Safety Analysis Plan

Safety evaluations will be based on the frequency and severity of reactogenicity events, AEs, and SAEs occurring from the time of first study product administration through the final study visit.

AEs and SAEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class. The rate and exact 95% CIs of AEs and SAEs in aggregate, as well as by MedDRA categories, will be computed. The number of SAEs will be reported by a detailed listing showing the type, MedDRA coding, relevant dates (treatment dosing date(s) and SAE onset and resolution dates), severity, relatedness, and outcome for each event.

Solicited local injection site and systemic reactogenicity will be summarized by severity for each day post injection (for each injection) and as the maximum severity over the entire assessment period (across injections). Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none vs. any severity) and using exact confidence intervals to summaries the proportion/percentage of subjects reporting each symptom any infection site symptom, and any systemic symptom. Solicited AEs will be summarized separately for each infection, and over both injections by study arm.

Vital sign and physical exam findings will also be summarized and/or providing in subject-level listings.

Missing safety data will not be imputed and no search for outliers will be performed.

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. The site will permit authorized representatives of 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. All eCRFs serve as the source documents.

Subject memory aids will not be 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 study 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 QA and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to source data/eCRFs 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 PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data will be entered electronically over the Internet by site study staff into Advantage eClinical, developed and maintained by the SDCC. The eCRFs serve as the source documents for data collected. Paper case report forms derived from the eCRF are provided by the SDCC and are to be used only when Advantage eClinical is unavailable. Details on data handling procedures, procedures for data monitoring, and instructions for use of the system and completion of the eCRFs are provided in the study MOP, eCRF Instructions, and Advantage eClinical User's Guide.

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 Coordinating Center/Biostatistician Responsibilities

All eCRFs and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. AEs must be recorded on the appropriate eCRF, assessed for severity and relationship, and reviewed by the site PI 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 PI. During the study, the site PI 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 eCRFs by the study personnel.

Site staff who are delegated the responsibility by the study PI will be the data originators for clinical data entered directly into the eCRF. A list of all authorized data originators, including site staff, will be included on the Study Personnel/Signature Responsibility List.

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 PI 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 (SOP). 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, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access through study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with the site PI 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 PI 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 result posting a copy of this protocol (and its amendments) and a copy of the SAP will be posted on ClincialTrials.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

Procedures	***Screening (Visit 00A) Day -56 to -1 (-56 to -28 for biopsy cohort)***	***Screening (Visit 00B) Day -7 to -1 (-34 to -28 for biopsy cohort)***	***Baseline Rectal Mucosal Biopsy, Visit 00C, Day -28 to -14***	***Study Visit 00P (Phone visit), Day -27 to -12***	***Enrollment/Baseline Visit 1, Day 1***	***Study Visit 2 Day 8 ± 1 day***	***Study Visit 3 Day 15 ± 1 days***	***Study Visit 4[e] Day 29 ± 1 days***	***Study Visit 5 Day 36 ± 1 days***	***Study Visit 6[f] Day 43 ± 3 days***	***Study Visit 6P (Phone visit) Day 44 + 1 days***	***Study Visit 7 Day 57 ± 1 days***	***Final Study Visit 8 Day 181 ± 7 days***	***Early Termination Visit***
Informed consent	X													
Assessment of Eligibility Criteria	X	X			X									
Demographics	X													
Medical history	X----------X				X----------X							X----------X		X
Sexual history	X	X	X		X			X		X		X	X	X
Randomization					X									
Concomitant medication review	X----------X				X----------X							X----------X		X
Review COVID-19 vaccine history					X			X						
Vital signs	X	(X)	X		X	(X)	(X)	X	(X)	X		(X)	(X)	(X)
Physical exam	X	(X)	(X)		(X)----------(X)									(X)
Administer study intervention					X			X[e]						

Procedures	***Screening (Visit 00A) Day -56 to -1 (-56 to -28 for biopsy cohort)***	***Screening (Visit 00B) Day -7 to -1 (-34 to -28 for biopsy cohort)***	***Baseline Rectal Mucosal Biopsy, Visit 00C, Day -28 to -14***	***Study Visit 00P (Phone visit), Day -27 to -12***	***Enrollment/Baseline Visit 1, Day 1***	***Study Visit 2 Day 8 ± 1 day***	***Study Visit 3 Day 15 ± 1 days***	***Study Visit 4[e] Day 29 ± 1 days***	***Study Visit 5 Day 36 ± 1 days***	***Study Visit 6[f] Day 43 ± 3 days***	***Study Visit 6P (Phone visit) Day 44 + 1 days***	***Study Visit 7 Day 57 ± 1 days***	***Final Study Visit 8 Day 181 ± 7 days***	***Early Termination Visit***
Pre-vaccine Administration Reactogenicity Assessment					X			X						
Post-vaccination Observation (for ≥15 minutes) to Assess Acute Reactions					X			X						
Evaluation of Vaccination Site to Assess for Reactogenicity and AE/SAEs after ≥15 minutes					X			X						
Memory Aid					X	X		X	X					(X)
Adverse event review and evaluation			X[d]	X[d]	X----------X						X[d]	X----------X		X
Sampling procedures														
Hematology (CBC, PT, aPTT)[a]	X	(X)	(X)											
Urine pregnancy test	X	(X)			X			X						(X)
STI screening[b]	X[c]				(X)----------(X)			X	(X)----------(X)			(X)	X	
Rectal mucosal biopsy[a]			X							X[f]				

