A Phase 2A Partially-blind Placebo Controlled Trial to Evaluate the Safety and Immunogenicity of Live Attenuated, Intranasal *B. pertussis* vaccine (BPZE1) in Healthy Adults

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STATEMENT OF COMPLIANCE

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

1. United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
2. 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)
3. International Council for Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
4. 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
5. National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
6. National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
7. 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:

Signed: _____ Date: _____

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AEFI	Adverse Event Following Immunization
AESI	Adverse Event of Special Interest
ALT	Alanine aminotransferase
aPV	Acellular Pertussis Vaccine
B. pertussis	*Bordetella pertussis*
CFR	Code of Federal Regulations
CI	Confidence Interval
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases
DNT	Dermonecrotic Toxin
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FHA	Filamentous Hemagglutinin
FIM	Fimbriae
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
GMFR	Geometric Mean Fold Rise
GMTR	Geometric Mean Titer Ratio
ICH	International Council for Harmonization
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MedDRA ®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NOCMC	New Onset Chronic Medical Condition
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PI	Principal Investigator

PRN	Pertactin
PT	Pertussis Toxin
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TCT	Tracheal Cytotoxin
US	United States
SWFI	Sterile Water for Injection
wPV	Whole cell Pertussis Vaccine

PROTOCOL SUMMARY

Title:	A Phase 2A Partially-blind Placebo Controlled Trial to Evaluate the Safety and Immunogenicity of Live Attenuated, Intranasal *B. pertussis* vaccine (BPZE1) in Healthy Adults
Phase:	2a
Population:	Approximately fifty healthy males and non-pregnant females, 18-49 years of age, in the US.
Number of Sites:	One Vaccine and Treatment Evaluation Unit (VTEU) site, Vanderbilt University Medical Center
Study Duration:	Approximately 12 months
Subject Participation Duration:	Approximately 6 months
Description of Agent or Intervention:	Lyophilized, live-attenuated *Bordetella pertussis* vaccine (BPZE1) reconstituted with water for injection (SWFI) and administered as a single intranasal dose of either 10^7 colony forming units (CFU) or 10^9 CFU Placebo: The same constituents, in the same quantities as BPZE1, absent attenuated *Bordetella pertussis*, reconstituted with water for injection (SWFI)
Objectives:	Primary: 1. To assess the safety and tolerability of a single intranasal dose of either 10^7 or 10^9 colony forming units (CFU) of lyophilized BPZE1 vaccine. Secondary: 1. To assess the humoral immunogenicity of lyophilized BPZE1 vaccine at Day 15, Day 29 and Day 181 following receipt of one intranasal dose of 10^7 or 10^9 CFU of BPZE1.

2. To assess mucosal immunogenicity of lyophilized BPZE1 vaccine at Day 29 and Day 181 following receipt of one intranasal dose of 10^7 or 10^9 CFU of BPZE1.
3. To evaluate nasal clearance of BPZE1 by culture at Day 29 (and if still positive, at Day 46) following receipt of one intranasal dose of lyophilized BPZE1 vaccine of 10^7 or 10^9 CFU of BPZE1.

Description of Study Design:

This is a phase 2a, single center, randomized, partially blind, placebo controlled, clinical trial evaluating a single intranasal dose of either 10^7 CFU or 10^9 CFU of BPZE1 in healthy adults (18-49 years of age, inclusive). The study will evaluate a lyophilized formulation of the product, defining the optimal dose for subsequent clinical trials. Since it is anticipated that the VaxINator device will be the primary mechanism of delivery in subsequent clinical trials, the majority of volunteers will receive vaccine or placebo via VaxINator device. Five individuals will receive 10^9 CFU of BPZE1 by needleless tuberculin syringe in order to compare immunogenicity to previous Phase 1 clinical trials of BPZE1.

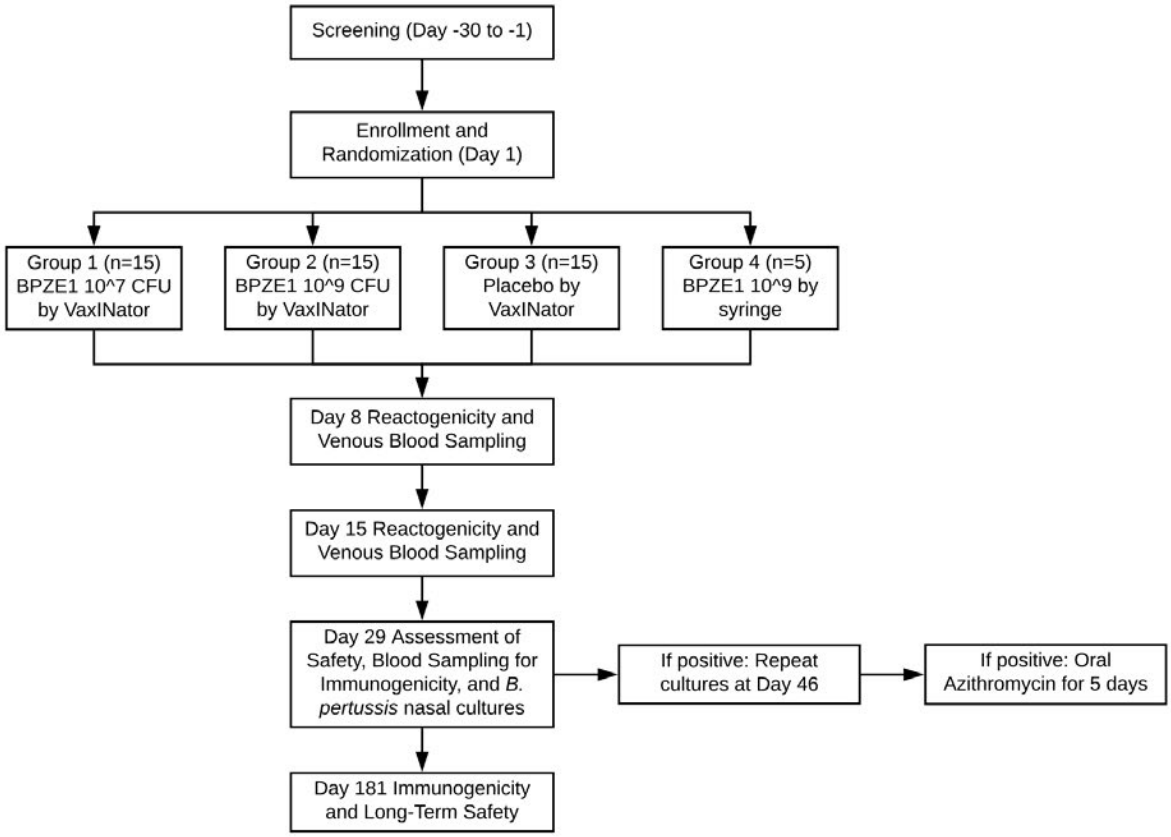
Fifty healthy adults, 18-49 years of age will be randomized to one of four treatment groups in a 3:3:3:1 ratio, as described in the table below.

Table 1: Treatment Groups and Intranasal Vaccine to be Administered

Group	N	Intranasal Vaccination	Device Used for Administration
1	15	BPZE1 10^7 CFU	VaxINator
2	15	BPZE1 10^9 CFU	VaxINator
3	15	Placebo	VaxINator
4	5	BPZE1 10^9 CFU	Needleless Tuberculin Syringe

Estimated Time to Complete Enrollment: 12 months

Figure 1: Schematic of Study Design



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Bordetella pertussis (B. pertussis) is a gram-negative bacterium and a causative agent of pertussis colonizing infection, a precursor to the disease more commonly known as whooping cough. It is a microbe that is transmitted from person to person via airborne droplets from the cough or sneeze of an infected host. A recent publication modeling pertussis cases and deaths estimated there were 24.1 million pertussis cases and 160,700 deaths in children younger than 5 years in 2014 worldwide.[1]Despite the dramatic decline in Whooping Cough cases and deaths in industrialized nations during the 20th Century due to public health vaccine initiatives, recent decades have witnessed a sharp increase in cases and it is generally agreed that there is a critical need for a new and more effective vaccine targeting *B. pertussis*.

Two types of pertussis vaccines are available worldwide: whole cell vaccine (wPV) and acellular vaccine (aPV). Although aPV overcomes several potential adverse effects of wPV (e.g. fever and seizures) and is the only pertussis vaccine marketed in the U.S., the protective efficacy of aPV appears to be lower than wPV with induced immunity waning in about 4-12 years. Additionally, the high cost of aPV limits its use in developing countries.

Acellular pertussis vaccines (aPV) have several deficiencies that have contributed to pertussis outbreaks across the US and around the world. Young infants are especially vulnerable, but are not fully protected until they complete a 3 dose aPV vaccination series, at about 7 months of age. The clinical and regulatory path to a vaccine to prevent pertussis in the most vulnerable infants, however, will be lengthy and a more immediate impact can be made with an adult and adolescent vaccine that prevents transmission of *B. pertussis* to vulnerable infants.

In addition to rapidly waning immunity after aPV immunization, preclinical baboon studies suggest that aPV does not prevent nasopharyngeal colonization and, hence, does not prevent transmission from adults and adolescents to vulnerable infants.[2] Multiple published studies in various countries have demonstrated high rates of subclinical (asymptomatic) *B. pertussis* colonizing infections. In a study that took nasal swabs from 629 asymptomatic school children in a Chinese province with 99% aP vaccination coverage, 4.8% were confirmed to be PCR positive for *B. pertussis.*[3] Based on data on controls in the adult and adolescent aP clinical trials by the APERT Study Group, the incidents of asymptomatic pertussis in the U.S. was predicted to be 5 - 10 million cases per year in a year when the total number of diagnosed cases was only 7,000.[4] Surveillance studies using seroprevalence of pertussis to identify recently infected asymptomatic

individuals demonstrate high rates of circulating *B. pertussis*, including 9.3% in the Netherlands[5] and 14.1% in Italy[6]. Mathematical models predict that asymptomatic *B. pertussis* colonization is the most probable cause of the surge in pertussis rates.[7] Thus, a need exists for an adult and adolescent vaccine that induces potent and durable systemic immunity, but also mucosal immunity to prevent nasopharyngeal colonization and transmission to infants.

Since the best-known method of inducing both potent systemic and mucosal immunity is by natural *B. pertussis* infection, BPZE1 was constructed [redacted] Over 20 peer-reviewed publications have demonstrated the preclinical safety and efficacy of the live-attenuated vaccine[8-11], and two Phase 1 clinical studies [12, 13] have demonstrated safety and immunogenicity at various doses in healthy adults. Despite the low dose and low volume used in the original Phase-1a (low dose) study, BPZE1 demonstrated the ability to transiently colonize healthy adult volunteers and, upon colonization, induce *B. pertussis* targeted immune responses in all colonized individuals[12]. In the Phase 1b study, discussed in detail below, 80% of subjects were transiently colonized with BPZE1 and 78% met criteria for serologic response, including all subjects who received high-dose investigational BPZE1 liquid vaccine.

The investigational BPZE1 [redacted] Preclinical studies, including a 10^{10} CFU dose (10x higher than the highest dose in this study) in baboons, showed no safety concerns.[14] In the non-human primate model, BPZE1 also demonstrated the ability to reduce the total burden of *B. pertussis* colonization by 99.9% compared to non-vaccinated controls and 99.8% relative to previous studies with aPV.

This study allows the new lyophilized formulation of the investigational BPZE1 vaccine to be evaluated in US adults and provides an opportunity to characterize the immunological response of two different dosages delivered by nasal immunization. The main advantage of the lyophilized BPZE1 formulation over the liquid formulation is long-term stability and storage at refrigeration temperature instead of storage at very low freezing temperature. For the Phase 1 clinical studies, the liquid BPZE1 formulation required freezing at -70° C to maintain long-term stability. Since the lyophilized product is reconstituted before administration, both formulations are delivered as

liquids and the lyophilized product simply provides improved storage temperature and time. [redacted] Thus, the primary purpose of this Phase 2a study is to evaluate the safety and immunogenicity of the lyophilized formulation.

The working cell bank (WBC) for both the liquid (Phase 1b) and lyophilized formulations were grown in THIJS medium.[15] Thus, the BPZE1 *B. pertussis* strain is the same active ingredient for both formulations, and the same WCB is used to inoculate the first series of shake-flask pre-cultures.

The lyophilized formulation consists of BPZE1 bacteria suspended in a lyophilization buffer (Section 6). [redacted].

Two Phase 1 clinical studies have been performed at the Karolinska University Hospital (Stockholm, Sweden). Both studies were single center, dose-escalating, placebo-controlled studies of liquid BPZE1, given as a single intranasal dose to healthy adult volunteers. A Phase 1a clinical study[12], NCT01188512, has been completed using a BPZE1 low dose liquid suspension (i.e. 10^3, 10^5 and 10^7 CFU) formulation in Sweden, which had no vaccine related serious adverse events but only induced 42% of the healthy adult male subjects in the 10^7 CFU group to transiently colonize with BPZE1. To improve the percentage of subjects transiently colonizing with BPZE1 after vaccination, a Phase 1b study [13], NCT02453048, was performed in Sweden to evaluate a higher dose liquid suspension (i.e., 10^7, 10^8 and 10^9 CFU) in a greater volume (i.e., 400 µL per nostril). The Phase 1b study included both adult males and females and excluded subjects with high baseline anti-pertactin antibody titers from randomized study groups. The randomized portion of both Phase 1 studies included 48 volunteers, 12 in each dose group and in one placebo group. The 12 subjects in the placebo group were evenly distributed over the whole study period in both studies to enable evaluation of apparent differences in the frequency of adverse events or immunogenicity over time.

2.1.1 Phase 1a Clinical Study Results

The investigational BPZE1 liquid vaccine was safe in the 10^3, 10^5 and 10^7 CFU doses used for nasal administration and BPZE1 colonization was dose-dependent, with 42% of the subjects in the 10^7 CFU group having positive BPZE1 colonization.

There were no vaccine related serious adverse events (SAE). There were no detectable differences in adverse events (AE) between placebo and the different dosage groups. The most frequent AEs were rhinorrhea (n=43 episodes in 26 subjects), sneezing (n=41 episodes in 19 subjects), nasal congestion (n=38 episodes in 29 subjects), headache (n=34 episodes in 21 subjects), and tiredness (n=32 episodes in 23 subjects). Many of the volunteers had uncomplicated upper respiratory tract infections (i.e., common cold) during the follow up period, which may explain at least a portion of their symptoms. The efficacy results, included:

- *Colonization of mucosal surface:* The vaccine strain BPZE1 was isolated in a total of seven subjects, one in each of the 10^3 and 10^5 dose group and five subjects in the 10^7 dose group. The colonization was first detected on day 4-14 and lasted to day 11-28 after vaccination
- *Immunogenicity:* Anti-pertussis IgG appeared only in the seven subjects who were colonized at the nasopharyngeal mucosa. The antibodies appeared after day 14 and remained at high levels after the 5-6 months follow up.

In summary, this Phase 1a study showed that BPZE1 used for nasal administration in humans can colonize the nasopharyngeal mucosa was both immunogenic and safe, without eliciting any symptoms of either mild or severe whooping cough. The results of this study suggested the possibility that a higher degree of colonization and immune response can be achieved by using a higher vaccine dose, higher vaccine volume, an improved method for application, or by excluding subjects with high baseline anti-pertactin antibody titers.

2.1.2 Phase 1b Clinical Study Results

The BPZE1 investigational liquid vaccine was safe in the higher doses and volumes used for nasal administration. Proportion of subjects having transiently BPZE1 colonized nasopharyngeal mucosa was not dose-related at 10^7 CFU or higher, but instead all dosage groups achieving 75% colonization rate or greater, and an overall combined colonization rate was observed for subjects from all randomized groups of over 80%. A dose response trend was observed, however, in the rate of colonization and clearance, with faster colonization rate and faster clearance rate being correlated with higher dosage. The serum antibody responses were strongly correlated to positive colonization, but there was no dose response. A small non-randomized group of subjects with

pre-existing high antibody titers for pertactin had a 33% rate of BPZE1 colonization after immunization with the 10^9 CFU dose.

There were no SAEs and any differences in AEs within randomized study groups, between placebo and the different dosages, were not detectable. The most frequent AEs were sneezing (n=55 episodes in 24 subjects), rhinorrhea (n=35 episodes in 22 subjects), nasal congestion (n=31 episodes in 19 subjects), cough (n=21 episodes in 16 subjects), and fatigue (n=21 episodes in 16 subjects). Efficacy results, included:

- *Colonization of mucosal surface:* The vaccine strain BPZE1 was isolated in a total of 29 randomized subjects, 10 in the 10^7 CFU dose group, 9 in the 10^8 CFU dose group, and 10 subjects in the 10^9 CFU dose group. Colonization was first detected on day 4-14 and lasted through day 11-28 after vaccination.
- *Immunogenicity:* Serologic responses (IgG and IgA) were measured to PT, PRN, FIM, and FHA and reported individually. In addition, responses were grouped by either:
    - ≥2-fold rise in PT-specific IgG or IgA that was at least 4x greater than the minimum limit of detection (MLD) of the assay, or
    - ≥2-fold rise in at least 2 antibodies targeting PRN, FIM, or FHA (IgG or IgA) that were at least 4x greater than the MLD

  Greater than 92% of subjects in the 10^7 CFU dose group and 100% of subjects in the 10^9 CFU dose group had a two-fold rise in at least one antibody titer (either PT, PRN, FIM, or FHA, IgG or IgA) that was at least 4x greater than the MLD (i.e., seropositive). All subjects receiving the 10^9 CFU BPZE1 dose were seropositive for at least 2 or more antibodies (IgG or IgA) or PT (IgG or IgA). No placebo subjects had 2 seropositive antibodies (IgG or IgA) or seropositive for PT antibodies (IgG or IgA). In the vast majority of subjects, an increase in serum antibody titers (IgG/IgA) occurred at day 14 or 21, peaked at day 28, and remained at high levels at the 6 months follow up visit (sometimes rising further at 6-months).
- Despite 33% BPZE1 colonization rate in the non-randomized pre-existing high pertactin group, 66% of the subjects experienced a seropositive response to ≥ 2 antibodies (IgG or IgA) or PT (IgG or IgA); in other words, multiple subjects generated an immunological response despite unrecognized BPZE1 colonization.

In summary, the investigational BPZE1 liquid vaccine was safe in the doses used for nasal administration. Colonization occurred in > 80% of the subjects across the three dosage groups and BPZE1 groups, especially the 10^9 CFU group, had a high proportion of subjects that reached the pre-specified threshold of seropositivity. Additionally, subjects with high baseline antibodies were capable of further seropositive antibody boosting, but at a lower rate and lower percent increase from baseline.

2.2 Rationale

The availability of a cost-effective pertussis vaccine that provides improved efficacy, durable protection, and the potential to stop transmission would represent a breakthrough in the prevention of pertussis infections and may lead to a reduction in the population reservoir of *B. pertussis* in undiagnosed individuals. The ability to prevent colonization and transmission could even facilitate global eradication of *B. pertussis* disease.

Intranasal administration provides an opportunity to generate a locally effective mucosal antibody response at the site of exposure. Preclinical data, as described above, supports the ongoing development of BPZE1 (either 10^7 CFU or 10^9 CFU), with the transition to a lyophilized formulation being the goal of this study. The hypothesis for this trial is that lyophilized BPZE1, administered intranasally, will be sufficiently safe and immunogenic. Given the safety profile of liquid BPZE1 in previous clinical trial recipients, we hypothesize that lyophilized BPZE1 will share similar safety characteristics. Thus, the goals of this clinical trial are to assess the safety, reactogenicity and immunogenicity of investigational, lyophilized BPZE1 vaccine administered intranasally by VaxINator device at a dose of either 10^7 CFU or 10^9 CFU to healthy adult volunteers. To aid in the transition from earlier Phase 1 studies, we will enroll a small number of subjects who will receive lyophilized BPZE1 by needleless tuberculin syringe, as this was the method of administration used previously.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Potential risks include risks of study participation (in general), specific risks to study subjects, and theoretic risks to the environment through the introduction of an attenuated *B. pertussis* strain into human hosts.

2.3.1.1 Risks of Study Participation

The risks of study participation include exposure to the study product, maintenance of confidentiality, and side effects of phlebotomy. All risks will be minimized to every extent possible.

2.3.1.2 Risk to Study Subjects

B. pertussis colonization is strictly limited to respiratory epithelium without dissemination of the bacteria outside the respiratory tract, which also excludes systemic bacteremia of the BPZE1 strain.

B. pertussis is spread mainly by aerosol formed by coughing of infected persons. The coughing is induced by the tracheal cytotoxin (TCT), which is more than 95% reduced in BPZE1. The BPZE1 strain is not expected to induce coughing, therefore transmission is highly unlikely. *Bordetella* species have fastidious growth requirements and have limited survival time outside the human body.

B. pertussis has not been shown to be allergenic in any preclinical or clinical studies to date, nor have any of the excipients in the lyophilized formulation. BPZE1 has been shown to protect against airway inflammation induced by allergens or viral infections in a murine model. [7] BPZE1 has also been shown to protect against infection with wild type *B. pertussis* infection 3 hours after immunization in a murine model.[8] However, there remains a theoretical risk of allergic reaction, as is present with any vaccine product.

BPZE1 vaccine or a placebo will be administered nasally via the VaxINator atomization device attached to a syringe to healthy adult volunteers under strictly controlled conditions. The VaxINator atomizes the liquid vaccine as it exits the syringe. There are no additional risks to study subjects based on the use of the VaxINator device, which is described in more detail in Section 6; similarly, there are no independent risks associated with the use of the tuberculin syringe used to drop BPZE1 vaccine into the nose.

To minimize the risk of transmission, the volunteers will stay at the study center for 2 hours after administration of the vaccine. In addition, volunteers with household or professional contact with infants below one year of age or household/professional contact with individuals with immunodeficiency will be excluded from participation in the study. The attenuated BPZE1 bacteria colonize the upper respiratory tract similarly to wild-type *B. pertussis*. Colonization of live organism will be assessed from a nasopharyngeal swab performed 28 days after administration to ensure all subjects are cleared of colonization. If a subject is not clear at day 29, a repeat nasal swab and culture will be performed at day 46. Chronic carriage of BPZE1 has not been reported (i.e., the vast majority of subjects have been clear at day 29 and no subject has had positive cultures at day 46) and is therefore not expected. Any subject who remains positive at day 46 will be provided a short course of azithromycin, which is clinically used to eradicate *B. pertussis* from the nasopharynx. Azithromycin will be prescribed by a study physician and filled by the hospital pharmacy or the patient's desired pharmacy at no cost to the subject.

Furthermore, in animal studies, when female mice were vaccinated with BPZE1 shortly before mating, no negative effect on either the pregnancy or their offspring was observed. The offspring were protected against *B. pertussis* challenge, based on evaluation of the lungs at necropsy.[16]

[redacted]

2.3.1.3 Risks to the Environment or Potential for Interaction with wild-type *B. pertussis* strains

Detailed information about the [redacted] BPZE1 is available in the environment risk assessment located in the investigator's brochure.

To avoid accidental exposure to the environment and to study staff, actions should be taken to minimize generation of aerosols, since the bacteria are strictly respiratory tract organisms. Staff members should wear eye-protective glasses during the vaccination. Persons handling BPZE1 should wear gloves and must practice standard hand hygiene before touching their skin or eyes.

[redacted]

There is no known animal vector or reservoir for *B. pertussis*. BPZE1 bacteria are not invasive and have no selective advantage in the environment. The potential for exchange of genetic material is virtually inexistent, since *B. pertussis* does not harbor plasmids or conjugative transposons. In addition, *B. pertussis* Tohama I (background used for the BPZE1 strain) does not harbor intact prophage genomes and is therefore incapable of producing functional phage particles.

[redacted]

proliferation power of the BPZE1 cells in the trachea and lungs and no weight

Based on these data, BPZE1 has been classified as a Biosafety level 1 organism by French authorities Republique Francaise Ministere De L'enseignement Superieur Et De La Recherche (French Ministry of Higher Education and Research), http://www.enseignementsup-recherche.gouv.fr/. Germany, Belgium, and Sweden have accepted the French Authority's BSL1 rating for the purpose of manufacturing and clinical studies.