Procedures	***Screening (Visit 00A) Day -56 to -1 (-56 to -28 for biopsy cohort)***	***Screening (Visit 00B) Day -7 to -1 (-34 to -28 for biopsy cohort)***	***Baseline Rectal Mucosal Biopsy, Visit 00C, Day -28 to -14***	***Study Visit 00P (Phone visit), Day -27 to -12***	***Enrollment/Baseline Visit 1, Day 1***	***Study Visit 2 Day 8 ± 1 day***	***Study Visit 3 Day 15 ± 1 days***	***Study Visit 4[e] Day 29 ± 1 days***	***Study Visit 5 Day 36 ± 1 days***	***Study Visit 6[f] Day 43 ± 3 days***	***Study Visit 6P (Phone visit) Day 44 + 1 days***	***Study Visit 7 Day 57 ± 1 days***	***Final Study Visit 8 Day 181 ± 7 days***	***Early Termination Visit***
Vaginal secretion sampling					X			X		X		X	X	(X)
Rectal secretion sampling					X			X		X		X	X	(X)
Oropharyngeal secretion sampling					X			X		X		X	X	(X)
Blood draw for serum antibody assays					X			X		X		X	X	(X)
Blood draw for systemic immunity assays					X	X	X	X	X	X		X	X	(X)

[a]Only to be performed in the rectal mucosal biopsy cohort (approximate N=20)

[b]STI screening will include GC/CT NAAT of urine, pharynx, and rectum, and RPR. HIV antibody testing will occur at Visit 00A and 08 only

[c]Baseline STI screening in non-pregnant females will also include NAAT for Trichomonas

[d]AE review and evaluation at Visit 00C will include only AEs from the baseline biopsy procedure

[e] The following will be reviewed for safety prior to administering the second dose: interim medical history, concomitant medications, COVID-19 vaccination status, vital signs, and negative pregnancy test result for females of childbearing potential.

[f] The following will be reviewed for safety prior to the rectal mucosal biopsy procedure: interim medical history, concomitant medications, and vital signs.

Procedures	***Screening (Visit 00A) Day -56 to -1 (-56 to -28 for biopsy cohort)***	***Screening (Visit 00B) Day -7 to -1 (-34 to -28 for biopsy cohort)***	***Baseline Rectal Mucosal Biopsy, Visit 00C, Day -28 to -14***	***Study Visit 00P (Phone visit), Day -27 to -12***	***Enrollment/Baseline Visit 1, Day 1***	***Study Visit 2 Day 8 ± 1 day***	***Study Visit 3 Day 15 ± 1 days***	***Study Visit 4[e] Day 29 ± 1 days***	***Study Visit 5 Day 36 ± 1 days***	***Study Visit 6[f] Day 43 ± 3 days***	***Study Visit 6P (Phone visit) Day 44 + 1 days***	***Study Visit 7 Day 57 ± 1 days***	***Final Study Visit 8 Day 181 ± 7 days***	***Early Termination Visit***
Abbreviations: X, study procedure for all participants; (X), study procedures to be determined by study staff; CBC, complete blood count; PT, prothrombin time; aPTT, activated partial thromboplastin time; STI, sexually transmitted infection														

Appendix B. TABLE OF STUDY ASSAYS AND BLOOD VOLUME COLLECTION

		1st screen (V00A)	**2nd screen (V00B)**	**V00C[a]**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**
Study Day relative to Vaccination #1 (D1)		**D-56 to -1 (D-56 to -28 for rectal biopsy cohort)**	**D-7 to -1 (D-34 to -28 for rectal biopsy cohort)**	-28 to -14	1	8	15	29	36	43	57	181
Sampling Procedures (blood volumes in mL)												
Clinical Laboratory	Screening serology (RPR)	8						8				8
	Hematology (CBC)[a]	4	4									
	Coagulation testing (PT/aPTT)[a]	4	4									
	GC/CT NAAT x3 mucosal sites	X						X		X[b]		X
	Trichomonas NAAT	X[c]										
Mucosal Immunity	Rectal mucosal IgG against Ng antigens				X			X		X	X	X
	Vaginal mucosal IgG against Ng antigens				X			X		X	X	X