No cross-contamination between the volunteers was observed in the previous phase 1 clinical trials of BPZE1, nor was any risk to the family members of study participants observed. In summary, the preliminary risk assessment for this study suggests there is an extremely low risk for potential environmental impact associated with administering the BPZE1 to study subjects.

2.3.2 Known Potential Benefits

The benefits of the study lie primarily in the opportunities to science and humanity. No direct personal benefit from participation in the study can be guaranteed, as the vaccine may or may not confer protection in humans.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

Primary:

1. To assess the safety and tolerability of a single intranasal dose of either 10^7 or 10^9 colony forming units (CFU) of lyophilized BPZE1 vaccine.

Secondary:

1. To assess the humoral immunogenicity of lyophilized BPZE1 vaccine at Day 15, Day 29 and Day 181 following receipt of one intranasal dose of 10^7 or 10^9 CFU of BPZE1.
2. To assess mucosal immunogenicity of lyophilized BPZE1 vaccine at Day 29 and Day 181 following receipt of one intranasal dose of 10^7 or 10^9 CFU of BPZE1.
3. To evaluate nasal clearance of BPZE1 by culture at Day 29 (and if still positive, at Day 46) following receipt of one intranasal dose of lyophilized BPZE1 vaccine of 10^7 or 10^9 CFU of BPZE1.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

1. Occurrence of solicited local and systemic reactogenicity from the time of the study vaccination through 14 days post-vaccination
2. Occurrence of unsolicited non-serious adverse events from the time of the study vaccination through 28 days post-vaccination
3. Occurrence of serious adverse events (SAEs) and new onset chronic medical conditions (NOCMCs) from the time of the study vaccination through approximately Day 181 post vaccination
4. Occurrence of adverse events of special interest through approximately Day 29 post vaccination

3.2.2 Secondary Outcome Measures

1. Humoral Immune Response
    - Geometric mean titers (GMTs) by serum IgG and IgA ELISA to PT, FHA, PRN, FIM 2/3 on Days 1, 15, 29, and 181

- Geometric mean fold rise (GMFR) by serum IgG and IgA ELISA to PT, FHA, PRN, FIM 2/3 on Days 15, 29 and 181 relative to baseline (Day 1)
- The proportion of subjects that achieve seroconversion (defined as ≥2-fold rise from baseline) to individual pertussis antigens (PT, FHA, PRN, and FIM 2/3) by serum IgG or IgA ELISA at Days 15, 29, 181 or any time point relative to baseline (Day 1)
- Proportion of subjects achieving seroconversion (serum IgG or IgA ELISA) to one or more pertussis antigens at Days 15, 29, 181 or any time point, relative to baseline (Day 1)
- Proportion of subjects achieving seroconversion (serum IgG/IgA ELISA) to two or more pertussis antigens at Days 15, 29, 181 or any time point, relative to baseline (Day 1)

2. Mucosal Immune Response
   - Geometric mean titer ratios (GMTRs) and ratio of antigen-specific IgA ELISA (PT-IgA, FHA-IgA, PRN-IgA, FIM-IgA) to total IgA by nasal aspirate at screening, and Days 29 and 181.
   - Fold rise of the ratio of antigen-specific IgA (PT-IgA, FHA-IgA, PRN-IgA, FIM-IgA) to total IgA by nasal aspirate from the screening visit to Days 29 and 181
   - The proportion of subjects that achieve seroconversion (defined as ≥2-fold rise of the ratio from baseline) to individual pertussis antigens (PT, FHA, PRN, and FIM 2/3) by nasal aspirate IgA ELISA at Days 29 and 181 or any time point relative to baseline (screening)
   - Proportion of subjects that achieve seroconversion (≥2-fold rise of the ratio from baseline) to at least 1 antigen (PT, FHA, PRN, or FIM 2/3) by nasal aspirate IgA ELISA from the screening visit to Days 29 and/or 181.
3. Proportion of subjects with detectable *B. pertussis* from nasopharyngeal cultures at Day 29.
   - For those who remain positive at Day 29, the subjects with detectable *B. pertussis* at Day 46 will also be evaluated and reported. Detection of *B. pertussis* in culture will be assumed to be due to study-product administration.

4 STUDY DESIGN

This is a phase 2a, single center, randomized, partially blind, placebo controlled, clinical trial evaluating a single intranasal dose of either 10^7 CFU or 10^9 CFU of BPZE1 in healthy adults (18-49 years of age, inclusive). The study will evaluate a lyophilized formulation of the product, with the goal of testing for the optimal dose for subsequent clinical trials. Since it is anticipated that the VaxINator device will be the primary mechanism of delivery in subsequent clinical trials, the majority of volunteers will receive vaccine or placebo via VaxINator device. Five individuals will receive 10^9 CFU of BPZE1 by needleless tuberculin syringe in order to compare immunogenicity to previous Phase 1 clinical trials of BPZE1.

We anticipate that enrollment will require approximately 12 months, with each subject participating for 6 months. Fifty healthy adults, 18-49 years of age will be randomized to one of four treatment groups in a 3:3:3:1 ratio, as described in the table below. Safety will be assessed through an Independent Safety Monitor (ISM) and a Safety Monitoring Committee (SMC) described in Section 9.6.

Table 2: Treatment Groups and Intranasal Vaccine to be Administered

Group	N	Intranasal Vaccination	Device Used for Administration
1	15	BPZE1 10^7 CFU	VaxINator
2	15	BPZE1 10^9 CFU	VaxINator
3	15	Placebo	VaxINator
4	5	BPZE1 10^9 CFU	Needleless Tuberculin Syringe

5 STUDY ENROLLMENT AND WITHDRAWAL

Approximately 50 healthy male and non-pregnant female subjects 18-49 years of age, inclusive, who meet all eligibility criteria will be enrolled in this study from one VTEU site. Enrollment will occur over an approximately 6-month period, and the target population will be the general Nashville community. Information regarding this study may be mailed or emailed to subjects who have previously participated in vaccine trials conducted at the VTEU site; other forms and/or mechanisms of recruitment may also be used. The local IRB will approve all materials prior to use.

Subject eligibility criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

No exemptions are granted on Subject Inclusion or Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed toward the DMID Medical Officer. Subjects otherwise eligible with grade 1 abnormalities in hematology or chemistry values may be considered for enrollment if, in the opinion of the investigator (or clinician on the 1572), the abnormalities are not clinically significant and do not pose additional risk to the study or the volunteer.

5.1 Subject Inclusion Criteria

Subjects eligible to participate in this study must meet all inclusion criteria:

1. Provide written informed consent prior to initiation of any study procedures
2. Able to understand and comply with planned study procedures and be available for all study visits
3. Males or non-pregnant females, 18-49 years of age, inclusive
4. In good health[1]

[1]*As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days that would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days. This includes no change in chronic prescription medication, dose, or frequency because of deterioration of the chronic medical diagnosis or condition in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, if it is in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition. Similarly,*

medication changes after enrollment and study vaccination are acceptable provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination. Note: Topical and inhaled medications (with the exception of inhaled or nasal corticosteroids within 30 days prior to enrollment), herbals, vitamins, and supplements are permitted.

5. Oral temperature is less than or equal to 100°F
6. Pulse is 45 to 100 bpm, inclusive[2]

[2]Pulse can be 45 to 50 bpm, inclusive, if no other symptoms are present. Otherwise, pulse should be 50-100 bpm.

7. Systolic blood pressure is 85 to 150 mm Hg, inclusive
8. Diastolic blood pressure is 55 to 95 mm Hg, inclusive
9. White blood cell count is 3,900 cells/µL or greater[3]
10. Hemoglobin is 13.0 g/dL or greater (men) or 11.8 g/dL or greater (women)[3]
11. Platelet count is 135,000 cells/µL or greater[3]
12. Alanine aminotransferase is less than 45 U/L (women) or 62 U/L (men)[3]
13. Serum creatinine is less than or equal to 1.25 mg/dL (men) or 1.11 mg/dL (women)[3]

[3] Abnormalities in white blood count, hemoglobin, platelet count, alanine aminotransferase, and serum creatinine that are suspected to be due to laboratory anomalies may be repeated once to ensure accuracy; additionally, otherwise eligible subjects with grade 1 abnormalities in these values may be considered for enrollment if, in the opinion of the investigator (or clinician on the 1572), the abnormalities are not clinically significant and do not pose additional risk to the study or the volunteer.

14. Negative serum HIV antibody assay
15. Women of childbearing potential[4] must use an acceptable contraception method[5] from 30 days before study vaccination until 60 days after vaccination.

[4]Not sterilized via tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year has passed since the last menses if menopausal.

*[5]Includes, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving study vaccination, barrier methods such as condoms or diaphragms/cervical caps **with** spermicide, effective intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill").*

16. Women of childbearing potential must have a negative urine or serum pregnancy test within 24 hours prior to study vaccination.

5.2 Subject Exclusion Criteria

1. Have immunosuppression as a result of an underlying illness or treatment, or use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination
2. Have known or suspected active chronic autoinflammatory condition
3. Have known active neoplastic disease (excluding non-melanoma skin cancer) or a history of any hematologic malignancy
4. Have a history of persistent asthma, major anatomic nasopharyngeal abnormality, or sinus polyp disease due to chronic sinusitis[6]

[6] *If a patient has a history of nasopharyngeal surgery such as, but not limited to rhinoplasty, tonsillectomy or sinus surgery, adequate healing time per the judgment of the investigator must occur prior to enrollment.*

5. Have known hepatitis B or hepatitis C infection
6. Have a history of alcohol or drug abuse within 5 years prior to study vaccination
7. Currently untreated or clinically unstable (in the opinion of the investigator) schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations
8. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 5 years prior to study vaccination
9. Have received corticosteroids (including oral, parenteral, inhaled, nasal, or intra-articular) of any dose within 30 days prior to study vaccination
10. Individual with PT serum IgG antibodies ≥ 20 IU/mL and/or PRN serum IgG antibodies ≥ 125 IU/mL
11. Unwilling to refrain from smoking tobacco for 28 days post vaccination
12. Receipt of immunoglobulin or blood derived products within 90 days of enrollment
13. Receipt of a vaccine against pertussis in the past 2 years
14. Receipt of a live vaccine within 30 days of study vaccination or an inactivated vaccine within 14 days of study vaccination
15. Planned vaccination with a licensed vaccine within 28 days of study vaccination
16. History of severe allergic reaction (e.g., anaphylaxis) or Bell's palsy, or Guillain-Barré syndrome, after a previous dose of any diphtheria toxoid-tetanus toxoid-, or pertussis-containing vaccine, or encephalopathy within 7 days of administration of a previous pertussis containing vaccine.
17. History of a progressive neurologic disorder
18. In close contact[7] with children less than 1 year of age or contact with persons with known immunocompromising conditions

[7] *Close contact includes sharing a household, serving as a healthcare worker, or working professionally in settings with repeated exposures.*

19. Receipt of *B. pertussis*-active antibiotics[8] within 7 days prior to vaccination

[8] *B. pertussis active antibiotics include macrolides, fluoroquinolones, trimethoprim-sulfamethoxazole, tetracyclines*

20. Known hypersensitivity to any component of the study vaccine.
21. Hypersensitivity to azithromycin, which may be used in the event of ongoing BPZE1 colonization
22. Any condition that, in the opinion of the investigator, might interfere with objectives of the study or safety to the individual
23. Acute illness, including temperature >100˚F within one week prior to vaccination[9]

[9] *Enrollment may be postponed if acute illness occurs; subjects must remain within the screening window, however, and must be rescreened if >30 days elapses prior to enrollment.*

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Once consented and upon entry of demographic data and confirmation of eligibility for this study, the subject will be enrolled. Approximately 50 subjects will be assigned randomly to 1 of 4 treatment arms, in a 3:3:3:1 ratio. The first treatment arm (n=15) will be vaccinated with one dose of 10^7 CFU BPZE1 using the VaxINator device. The second treatment arm (n=15) will be vaccinated with one dose of 10^9 CFU BPZE1 using the VaxINator device. The third treatment arm (n=15) will be vaccinated with one dose of placebo via VaxINator device. The fourth treatment arm (n=5) will be vaccinated with one dose of 10^9 CFU BPZE1 using a needleless tuberculin syringe.

Enrollment of subjects will be done online using the enrollment module of AdvantageEDC^{SM}. The randomization code will be prepared by statisticians at the DCC and included in the enrollment module for this study. AdvantageEDC^{SM} will assign each subject to a treatment arm after the demographic and eligibility data have been entered into the system. A designated individual at the participating VTEU site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure location.

Instructions for use of the enrollment module are included in the AdvantageEDC^{SM} User's Guide. Manual back-up procedures and instructions are provided for use in the event that the

participating VTEU site temporarily loses access to the Internet or the online enrollment system is unavailable.

5.3.2 Masking Procedures

This is a partially-blind clinical study as the method of immunization (i.e., VaxINator vs. needleless syringe) will be known. After reconstitution and transfer to a 1-mL syringe, the 10^7 CFU dose investigational vaccine and the placebo are transparent and indistinguishable while the 10^9 CFU dose can be discriminated from the placebo and the 10^7 CFU dose due to its opaque appearance. Therefore, syringes will be wrapped with a colored sleeve to avoid potential unblinding of study personnel or the subject. Investigators, the subject, and study personnel will remain blinded to allocation of BPZE1 or placebo.

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

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

Laboratory personnel performing immunogenicity assays will be blinded to study assignment. Upon request by the participating VTEU site, and at the discretion of DMID, unmasking may occur after all data have been locked at Day 181, analyzed, interpreted, and the final CSR has been completed.

The Safety Monitoring Committee (SMC) may receive data in aggregate and presented by treatment arm. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion and may request the treatment arm be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only.

5.3.3 Reasons for Withdrawal

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

An investigator may also withdraw a subject from receiving the study product for any reason. Follow-up safety evaluations 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, might include, but are not limited to the following:

1. Subject no longer meets eligibility criteria
2. Subject meets individual halting criteria
3. Subject becomes noncompliant
4. 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
5. Subject lost to follow-up
6. Subject becomes pregnant, if applicable
7. 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 performed. If the subject consents, every attempt will be made to follow all AEs through resolution. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent or the investigator may seek subsequent informed consent using an IRB/IEC-approved consent form with the revised procedures.

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

5.3.4 Handling of Withdrawals

The primary reason for withdrawal from this study will be recorded on the appropriate data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.5.

Although subjects are free to withdraw at any time or may be withdrawn by the site principal investigator or appropriate sub-investigator at any time, subjects will be encouraged to remain in this study for follow-up safety assessments (may be conducted by phone rather than in person) continuing through approximately 6 months after study vaccination. These subjects will also be

encouraged to provide a venous blood sample for serological assessment at approximately 28 days after study vaccination. See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Every attempt will be made to follow all adverse events, including systemic reactions, unsolicited non-serious adverse events, and serious adverse events ongoing at the time of early withdrawal through resolution.

For subjects who fail to appear for a follow-up safety assessment, extensive effort (e.g., three documented contact attempts via phone calls, e-mails, etc., made on separate occasions and followed by a certified letter) will be made to locate them. Any questions to determine their health status will be made by phone or visit by the subject. These efforts will be documented in the subject's study records.

5.3.5 Subject Replacement

Subjects who withdraw, are withdrawn/terminated from this study, or are lost to follow-up after signing the informed consent form and randomization but before receipt of study vaccine may be replaced. Subjects who are vaccinated, but do not complete the Day 15 visit, will be replaced. Subjects who are vaccinated and subsequently, after the Day 15 visit, withdraw, are withdrawn or terminated from this study, or are lost to follow-up will not be replaced. Subjects who were previously screened and found to be ineligible due to initial serum concentrations of PRN IgG ≥ 20 IU/ml in protocol version 3.0 may be rescreened per the revised exclusion criterion of protocol version 4.0.

5.3.6 Termination of Study

Although the sponsor has every intention of completing this study, it reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to SMC recommendation and at the discretion of DMID.

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 INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

BPZE1 investigational vaccine

The investigational BPZE1 lyophilized vaccine is for nasal administration and contains

[redacted]

Two BPZE1 investigational vaccines with different strengths (expressed as colony forming units (CFU) per mL or per dose), composed of BPZE1 at 10^7 and 10^9 CFU/dose, are to be evaluated. Each individual investigational vaccine vial, after reconstitution, contains 1 mL of BPZE1 suspension.

Placebo

The placebo consists of the same constituents in the same quantities as the BPZE1 investigational vaccines, absent the attenuated *B. pertussis* cells.

VaxINator atomization device

BPZE1 investigational vaccine or placebo will be administered nasally via the VaxINator atomization device attached to a 1 mL syringe. The VaxINator is manufactured by Teleflex Medical. Teleflex has a Master File (MF #16712) for the VaxINator. The VaxINator is a conical shape plastic component that Luer-locks onto a standard syringe. The VaxINator atomizes the liquid vaccine as it exits the syringe. The conical shape forms a plug in the nostril and high applied-pressure atomizes the liquid into a fine mist.

6.1.1 Acquisition

6.1.1.1 BPZE1 vaccine and Placebo

Individual boxes of packaged and labeled investigational vaccine/placebo contain investigational vaccine/placebo. The boxes shall be shipped from [redacted] for distribution to the clinical site. All boxes of investigational vaccine/placebo will be shipped at below -20 C with temperature logging devices and should be stored at -10˚C or below after arrival to the site.

6.1.1.2 Additional Components

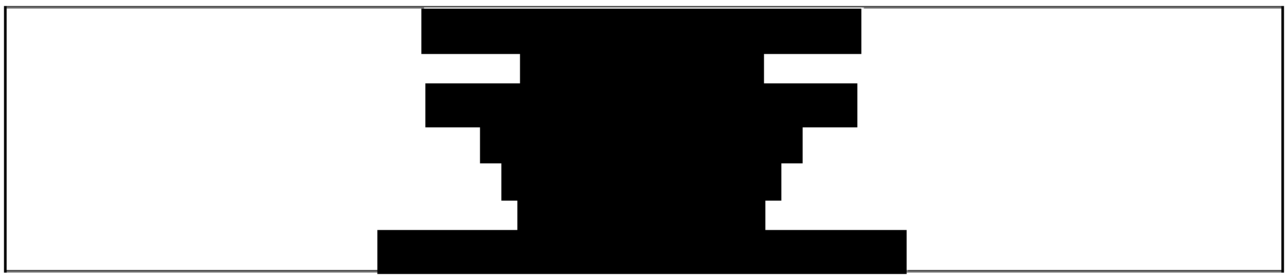
VaxINator kits are supplied in individual sterile pouches containing a VaxINator atomization device, a 1-mL single-use syringe, and other accessories not utilized in the current protocol that will be discarded. VaxINator kits will be shipped from [redacted] for distribution to the clinical site.

The following additional components will be sourced by [redacted] for distribution to the clinical site:
1) 18G x 1 ½", sterile needles in a box containing 100 units in total
2) 1-mL, sterile, tuberculin syringes in a box containing 100 units in total

All boxes of components and VaxINators (excluding investigational vaccine or placebo) should be stored at ambient temperature.

Upon request by DMID, BPZE1 vaccine, Placebo and VaxINator atomization device and additional components will be transferred to the following address:

The Investigational Drug Service Pharmacy will provide sterile Water for Injection (SWFI) needed for study drug dilution.



BPZE1 vaccine, Placebo and VaxINator atomization device and additional components will be provided through the DMID CMS to the participating sites prior to the start of this trial upon request and with prior approval from DMID.

6.1.2 Formulation, Packaging, and Labeling

BPZE1 vaccine and Placebo
The primary packaging is a sterile glass 2R DIN vial with a chlorobutyl lyophilization stopper. The vial closure system is a combination of the stopper and the aluminum cap. The vials are crimped directly after the lyophilization process using the automated Fill & Finish unit that filled the vials with 1.0 mL suspension.

One vial for administration will be prepared for each subject in the clinical trial, typically packaged in a box of 10 vials (sufficient for 10 subjects). The box complies with the safety regulations for GMO storage and shipment. The vials and the boxes will be labeled with the required information applicable to investigational vaccine or placebo product used in clinical trials. The boxes either contain the vaccine or the placebo, typically as 10 vials per box.

A single VaxINator kit (sterile pouch containing a VaxINator atomization device, a 1-mL single-use syringe, and other accessories not used in this protocol to be discarded) is provided for each subject in groups 1, 2, and 3. The boxes will be labeled with the required information applicable to a medical device accessory, for clinical trial investigational use only.

The 18G x 1 ½", and tuberculin syringes to reconstitute the investigational vaccine or placebo vials will be stored separately from the investigational vaccine pouches at ambient temperature. For each individual subject, the participating site research pharmacist will fill a 1-mL tuberculin syringe with SWFI for reconstitution.

The placebo consists of the same constituents in the same quantities as the BPZE1 investigational vaccines, absent the attenuated *B. pertussis* cells. After reconstitution and transfer to a 1-mL syringe, the 10^9 CFU dose can be discriminated from the placebo and the 10^7 CFU dose due to the opaque appearance of the 10^9 CFU dose BPZE1 investigational vaccine, while the placebo and the 10^7 CFU dose investigational vaccine are transparent. The 10^7 CFU dose investigational vaccine and the placebo are indistinguishable after reconstitution.

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

Further details are included in the *Bordetella pertussis* vaccine (BPZE1) IB, as well as in the protocol-specific MOP.

6.1.3 Product Storage and Stability

BPZE1 vaccine and Placebo
Both BPZE1 and placebo product will be stored at -10˚C or less until ready for use in order to maintain viability of BPZE1 prior to reconstitution with SWFI. The exposure of reconstituted BPZE1 to room temperature prior to vaccination should not exceed 60 minutes. A detailed description regarding the storage, reconstitution and handling the investigational vaccines is given in the Manual of Procedures (MOP).

VaxINator devices are stored at ambient temperature.

Additional details of product storage and stability can be found in the MOP.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

See the protocol-specific MOP for detailed information on the preparation, labeling, storage, and administration of study vaccine for each treatment arm. Study vaccine preparation will be performed by the participating site research pharmacist.

For those randomized to the VaxINator groups, a full-dose (i.e., 800 µL containing 10^7 or 10^9 CFU) will be drawn into one 1 mL syringe from the vial, the VaxINator tip connected, and 400 µL volume will be administered to each nostril. The VaxINator provides a uniform, controlled delivery, which allows the clinician to accurately deliver 400 µL of vaccine to the initial nostril and then administer the remaining 400 µL to the opposite nostril.

For those randomized to administration via needleless tuberculin syringe, the same procedures will be followed, deleting the connection of a VaxINator tip.

The dose of BPZE1 active ingredient or placebo will be administered to the volunteer within 60 minutes of reconstitution.

For the 10^9 CFU vaccine dose, one mL of SWFI is used to reconstitute the 1 mL vial of lyophilized BPZE1, but only 800 µL of vaccine are used for administration into both nostrils. The SWFI will be drawn into 1 mL syringes, the 1 mL SWFI will be injected into the lyophilized BPZE1 vial for reconstitution, 900 µL vaccine dose will be withdrawn into a separate 1 mL syringe, the plunger will be expelled to the 0.8-mL mark, the VaxINator shall be attached to the syringe, and 400 µL volume will be administered to each nostril. The VaxINator provides a uniform, controlled delivery, which allows the vaccinator to accurately deliver 400 µL of vaccine to the initial nostril and then administer the remaining 400 µL to the opposite nostril.

For the 10^7 CFU vaccine dose, three 1 mL SWFI-filled syringes and two placebo cryovials are used to reconstitute and dilute the 1 mL vial of lyophilized 10^9 BPZE1. The SWFI will be injected into each of the placebo cryovials and the 1 mL vial of lyophilized BPZE1. Two serial dilutions (10-fold and 100-fold) of the reconstituted 10^9 CFU vaccine will be done to prepare a 10^7 CFU vaccine dose. A 900 µL vaccine dose will be withdrawn into a separate 1 mL syringe, the plunger will be expelled to the 0.8-mL mark, the VaxINator shall be attached to the syringe, and 400 µL volume will be administered to each nostril. The VaxINator provides a uniform, controlled delivery, which allows the vaccinator to accurately deliver 400 µL of vaccine to the initial nostril and then administer the remaining 400 µL to the opposite nostril.To avoid accidental exposure to the environment and to study staff, actions should be taken to minimize generation of aerosols, since the bacteria are strictly respiratory tract organisms. Staff members should wear eye-protective glasses during the vaccination. Persons handling BPZE1 should wear gloves and must practice standard hand hygiene before touching their skin or eyes.