		1st screen (V00A)	2nd screen (V00B)	V00C[a]	V1	V2	V3	V4	V5	V6	V7	V8
Study Day relative to Vaccination #1 (D1)		D-56 to -1 (D-56 to -28 for rectal biopsy cohort)	D-7 to -1 (D-34 to -28 for rectal biopsy cohort)	-28 to -14	1	8	15	29	36	43	57	181
	Oropharyngeal mucosal IgG against Ng antigens				X			X		X	X	X
	Rectal mucosal IgA against Ng antigens				X			X		X	X	X
	Vaginal mucosal IgA against Ng antigens				X			X		X	X	X
	Oropharyngeal mucosal IgA against Ng antigens				X			X		X	X	X
	Rectal mucosal T cell phenotyping (ICS)[a]			X						X		
Systemic Immunity	Serum IgG against Ng antigens (ELISA)				8			8		8	8	8
	Serum IgA against Ng antigens (ELISA)				8			8		8	8	8
	Ng specific MBC (ELISPOT)				24			24		24		24
	Ng specific T cell (ELISPOT)				16	16	16	16	16	16	16	

		1st screen (V00A)	2nd screen (V00B)	V00C[a]	V1	V2	V3	V4	V5	V6	V7	V8
Study Day relative to Vaccination #1 (D1)		D-56 to -1 (D-56 to -28 for rectal biopsy cohort)	D-7 to -1 (D-34 to -28 for rectal biopsy cohort)	-28 to -14	1	8	15	29	36	43	57	181
	OMV and peptide antigen specific T cell (ICS)				32		32	32		32	32	
	Serum bactericidal Ab				8			8		8	8	8
	Serum IgG against Nm antigens (ELISA)				8					8		8
	Nm specific MBC (ELISPOT)				16			16		16		
	Nm specific T cell (ELISPOT)				16		16			16		
Blood volumes (mL)												
Per visit		16	8	0	136	16	64	112	16	136	72	56
Cumulative		16	24	24	160	176	240	352	368	504	576	632

[a]*Only for subjects in the rectal mucosal biopsy cohort*

[b]*Only if indicated based on interim sexual history*

[c]*Female subjects only*

Abbreviations: Ng, Neisseria gonorrhoeae; Nm, Neisseria meningitidis

Appendix C. TOXICITY TABLE

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)	Subject is aware of pain, but it does not interfere with daily activity, **and** it does not require pain medication or it requires use of a non-narcotic pain reliever ≤24hours	Subject is aware of pain; there is interference with daily activity **or** it requires repeated use of a non-narcotic pain reliever for >24 hours	Subject is aware of pain, **and** it prevents daily activity or requires any use of a narcotic pain reliever
Tenderness – hurts only when injection site is touched or the arm is moved	The area immediately surrounding the injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it prevents daily activity
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
Erythema (Redness)*	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

* Size will also be measured in mm but will not be used as a halting criterion.

Ecchymosis (bruising), erythema (redness), and induration (hardness)/swelling as analyzed by measurement will be graded as follows:

Local (Injection Site) Reactogenicity Measurements

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Ecchymosis (Bruising)*	25 mm – 50 mm	51 mm – 100 mm	>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	>100 mm

* Will not be used as halting criteria.

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
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	Some interference with daily activity	Significant interference, prevents daily activity
Nausea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

* Not at injection site.

Oral temperature[#] will be graded as follows:

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 vaccine 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.

Additional Adverse Event Severity Grading

Pulse and blood pressure[#] will be graded as follows:

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia - beats per minute	50 – 54	45 – 49	<45
Tachycardia - beats per minute	101 – 115	116 – 130	>130
Hypotension (systolic) mmHg	85 – 89	80 – 84	<80
Hypotension (diastolic) mmHg	50 – 54	45 – 49	<45
Hypertension (systolic) mmHg	141 – 150	151 – 155	>155

Hypertension (diastolic) mmHg	91 – 95	96 – 100	>100

[#] Pulse and blood pressure assessed on Day 1 prior to the first study vaccination will be considered as baseline.

Clinical safety laboratory results* will be graded as follows:

Clinical Safety Laboratory Adverse Event*

Hematology	Normal Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC $10^3/\mu L$ (Low)	3.8 – 10.8	2.5 – 3.7	1.5 – 2.4	<1.5
WBC $10^3/\mu L$ (High)	3.8 – 10.8	10.9 – 15.0	15.1 – 20.0	>20.0
Hgb g/dL (Low) (Female)	11.7 – 15.5	11.0 – 11.6	9.5 – 10.9	<9.5
Hgb g/dL (Low) (Male)	13.2 – 17.1	12.0 – 13.1	10.0 – 11.9	<10.0
Platelets $10^3/\mu L$ (Low)	140 – 400	125 – 139	100 – 124	<100
Platelets $10^3/\mu L$ (High)	140 – 400	401 – 550	551– 750	>750
PT (seconds) (increase by factor) (prothrombin time)	9.0 – 11.5	>1.0 – 1.1 x ULN	>1.1 – 1.2 x ULN	> 1.2 x ULN
aPTT (seconds) (increase by factor) (activated partial thromboplastin time)	22.0 – 34.0	>1.0 – 1.2 x ULN	>1.2 – 1.4 x ULN	>1.4 x ULN

*Clinical laboratory evaluations assessed at the screening visit will be considered as baseline