6.3 Modification of Study Intervention/Investigational Product for a Participant

No modifications of the investigational product are anticipated as this is a single dose study.

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

Study products will be stored and shipped from the DMID-CMS, [redacted], to the participating VTEU. Once received, study products will be stored in and dispensed by the Investigational Pharmacy. Unused product will be handled in accordance with the MOP.

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

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

Used and unused vials of vaccine and placebo will be retained until clinical trial accountability is completed, monitored and released for disposition as applicable. At study termination, all unused investigational product and VaxINator devices will be handled in accordance with the MOP following complete drug accountability and monitoring.

6.5 Assessment of Subject Compliance with Investigational Product

Subjects will be directly observed at the time of dosing by a member of the clinical research team who is licensed to administer the study product. Administration will be documented on the appropriate data collection form and entered into the eCRF.

6.6 Concomitant Medications/Treatments

Administration of medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications and medications taken within 30 days prior to signing the informed consent form through approximately 28 days after study vaccination (or through the early termination visit if prior to that time). Prescription and over-the-counter drugs will be included, as well as herbals, vitamins and supplements. In addition, receipt of non-study vaccines will be solicited through approximately 181 days after study vaccination and reported in the eCRF.

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 investigational product should not be used during the study-reporting period unless clinically indicated as part of the subject's health care. Use of bronchodilator medication due to wheezing (new prescription or daily use) will be used along with signs and symptoms to define a medically significant wheezing adverse event.

7 STUDY SCHEDULE

7.1 Screening Visit (Visit 00, Screening, Clinic Visit [Window: Day -30 to Day -1])

Potential subjects will be screened for eligibility up to 30 days prior to the administration of the study vaccine. The following activities will be performed:

- Subjects will be provided with a description of this study (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures, including any screening procedures.
- Demographic information will be obtained by interview of subjects.
- Eligibility criteria will be reviewed with subjects.
- Complete medical history will be obtained by interview of subjects to ensure eligibility.
- All concomitant medications taken and vaccines received within 30 days prior to signing the informed consent form will be reviewed with subjects and reported in the electronic Case Report File (eCRF).
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained to ensure eligibility. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- A urine or serum pregnancy test will be performed on all women of childbearing potential and must be negative to ensure eligibility.
- Approximately 10 mL of venous blood will be collected for complete blood count, serum chemistries and HIV antibody testing.
- Approximately 5 mL of venous blood will be collected for screening antibody assays (PT and PRN serum IgG).
- Nasal samples will be obtained for baseline mucosal antibody assays.

7.2 Enrollment/Vaccination (Visit 1, Clinic Visit [Day 1])

- Subject's willingness to participate will be reconfirmed and documented in the subject's study records prior to performing any further study procedures, including administration of the study vaccination.

- Eligibility criteria will be reviewed with subjects prior to study vaccination to ensure continued eligibility.

- Complete medical history and any updates obtained by interview of subjects since the screening visit will be reviewed with subjects prior to study vaccination to ensure continued eligibility.

- All concomitant medications will be reviewed with subjects prior to study vaccination for accuracy and completeness. Any new concomitant medications taken since the screening visit will be reviewed with subjects and assessed for continued eligibility prior to study vaccination. Medications reported in the eCRF are limited to those taken within 30 days prior to study vaccination.

- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to study vaccination. Vital signs assessed on Day 1 will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A limited physical examination will be performed, focused on the nasopharynx, oropharynx, and lungs prior to study vaccination; in addition, a targeted physical examination will be performed, if indicated, based on review of interim medical history, by a study clinician.

- A urine or serum pregnancy test will be performed within 24 hours prior to study vaccination on all women of childbearing potential. Results must be negative and known prior to randomization and first study vaccination.

- Approximately 10 mL of venous blood will be collected immediately prior to study vaccination for baseline antibody assays.

    - For subjects who consent to additional future use samples, approximately 24 mL of venous blood will be collected immediately prior to study vaccination for PBMC isolation and possible future research.

- Subjects will be enrolled in AdvantageEDC^{SM} and assigned randomly to a treatment arm prior to study vaccination.

- Pre-administration reactogenicity assessments will be performed prior to vaccination to establish baseline. Subjects will then receive a single dose of study vaccine via intranasal application. Subjects will be observed in the clinic for two (2) hours to record post-administration reactogenicity and any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.

- Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited mucosal and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their oral temperature around the same time each day. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Subjects will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the subject on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

7.3 Follow-up Visits

7.3.1 Visit 2, Day 8, Clinic Visit (Window: Day 8 ± 2 days)

- Interim medical history, including an assessment for new medical conditions, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with subjects.

- All concomitant medications and vaccines will be recorded on the appropriate data collection form.

- All AE/SAEs will be recorded on the appropriate data collection form.

- A limited physical examination will be performed, focused on the nasopharynx, oropharynx, and lungs; in addition, a targeted physical examination will be performed, if indicated, based on review of interim medical history, by a study clinician.

- Approximately 10 mL of venous blood will be collected for safety laboratory studies, to include a complete blood count (white blood cell count, platelet count, hemoglobin concentration) and serum chemistries (ALT and creatinine)

- For subjects who consent to additional future use samples, approximately 24 mL of venous blood will be collected for PBMC isolation and possible future research.

7.3.2 Visit 3, Day 15, Clinic Visit (Window: Day 15 ± 3 days)

- Interim medical history, including an assessment for new medical conditions, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- All concomitant medications and vaccines will be recorded on the appropriate data collection form.

- Memory aid information will be reviewed with subjects.

- All AE/SAEs will be recorded on the appropriate data collection form.

- A limited physical examination will be performed, focused on the nasopharynx, oropharynx, and lungs; in addition, a targeted physical examination will be performed, if indicated, based on review of interim medical history, by a study clinician.

- Approximately 10 mL of venous blood will be collected for antibody assays.

7.3.3 Visit 4, Day 29, Clinic Visit (Window: Day 29 ± 3 days)

- Interim medical history, including an assessment for new medical conditions, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- All concomitant medications and vaccines will be recorded on the appropriate data collection form.

- All AE/SAEs will be recorded on the appropriate data collection form.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician.

- Approximately 10 mL of venous blood will be collected for antibody assays.

- For subjects who consent to additional future use samples, approximately 24 mL of venous blood will be collected for PMBC isolation and possible future research.

- Nasal samples will be obtained for *B. pertussis* culture and immunological assays. If culture is positive for *B. pertussis*, the isolate will be cryopreserved as detailed in the MOP.

7.3.4 Visit 4a (If needed, based on *B. pertussis* positive culture on Day 29), Day 46, Clinic Visit (Day 46 ± 3 days)

- Interim medical history, including an assessment for new medical conditions, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- All concomitant vaccines will be recorded on the appropriate data collection form.

- All SAEs will be recorded on the appropriate data collection form.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician.

- Nasal samples will be obtained for *B. pertussis* culture (if necessary-positive from day 29) and immunological assays. If culture is positive for *B. pertussis*, the isolate will be cryopreserved as detailed in the MOP.

7.3.5 Visit 4b (if needed, based on positive *B. pertussis* culture from Day 46), Day 50, Phone Call (Day 50 ± 3 days)

- If a participant's sample from Visit 4a is positive for *B. pertussis*, a study investigator will prescribe a 5-day course of azithromycin (500 mg orally once per day).

7.4 Final Study Visit, Visit 5, Day 181, Clinic Visit (Day 181 ± 21 days)

- Interim medical history, including an assessment for new medical conditions, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- All concomitant vaccines will be recorded on the appropriate data collection form.

- All SAEs will be recorded on the appropriate data collection form.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician.

- Approximately 10 mL of venous blood will be collected for antibody assays.

- For subjects who consent to additional future use samples, approximately 24 mL of venous blood will be collected for PBMC isolation and possible future research.

- Nasal samples will be obtained for immunological assays

7.5 Early Termination Visit

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

- Interim medical history, including an assessment for new medical conditions, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with subjects (if within 15 days after study vaccination).

- All concomitant medications will be recorded on the appropriate data collection form (if prior to 29 days after study vaccination).

- All non-study vaccines will be recorded on the appropriate data collection form (if prior to 181 days after study vaccination).

- All AE/SAEs will be recorded on the appropriate data collection form. Only SAEs will be recorded after 28 days following study vaccination.

- Vital signs, including oral temperature, pulse, and blood pressure, may be obtained if indicated based on clinical history. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician.

- Post-administration reactogenicity assessments will be performed (if within 15 days after study vaccination).

- Venous blood will only be obtained if the early termination visit occurs: 1) prior to Visit 2, 2) after day 14 post-vaccination but before Visit 3, or 3) at least 28 days following Visit 3.

    - Approximately 10 mL of venous blood will be collected for antibody assays.

    - For subjects who consent to additional future use samples, approximately 24 mL of venous blood will be collected for PBMC isolation and possible future research.

7.6 Unscheduled Visit

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

- Interim medical history, including an assessment for new medical conditions, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.

- Memory aid information will be reviewed with subjects (if within 15 days after study vaccination).

- All concomitant medications will be recorded on the appropriate data collection form (if prior to 29 days after study vaccination).

- All non-study vaccines will be recorded on the appropriate data collection form (if prior to 181 days after study vaccination).

- All AE/SAEs will be recorded on the appropriate data collection form. Only SAEs will be recorded after 28 days following study vaccination.

- Vital signs, including oral temperature, pulse, and blood pressure, may be obtained if indicated based on clinical history. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician.

- Post-administration reactogenicity assessments will be performed (if within 15 days after study vaccination).

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Complete medical history will be obtained by interview of subjects at the screening visit and will be updated on Day 1 prior to the study vaccination. 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, persistent asthma, nasopharyngeal symptoms (e.g., sinusitis) cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits after study vaccination, an interim medical history will be obtained by interview of subjects noting any changes since the previous clinic visit or contact. The interim medical history should include an assessment for new medical conditions.

Medications history (concomitant medications) will include a review of all current medications and medications taken within 30 days prior to signing the informed consent form through approximately 28 days after study vaccination or through the early termination visit (if prior to 28 days after the last study vaccination), whichever occurs first. All medications will be recorded on the appropriate data collection forms. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. In addition, receipt of non-study vaccines will be solicited from 28 days prior to enrollment through approximately 181 days after study vaccination and reported in the eCRF. Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition. Assessment of eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Sections 5.1and 5.2). In addition, the site principal investigator 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.

At the screening visit, a physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. On Visit 1, prior to study vaccination, and on Visits 2 and 3, a limited physical examination will be performed, focused on the nasopharynx, oropharynx, and lungs; in addition, a targeted physical examination will be performed, if indicated, based on review of interim medical history, by a study clinician. At follow-up visits 4, 4a and 5, a targeted physical examination may be performed, if indicated based on subject's interim medical history, by a study clinician.

Vital signs (oral temperature, pulse, and blood pressure) will be collected at the screening visit and prior to study vaccination. Vital signs assessed on Day 1 prior to vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

Reactogenicity assessments will include an assessment of solicited adverse events occurring from the time of each study vaccination through 14 days after study vaccination, which includes an assessment of mucosal reactions that include: runny nose, stuffy nose/congestion, nasal pain/irritation, epistaxis, sneezing, sinus pressure/pain, sore throat, cough, and shortness of breath, as well as systemic reactions that include fever, chills, fatigue, malaise, myalgia, arthralgia, headache, and hypersensitivity. Pre-administration reactogenicity assessments will be performed prior to study vaccination to establish baseline, then the study vaccination will be given.

Subjects will be observed in the clinic for approximately 2 hours after study vaccination. Post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.

All subjects will complete a subject memory aid from the time of study vaccination through 14 days after study vaccination. Subject memory aids will be reviewed with the subjects for adverse events (solicited injection site and systemic reactions and unsolicited AEs) at follow-up visits occurring through approximately 14 days after study vaccination.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Urine or serum pregnancy tests will be performed locally by the site at the screening visit and within 24 hours prior to study vaccination on all women of childbearing potential. Results must be negative and known prior to randomization on Day 1 to be eligible for participation in this study.

Clinical screening laboratory parameters to be evaluated to confirm study eligibility and receipt of study vaccination will include complete blood counts, serum chemistries and a negative serum HIV antibody assay. A venous blood sample will be collected from each subject at the screening visit.

8.2.2 Special Assays or Procedures

The following assays will be performed in the Vanderbilt Vaccine Research Program Laboratory. Details regarding each of the following assays are found in the MOP.

Venous blood for baseline pertussis antibodies
Venous blood will be obtained at screening to confirm PT IgG< 20 IU/mL and PRN IgG< 125 IU/mL serum. Standard ELISA will be used to measure serum concentrations of antibodies.

Venous blood for immune response to vaccination
Venous blood will be taken prior to and after vaccination to measure serum IgG and IgA by ELISA to four pertussis antigens (PT, FHA, PRN, FIM)

Nasal aspiration samples to evaluate mucosal immune response
Nasal aspiration samples will be used to measure mucosal IgA levels by ELISA to PT, FHA, PRN and FIM following vaccination. Procedures for collection of nasal aspiration samples are described in the Manual of Procedures.

***B. pertussis* Culture**
Standard microbiologic techniques will be used to assess the presence of *B. pertussis* by culture at Day 29 following vaccination.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP.

8.2.3.2 Specimen Shipment

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

Specimens for pertussis-specific immunology studies will remain at the Vanderbilt VTEU site as experiments are being conducted. Any specimens remaining once the protocol-defined studies are completed will be shipped to the DMID CAR.

Further instructions for specimen shipment are included in the protocol-specific MOP.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Serious adverse events (SAE) occurring from the time of vaccination through approximately 6 months after vaccination.

2. Solicited Adverse Events – reactogenicity events occurring from the time of each study vaccination through 14 days after study vaccination:

   a) Mucosal reactions including runny nose, stuffy nose/congestion, nasal pain/irritation, epistaxis, sneezing, sinus pressure/pain, sore throat, cough, and shortness of breath, cough, rhinorrhea, and sneezing

   b) Systemic reactions including fever, chills, fatigue, malaise, myalgia, arthralgia, headache, and hypersensitivity.

3. Unsolicited Adverse Events – study vaccine-related non-serious adverse events occurring from the time of study vaccination through approximately 28 days after study vaccination.

4. New onset chronic medical conditions (NOCMCs) and adverse events of special interest (AESI) – NOCMC's will be assessed throughout the follow-up period, while medically attended wheezing events (an AESI) will be collected through Day 29 post-vaccination.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event (AE): International Council for Harmonisation (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. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited mucosal (vaccination site) and systemic (subjective and quantitative) reactions, not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited non-serious AEs includes event description, date of onset, licensed study physician's assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through stabilization or resolution.

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

AEs must be graded for severity and assessed for relationship to study product (see definitions below). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator using a protocol-defined grading system (see Sections 9.2.2 and Appendix B). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): Events interrupt the subject's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Relationship to Study Product: The licensed study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be

assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2 Reactogenicity

Reactogenicity events are AEs that are common and known to occur following administration of these types of study vaccines. The following Toxicity Grading Scales will be used to grade solicited mucosal (vaccination site) and systemic (subjective and quantitative) reactions:

Mucosal Reactogenicity Grading

Local Reaction	***Mild (Grade 1)***	***Moderate (Grade 2)***	***Severe (Grade 3)***
Runny nose	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical care
Stuffy nose/congestion	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with breathing from nose	Unable to breathe through nose or seeks medical care
Nasal pain/Irritation	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort that prevents daily activity or seeks medical care
Epistaxis	Total duration of all episodes in a 24-hour period <30 minutes	Total duration of all episodes in a 24-hour period >30 minutes	Any bleeding that required visit for medical encounter
Sneezing	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort; prevents daily activity
Sinus pressure/pain	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort that prevents daily activity or seeks medical care
Sore/irritated throat	Noticeable but does not interfere with eating or drinking	Moderate discomfort that interferes with eating or drinking	Significant discomfort that prevents eating or drinking or seeks medical care
Cough	Noticeable but does not interfere with daily activity or sleeping	Frequent cough that interferes with daily activity or sleeping	Prevents daily activity, prevents sleep, or seeks medical care

Shortness of breath/wheezing	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort/ prevents daily activity or seeks medical encounter

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
Rash/Hypersensitivity	Pruritus with or without rash	Localized urticaria	Generalized urticaria, anaphylaxis, or angioedema or localized urticaria that requires medical encounter.

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 study vaccination will be considered as baseline.*

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

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

9.2.3 Serious Adverse Events

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

- Death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require 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.
- All events described as Guillain-Barré syndrome will also be considered SAEs.

** Life-threatening adverse event. An adverse event 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 adverse event that, had it occurred in a more severe form, might have caused death.*

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE form and eCRF.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Reviewed and evaluated by an Independent Safety Monitor (ISM), the SMC (periodic review unless related), DMID, and the IRB.

9.2.4 New Onset Chronic Medical Conditions

The development of new onset chronic medical conditions will be assessed through Day 181. These are defined as new medical conditions, not present at the time of screening or enrollment, that require ongoing medical care and intervention.

9.2.5 Adverse Events of Special Interest

Medically attended wheezing events will be considered adverse events of special interest given the route of study product administration and the nature of the study product (i.e., a live attenuated bacterial vaccine). Though subjects with a history of persistent asthma will be excluded from the study, it is possible that new onset wheezing episodes could occur. These will be evaluated by a study investigator and relatedness to study product will be assessed. Medically attended wheezing events will be evaluated through Day 29.

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

Solicited mucosal site and systemic reactogenicity events will be documented and reported from the time of study vaccination through 14 days (Day 15) after study vaccination.

Unsolicited, non-serious AEs will be documented from the time of study vaccination through approximately 28 days (Day 29) after study vaccination.

SAEs will be documented from the time of study vaccination through approximately 6 months post vaccination.

In addition, if a subject has an abnormal laboratory test value (e.g., complete blood count or serum chemistries), it will be recorded as an AE and will be graded per toxicity table and will be followed up until resolution.

9.3 Reporting Procedures

9.3.1 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

[redacted]

In addition to the SAE form, selected SAE data fields must also be entered into AdvantageEDC[SM]. Please see the protocol-specific MOP for details regarding this procedure.

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

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

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

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site principal investigator or appropriate sub-investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating site principal investigators (i.e., all principal investigators to whom the sponsor is providing drug under its IND or under any principal investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of 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. 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 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 serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via AdvantageEDC^{SM} on the Pregnancy Report form. With the subject's permission, all protocol-required venous blood samples will be obtained, and the subject will continue to be followed for safety for the duration of this study. Efforts will be made to follow all pregnancies reported during the course of this study until approximately 1 to 2 months post-pregnancy pending the subject's permission.

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

AEs will be collected, assessed, and followed through resolution from the time of study vaccination through approximately 28 days after study vaccination.

SAEs will be collected, assessed, and followed from the time of study vaccination through resolution even if this extends beyond the study-reporting period (approximately Day 181).

Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

If the site principal investigator or appropriate sub-investigator becomes aware of an acute febrile illness and the site principal investigator or appropriate sub-investigator decides to bring the subject in for an evaluation to determine etiology, then the site principal investigator or appropriate sub-investigator, at their own discretion, can determine the specific testing that should be performed.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5 Halting Rules

Further enrollment and study vaccinations will be halted for SMC review/recommendation if any of the following are reported:

- Any subject experiences ulceration, abscess, or necrosis in the nose that is considered related to study product administration, through the subject's last study visit.
- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- Two or more subjects experience generalized urticaria within 3 days after administration of study product that is considered related to study product.
- Any subject experiences a study product-related SAE from the time of study vaccination through the subject's last study visit.
- Any subject experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study product, through the subject's last study visit.

This study will also be halted for SMC review/recommendation if, within 8 days after administration of each study vaccination, any of the following occurs:

- Two or more subjects experience the same severe (Grade 3) mucosal Reactogenicity adverse event (table in section 9.2.2).
- Two or more subjects experience the same severe (Grade 3) Subjective Systemic Reactogenicity adverse event. Grading scales for solicited reactions are included in Section 9.2.2.
- Two or more subjects experience the same severe (Grade 3) Quantitative Systemic Reactogenicity adverse event. Grading scales for solicited reactions are included in Section 9.2.2.
- Two or more subjects experience the same severe (Grade 3) study related laboratory adverse event (HGLT in MedDRA coding)
- Two or more subjects experience the same severe (Grade 3) study related unsolicited systemic adverse event (HGLT in MedDRA coding)

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

9.6 Safety Oversight (ISM plus SMC)

9.6.1 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs in real time and other AEs as needed and provide an independent medical assessment and recommendation to DMID. The participating VTEU site will have an ISM with experience in infectious diseases or internal medicine, in close proximity to the participating VTEU site, and have the authority to readily access study participant records.

9.6.2 Safety Monitoring Committee (SMC)

Safety oversight will be conducted by a SMC that is an independent group of experts that monitors subject safety and advises DMID. The SMC members will be separate and independent of study personnel participating in this study and should not have scientific, financial or other conflict of interest related to this study. The SMC will consist of members with appropriate expertise to contribute to the interpretation of the data from this study.

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

- Organizational meeting: Prior to start of the study
- Data Review Meeting (DRM): After 15-day reactogenicity data are available for the first 25 subjects
- Ad hoc meetings: When a halting rule is met, for immediate concerns regarding observations during this study, or as needed.

Final review meeting: Approximately 6 months after final clinical database lock to review the cumulative unblinded safety data for this trial. The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID. The SMC will operate under the rules of a DMID-approved charter that will be approved at the organizational meeting of the SMC. At this time, each data element that the SMC needs to assess will be clearly defined. Procedures for SMC reviews/meetings will be defined in the charter. The SMC will review applicable data to include, but not limited to, study progress and

participant, clinical, safety, and reactogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, and solicited and unsolicited AE/SAEs. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC may receive data in aggregate and presented by treatment arm. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request the treatment assignment be unblinded for an individual subject if required for safety assessment.

The SMC will review grouped and unblinded data in the closed session only. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this study as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this study.

DMID or the SMC chair may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. The DMID Medical Monitor is empowered to stop enrollment and study vaccinations if adverse events that meet the halting criteria are reported. The DMID Medical Monitor and the ISM will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during the study.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that 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 study is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan. Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol compliance. Site monitors will have reasonable access to the participating VTEU site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with the site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

The goals of this study are to assess the immunologic response, safety, and tolerability of lyophilized BPZE1 administered intranasally at 10^7 CFU compared to 10^9 CFU, in healthy volunteers 18-49 years of age. Subjects in three of four treatment arms (45 of 50 expected subjects) will be vaccinated using the VaxINator device. In addition, the study will enroll a small number of subjects (5 expected) who will receive lyophilized BPZE1 administered by needleless tuberculin syringe to allow for historical, informal comparisons to previous Phase 1 data.
This study is designed as a randomized, placebo controlled, partially-blind (i.e., the method of immunization [VaxINator vs. needleless syringe] will be known) clinical trial evaluating the safety and immunogenicity of a single dose of either 10^7 CFU or 10^9 CFU of BPZE1 delivered by intranasal VaxINator device or needleless tuberculin syringe.

11.1 Study Hypotheses

This Phase 2a study is not designed to test a formal null hypothesis. Rather, it is intended to obtain preliminary estimates of the safety and immune response induced by the BPZE1 lyophilized vaccine in healthy adults. See Section 3 for Study Objectives and Outcome Measures.

11.2 Sample Size Considerations

The study population for this clinical trial includes healthy male and non-pregnant female subjects 18-49 years of age, inclusive, who meet all eligibility criteria. Study subjects will be recruited from the general population at the Vanderbilt University Medical Center VTEU site
This study is planned to enroll approximately 50 healthy adults randomized into four treatment groups in a 3:3:3:1 ratio, as described in Table 2 (Section 4). The tables below describe the probability of observing safety events and the minimum detectable difference in seroconversion rates for the planned sample size.

Table 3 indicates the probability of observing one or more safety events (such as solicited local or systemic reactogenicity events, or an unsolicited non-serious AE of a particular type) for a treatment arm with the assumed event frequency and for the given sample size. For example, for any of the treatment arms with a sample size of 15 subjects, there is a 79.4% probability of detecting an AE that occurs at a frequency of 10%, and a 14% probability if the AE occurs at a rate of 1%.

Table 3: Probability (%) of Observing at Least One Adverse Event

Event Frequency	N = 5	N=15
≥0.01% Rare	<0.1	<0.1
≥0.1% Uncommon	0.5	1.5
≥1% Common	4.9	14.0
≥10% Very Common	41.0	79.4

One of the secondary outcome measures is the proportion of subjects that achieve seroconversion to individual pertussis antigens (PT, FHA, PRN, and FIM 2/3) by serum IgG or IgA ELISA at Days 15, 29 and 181 for humoral immune response. Table 4 shows the minimum detectable differences with 80% power between the proportion of subjects attaining seroconversion in the BPZE1 by VaxINator group versus Placebo using a two-sided Fisher's Exact test and alpha = 0.05.

Table 4: Minimum Detectable Difference in Proportion of Subjects Attaining Seroconversion comparing Placebo versus the BPZE1 (107 or 109 CFU)

Placebo by VaxINator	BPZE1 (10^7 or 10^9 CFU) by VaxINator	Assumed Proportion of Subjects Attaining Seroconversion (Placebo)	Proportion of Subjects Attaining Seroconversion (BPZE1)	Minimum Detectable Difference (Treatment – Placebo)
N=15	N=15	0.00	0.41	0.41
		0.01	0.43	0.42
		0.05	0.54	0.49
		0.10	0.63	0.53
		0.20	0.76	0.56

N=Sample Size

11.3 Planned Interim Analyses

Interim analyses would only be used to terminate this trial in the event that unanticipated safety events deemed to be of sufficient concern require such action by the sponsor. These interim assessments will not be made based on testing a formal statistical hypothesis; therefore, p-value adjustment will not be made to any analyses.

11.3.1 Interim Safety Review

An ISM and SMC will be convened by DMID to review study progress and participant, clinical, safety, and reactogenicity data, as described in Section 9.6. An interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, solicited and unsolicited AE/SAEs, and *B. pertussis* colonization data. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC may receive data in aggregate and presented by treatment arm, including expected and observed rates of the expected AEs. The SMC will review grouped data in the closed session only. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this trial as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

Additionally, this trial will be monitored to determine if any of the halting rules described in Section 9.5 are met.

A preliminary report prepared with data through Day 46 (or Day 29 if no subjects remain colonized with B. pertussis at the Day 29 visit) will be prepared as described in Section 11.4. This report will include analyses of safety data.

11.3.2 Interim Immunogenicity Review

A preliminary report of safety and immunogenicity and *B. pertussis* colonization data through Day 46 (or Day 29 if no subjects remain colonized with *B. pertussis* at the Day 29 visit) will be prepared. The analyses presented in this report will be released while subjects remain in the trial for long-term safety and immunogenicity follow-up, and will be included in the final analysis of these data.

11.4 Final Analysis Plan

The results obtained from this study will be used to inform the dose recommended for a subsequent Phase 2b study. Clinical, safety, reactogenicity, immunogenicity and *B. pertussis* colonization data through approximately 45 days after study vaccination, or 28 days after study vaccination if no subjects remain colonized with *B. pertussis* at Day 29, will represent the primary clinical database for this trial. Once the last subject completes the visit that occurs approximately 45 days after study vaccination (or approximately 28 days after study vaccination, if no subjects remain colonized with *B. pertussis* at the Day 29 visit), the primary clinical database will be cleaned, monitored, and frozen. Analyses of safety, reactogenicity, immunogenicity, and *B. pertussis* colonization data by treatment arm are planned, including descriptive summaries of 15-day reactogenicity data, 28-day adverse event data (including SAE, NOCMC and AESI), 28 and 45-day nasal clearance data, and humoral and mucosal immunogenicity data through Day 46 (or Day 29 if no subjects remain colonized with *B. pertussis* at the Day 29 visit). As it is anticipated that subjects will remain in long term immunogenicity and safety follow-up at the time of these analyses, the presentation of these data will not include any unblinded listings of individual subject data and aggregate summaries will be in a format that prevents inadvertent unblinding of the sponsor or clinical investigators to any individual subject's treatment assignment or immune response data.

A preliminary report will be prepared by the DCC after the primary clinical database is cleaned, monitored, and frozen and all immunogenicity and colonization data through Day 46 (or Day 29 if no subjects remain colonized with *B. pertussis* at the Day 29 visit) are received. These analyses may be made available to DMID and the vaccine company partner and to the lead principal investigator for planning subsequent trials. These analyses will not be used to make any decisions concerning the conduct of this trial. A formal statistical analysis plan that defines the analyses of data to be included in the preliminary report for early release and the CSR will be developed and finalized prior to the to the Day 46 data freeze (or Day 29 if no subjects remain colonized with *B. pertussis* at the Day 29 visit).

Analysis of Day 181 humoral and mucosal immunogenicity data may be performed and released as the data are available from the research laboratories. Any such analyses would be considered the final analysis for the endpoint, and included in the CSR.

Once the last subject completes the last visit, the final clinical database will be cleaned, monitored and locked. Unblinded analyses of safety, reactogenicity, colonization, and available immunogenicity data will then be performed by the DCC and will be included in the CSR. All safety analyses will be performed using the safety population, all immunogenicity analyses will be performed using the immunogenicity and the per-protocol (PP) populations, whereas all

colonization data will be summarized using per-protocol population. The analysis populations are defined below (Section 11.4.1.)

All analyses will be presented by treatment group (i.e. group by BPZE1 10^9 CFU, BPZE1 10^7 CFU, or Placebo). In addition, results for BPZE1 at dose of 10^9 CFU will be further grouped by vaccination device (group by VaxINator or needleless tuberculin syringe) to allow for comparison of immunogenicity results with previous results from Phase 1 clinical trials of BPZE1 (these previous trials used needleless tuberculin syringe as device for administration).

11.4.1 Analysis Populations

11.4.1.1 Safety Population

The Safety Population will consist of all subjects who have received the study vaccine and for whom any data on safety are available. Subjects will be classified according to the treatment received. The primary safety analysis will be done on this population.

11.4.1.2 Immunogenicity Population

The Immunogenicity Population will include all subjects who have received the study vaccination and contributed both pre-vaccination samples and either at least one post vaccination sample for humoral immunogenicity testing for which valid results were reported, or at least one post vaccination nasal sample for which valid results were reported. Subjects will be classified according to the treatment received.

11.4.1.3 Per Protocol Population

The Per Protocol (PP) Population will include all subjects in the immunogenicity population, with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline.
- Data from all visits subsequent to major protocol deviations, such as:
    - Receipt of non-study licensed live vaccine within 30 days before or after the study vaccination,
    - Receipt of non-study licensed inactivated vaccine within 14 days before or after the study vaccination,
    - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days before or after the study vaccination.

- Data from any visit that occurs substantially out of window. Whether a visit that occurs substantially out of window will be determined jointly by the principal investigator, the Sponsor, and the DCC.

For analyses using the PP population, subjects will be grouped based on study vaccinations received.

11.4.2 Safety Data

Summaries and analysis of safety data will be presented for the Safety Analysis Population. All summaries and analyses will be presented by treatment group.

Solicited AEs will be summarized by severity for each day after the study vaccination (Days 1-14 post study vaccination) and as the maximum severity over all 15 days. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the proportion of subjects reporting each symptom, any local symptom, and any systemic symptom. Summaries of solicited AEs will be presented separately for each study vaccination as well as overall study vaccinations by treatment arm. The proportion of subjects reporting symptoms may be compared between treatment arms using Chi-square or Fisher's exact test.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). SAEs will be reported by detailed listings showing the event description, MedDRA® preferred term and SOC, relevant dates (study vaccinations and AEs), severity, relatedness, and outcome for each event. Non-serious unsolicited AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA® preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the proportion of subjects and exact 95% confidence intervals of AEs in aggregate and by MedDRA® categories will be computed.

Subjects will be tested for *B. pertussis* colonization by culture at Day 29 and, if positive, again at Day 46. The proportion of subjects with positive *B. pertussis* culture will be reported for the PP population at each time point with corresponding 95% confidence intervals.

11.4.3 Immunogenicity Data

Summaries and analysis of immunogenicity data will be presented for the immunogenicity and PP populations by treatment group.

Immune responses in terms of toxin-specific serum IgG and IgA antibody titers (PT, FHA, PRN, FIM 2/3) will be summarized at each time point. Analyses will include the Geometric Mean Titers (GMTs) along with corresponding 95% confidence intervals and Geometric Mean Fold Rise (GMFR), as well as the proportion of subjects achieving seroconversion (defined as 2-fold increase over baseline of GMT) for each antigen (PT, FHA, PRN, and FIM 2/3) and the proportion of subjects that achieved seroconversion for ≥ 1 and ≥ 2 antigens (PT, FHA, PRN, and FIM 2/3).

Immune responses in terms of the ratio of toxin-specific (PT, FHA, PRN, FIM 2/3) nasal mucosal IgA antibody to total nasal mucosal IgA antibody will be summarized at each time point. Analyses will include the geometric mean titer ratios (GMTR) of antigen-specific IgA to total IgA and corresponding 95% confidence intervals, along with fold change of the GMTR from baseline. The proportion of subjects achieving seroconversion, defined as ≥2-fold rise of the GMTR from baseline, to each antigen (PT, FHA, PRN, and FIM 2/3) will be reported as well as the proportion of subjects that achieve seroconversion by nasal aspirate ELISA to one or more antigens (PT, FHA, PRN, and FIM 2/3) at either or both of Day 29 and Day 181.

11.4.4 Missing Values and Outliers

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

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The participating VTEU site will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. The participating VTEU site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical study records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the study. Source data are all information, original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required unless needed.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating VTEU site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigators will provide direct access to all study-related sites, source data/data collection forms, and reports for monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigators will ensure all study personnel are appropriately trained and applicable documentations are maintained on site. The DCC 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 VTEU site for clarification and resolution.

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site's principal investigator will obtain IRB approval for this protocol to be conducted at the research site and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, 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 must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

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

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

Informed consent, or IRB approved waiver of consent or altered consent process, is required for all subjects participating in a DMID-sponsored clinical trial, in compliance with applicable regulations and guidelines, including but not limited to 45 CFR 46, 21 CFR 50, 56, 312/812 for FDA-regulated studies, and ICH E6 Guidelines. Prior to the beginning of the trial, the investigator should have the IRB's written approval, favorable opinion of the written informed consent form(s), and any other written information to be provided to the subjects. The informed consent documents should meet the requirements of 21 CFR 50.20 and contain the information required of 21 CFR 50.25(a) and 21 CFR 50.25 (b). The relevant IRB will have the final authority for ensuring the adequacy of the information in the informed consent document.

Prior to enrollment of subjects into this study, the approved protocol and informed consent form will be reviewed and approved by the appropriate IRB listed on its FWA. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this study and a copy will be provided to DMID. The IRB FWA number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site principal investigator for submission to the IRB. A single IRB will be used for this study.

14.3 Informed Consent Process

The site principal investigators will choose subjects in accordance with the eligibility criteria detailed in Section 5.1. Before any study procedures are performed, subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB. 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.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a study and continuing throughout the individual's study participation. Before any study procedures are performed, including pre-screening of subjects for eligibility, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products. This will include the nature, risks and possible benefits of this study, alternate therapies, any known AEs, the investigational status of the study interventions/products, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum and urine samples. Subjects will be allowed sufficient time to consider participation in this research study, after having the nature, risks and possible benefits of this study explained to them, and can discuss this study with their family, friends or legally authorized representative or think about it prior to agreeing to participate.

Informed consent forms describing in detail the study interventions/products, study procedures, risks and possible benefits will be given to subjects. The informed consent form must not include any exculpatory statements. Informed consent forms will be IRB-approved and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain this research study to subjects and answer any questions that may arise. Subjects must sign the informed consent form, and written documentation of the informed consent process is required prior to starting any study procedures specifically for this study, including determining eligibility and administering study product.

By signing the informed consent form, subjects agree to complete all study procedures required by this study, unless the subject withdraws voluntarily, or is withdrawn or terminated from this study for any reason. The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this study.

DMID will provide the site principal investigators, in writing, any new information that significantly impacts the subjects' risk of receiving the investigational products. This new information will be communicated by the site principal investigators to subjects who consent to participate in this study in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

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

This study will be inclusive of all adults who meet the Subject Inclusion Criteria (see Section 5.1) and do not meet the Subject Exclusion Criteria (see Section 5.2), regardless of religion, sex, or ethnic background.

Women of childbearing potential who are not sterilized via tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year has passed since the last menses if menopausal must agree to practice highly effective contraception that may include, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the study vaccination, barrier methods such as condoms or diaphragms/cervical cap **with** spermicide, effective intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables or oral contraceptives (the “pill”), with use of a highly effective method of contraception for a minimum of 30 days prior to study product exposure and agree to practice highly effective contraception for the duration of study product exposure, including 60 days after vaccination. A highly effective method of contraception is defined as one that results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. In addition to contraceptive use, all women of childbearing potential will be required to have a negative urine or serum pregnancy test within 24 hours prior to study vaccination. If a female subject becomes pregnant while participating in this study, we will ask her permission to follow-up with her about her health and the health of her baby through pregnancy outcome.

Children will not be included in this study.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the site principal investigators, other study personnel, the sponsor, and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects. Subjects will have code numbers and will not be identified by name.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning this study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the sponsor and all data and information generated by the participating VTEU site as part of this study (other than a subject's medical records) will be kept confidential by the site principal investigators and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting this study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of this study; (3) information which is necessary to disclose to provide appropriate medical care to a study subject; or (4) study results which may be published as described in Section 16. The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site principal investigators. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The participating VTEU site will permit access to such records.

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

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

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

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

14.6 Study Discontinuation

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. If any subject's private information will continue to be collected for this study, the IRB must approve a consent form with the study procedures, any risks and discomforts, and applicable elements, and the investigator or designee will re-consent the subjects as approved by the IRB.

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

14.8 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining serum from venous blood samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria.

Additionally, subjects will be asked for permission to collect approximately 24 mL of additional venous blood for PBMC isolation and possible future research during Visits 1, 2, 4, 5 or, if applicable, during an early termination visit.

Samples will be stored indefinitely at a central clinical storage facility and may be shared with investigators at the participating VTEU site and with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on the samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality.

There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will not be kept in their health records.

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

15 DATA HANDLING AND RECORD KEEPING

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

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

Data reported in the eCRF derived from the data collection forms should be consistent with the data collection forms or the discrepancies should be explained.

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

15.1 Data Management Responsibilities

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

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

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

15.2 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, and physical assessments), reactogenicity, serologic immunogenicity, and mucosal immunogenicity data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the DCC. 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 and reactogenicity data will be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Data for this study will include clinical, safety, and outcome measures (e.g., reactogenicity, serologic immunogenicity/mucosal immunogenicity [antibody assays], and shedding of BPZE1 from nasal samples).

15.4 Timing/Reports

A preliminary analysis will be performed when all safety, reactogenicity, immunogenicity and *B. pertussis* colonization data through Day 46 (or Day 29 if no subjects remain colonized with *B. pertussis* at Day 29) are available. Analyses will be made available to DMID, the lead principal investigator, and vaccine company partner for planning subsequent clinical trials. These analyses will not be used to make any decisions concerning the conduct of this trial. As it is anticipated that subjects will remain in long term follow-up at the time of these analyses, blinded investigators who will not be involved in the analysis will be responsible for assessing SAEs and AESIs until all subjects have completed the final follow-up visit.

Other interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety and immunogenicity summary reports may be generated for the SMC, as well. See Section 9.3 for further reporting details.

The final clinical study report (CSR) will be completed when all clinical, safety, and reactogenicity data through approximately 180 days after the study vaccination are available. Once the last subject completes the last visit, the final clinical database will be cleaned, monitored and locked. Unblinded analyses of safety, reactogenicity, colonization, and available immunogenicity data will then be performed by the DCC and will be included in the CSR.

After full analysis and final reporting is complete, and upon request and DMID approval, the DCC will provide the participating VTEU site with a summary of results by treatment arm and/or subject treatment assignments. In this regard, the participating VTEU site requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5 Study Records Retention

Study records and reports, including, but not limited to, eCRFs, source documents, informed consent forms, and study drug disposition records, shall be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the drug, until 2 years after the investigation is discontinued and FDA has been so notified. The participating VTEU site must contact DMID for authorization prior to the destruction of any study records. Informed consent forms for future use will be maintained as long as the sample exists.

15.6 Protocol Deviations

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

These practices are consistent with ICH E6:

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

It is the responsibility of the site principal investigators and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the DCC's AdvantageEDC^{SM}.

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

16 PUBLICATION POLICY

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 Statistical Analysis Plan 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

17 LITERATURE REFERENCES

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APPENDIX A: SCHEDULE OF EVENTS

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04***	**V05**	**Early Termination (if needed)**	**Unscheduled (if needed)**
Study Day post study vaccination	**Screening (D-30 to D-1)**	**Enrollment and Dose 1 D1**	**D8±2d**	**D15±3d**	**D29±3d**	**D181±21d**		
Obtain Informed Consent∞	X	X†¬						
Collect Demographic Information	X							
Review Eligibility Criteria	X	X†¬						
Medical History@	X	X†¬	X	X	X	X	X	X
Concomitant Medications	X√	X√†¬	X	X	X	X√√	X (if prior to 28 days after study vaccination) X√√ (if prior to 181 days after study vaccination)	X (if prior to 28 days after study vaccination) X√√ (if prior to 181 days after study vaccination)
Vital Signs$ (Oral Temperature%, Pulse, and BP)	X	X†					X (may be obtained if indicated)	X (may be obtained if indicated)
Physical Examination!	X	X†1	X1	X1	(X)	(X)	(X)	(X)
Urine or Serum Pregnancy Test	X^	X^						
Venous Blood Collection for Screening Laboratory Assays2	X							
Venous Blood Collection for Safety Laboratory Assays			X					
Venous Blood Collection for Serum Antibody Assays-	X	X†		X	X	X	XΩ	
Venous Blood Collection for PBMC isolation and Future Research		X†	X		X	X	XΩ	
Nasal Sample Collection for Mucosal Antibody Assays	X				X	X		
Nasal Sample Collection for *B. pertussis* Culture					X*			
Enrollment in AdvantageEDC℠ and Randomization		X†						
Pre-Administration Reactogenicity Assessments		X†						

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04***	**V05**	**Early Termination (if needed)**	**Unscheduled (if needed)**
Study Day post study vaccination	**Screening (D-30 to D-1)**	**Enrollment and Dose 1 D1**	**D8±2d**	**D15±3d**	**D29±3d**	**D181±21d**		
Study Vaccination		X						
2-hour Evaluation After Study Vaccination		X						
Post-Administration Reactogenicity Assessments		X					X (if within 15 days after study vaccination)	X (if within 15 days after study vaccination)
Distribute Memory Aid and Study-Related Materials		X						
Review Memory Aid for Reactogenicity			X	X			X (if within 15 days after study vaccination)	X (if within 15 days after study vaccination)
AE/SAE Assessment		X[&]	X[&]	X[&]	X	X[**]	X[**](AEs if prior to 28 days after study vaccination)	X** (AEs if prior to 28 days after study vaccination)

∞ Prior to study procedures.
† Prior to study vaccination.
¬ Review/confirm information or activity in subjects previously consented and screened.
@ Complete medical history will be obtained by interview of the subjects at the screening visit and will be updated on Day 1 prior to study vaccination and interim medical history will be obtained by interview of the subjects at follow-up visits after study vaccination.
√ All concomitant medications taken and vaccines received within 30 days prior to signing the informed consent and/or prior to study vaccination. Medications reported in the eCRF for this visit are limited to those taken within 30 days prior to signing the informed consent and/or prior to study vaccination.
√√ Receipt of non-study vaccines will be solicited through approximately 181 days after study vaccination and reported in the eCRF.
$ Vital signs assessed on Day 1 prior to study vaccination will be considered as baseline.
% Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
! A physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
1 A limited physical examination will be performed, focused on the nasopharynx, oropharynx, and lungs; in addition, a targeted physical examination will be performed, if indicated, based on review of interim medical history, by a study clinician.
() Targeted physical examination if indicated based on review of complete and any updates obtained by interview of subjects since the screening visit, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
^ Will be performed on all women of childbearing potential at screening and within 24 hours prior to study vaccination and results must be negative and known prior randomization and study vaccination.
- PT and pertactin serum IgG
& Inclusive of reactogenicity assessments performed on the day of study vaccination through 14 days after study vaccination.
** Assessment of AE/SAE limited to new-onset chronic medical conditions, AESI, and SAEs if after 28 days following the study vaccination.

[2] To be performed locally by the site, and will include white blood cell count, hemoglobin, platelet count, alanine aminotransferase, serum creatinine, and HIV screening

Ω Venous blood will only be obtained if the early termination visit occurs: 1) prior to Visit 2, 2) after day 14 post-vaccination but before Visit 3, or 3) at least 28 days following Visit 3.

* See Schedule of Events secondary table for Visits 4a and 4b.

Study Visit Number	**V4a***	**V4b** (Phone call)**
Study Day post study vaccination	**D46±3 d**	**Day 50 ± 3 days**
Nasal Sample Collection for *B. pertussis* Culture and immunological assays	X	
Prescription of Azithromycin 500 mg by mouth once daily		X
Interim Medical History	X	
SAE Assessment	X	
Targeted Physical Examination, if indicated	X	
Concomitant Non-study Vaccines	X	

*Study visit only if nasal sample is positive for *B. pertussis* at Day 29

**Study visit only if nasal sample is positive for *B. pertussis* at Day 46

Venipuncture Volumes (mL)

Study Visit Number	**V00**	**V01**	**V02**	**V03**	**V04**	**V05**	**Early Termination (if needed)**	**Unscheduled (if needed)**
Study Day post study vaccination	**Screening (D-30 to D-1)**	**Enrollment and Dose 1 D1**	**D8±2d**	**D15±3d**	**D29±3d**	**D181±21d**		
Venous Blood Collection for Screening Laboratory Assays[2]	10	0	0	0	0	0	0	0
Venous Blood Collection for Safety Laboratory Assays	0	0	10	0	0	0	0	0
Venous Blood Collection for Serum Antibody Assays-	5	10	0	10	10	10	10	0
Venous Blood Collection for PBMC isolation and Future Research	0	24	24	0	24	24	24	0
Maximum Total*	**15**	**34**	**34**	**10**	**34**	**34**	**34**	**0**

*Maximum total for up to 6 study visits (161 mL).

APPENDIX B: CLINICAL SAFETY LABORATORY ADVERSE EVENT GRADING

Hematology	Protocol Range	Reference Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC 10^3/uL (Decrease)	≥3.9	3.9 – 10.7	2.5 – 3.8	1.5 – 2.4	<1.5
WBC 10^3/uL (Increase)	≥3.9	3.9 – 10.7	10.8 – 15.0	15.1 – 20.0	>20.0
Hemoglobin g/dL (Decrease, Male)	≥13.0	13.0 – 18.1	11.0 – 12.9	9.5 – 10.9	<9.5
Hemoglobin g/dL (Decrease, Female)	≥11.8	11.8 – 16.0	10.1 – 11.7	8.5 – 10.0	<8.5
Platelet count 10^3/uL (Decrease)	≥135	135 – 371	125 – 134	100 – 124	<100
Platelet count 10^3/uL (Increase)	≥135	135 – 371	372 – 550	551 – 750	>750

Chemistry	Protocol Range	Reference Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
ALT U/L (Increase, Female)	<45	0-44	45 – 100	101 – 200	>200
ALT U/L (Increase, Male)	<62	0-61	62 – 138	139 – 275	>275
Creatinine mg/dL (Increase, Female)	≤1.11	0.57-1.11	1.12 – 1.60	1.61 – 2.00	>2.00 or requires dialysis
Creatinine mg/dL (Increase, Male)	≤1.25	0.72-1.25	1.26 – 1.70	1.71 – 2.00	>2.00 or requires dialysis