ILiAD Biotechnologies

IB-200P

A Phase 2b, Multi-Center, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy Adults to Assess the Immunological Response and Safety Profile of Single Dose (Prime) and Two Dose (Prime + Boost) Schedule, and Compared to a Boostrix ™ Prime Dose With or Without a BPZE1 Boost Dose

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Statistical Analysis Plan

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List of Abbreviations

AE	adverse event
aP	acellular pertussis
ATC	Anatomical Therapeutic Chemical
eCRF	electronic case report form
CFU	colony-forming units
CSR	clinical study report
DNT	dermonecrotic toxin
ELISA	enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FHA	filamentous hemagglutinin
FIM	fimbriae
GMFR	Geometric Mean Fold Rise
GMT	Geometric Mean Titer
ICF	informed consent form
ICH	International Conference on Harmonisation
IgA	immunoglobulin A
IgG	immunoglobulin G
IM	intramuscular
ITT	intent-to-treat
MedDRA	Medical Dictionary for Regulatory Activities
PBMC	peripheral blood mononuclear cell
PP	per protocol
PRN	pertactin
PT	pertussis toxin
PT	preferred term
SAE	serious adverse event
SAP	statistical analysis plan
S-IgA	secretory immunoglobulin A
SMC	Safety Monitoring Committee
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse events
WHO	World Health Organization
WFI	water for injection

1. Introduction

Currently registered acellular pertussis (aP) vaccines protect against respiratory disease but not against colonizing *Bordetella pertussis* infection and transmission. A single intranasal administration of the highly attenuated live *B. pertussis* vaccine BPZE1 has demonstrated in the nonhuman primate model the ability to reduce the bacterial burden of a substantial *B. pertussis* challenge by more than 99.8% compared to prior studies challenging baboons immunized with 3 doses of aP vaccine (Locht et al 2017).

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*. In 2014, it was estimated there were 24.1 million pertussis cases and 160,700 deaths from pertussis in children younger than 5 years of age, with 53% of the estimated deaths being in infants younger than 1 year (Yeung et al 2017). With the current acellular vaccines being ineffective against colonization (and therefore transmission) there is little to any herd immunity that can develop, leaving infants at risk for acquiring pertussis from close contacts. As a potential solution to this problem, the BPZE1 vaccine has been developed to be given as a single intranasal administration. Unlike existing vaccines, BPZE1 has the potential to prevent transmission of *B. pertussis* from siblings and adults to neonatal infants. While ultimately intended for vaccinating neonatal infants, a nearer term solution is to immunize adults and adolescents with BPZE1 to prevent transmission of *B. pertussis* to vulnerable infants.

BPZE1 was engineered by genetically altering or removing 3 *B. pertussis* toxins: pertussis toxin (PT), tracheal cytotoxin (TCT), and dermonecrotic toxin (DNT). Genetic stability of liquid BPZE1 formulation was demonstrated in vitro and in vivo.

A Phase 2a clinical study is being conducted to compare 2 doses (10^7 and 10^9 CFU) of the lyophilized formulation of BPZE1, delivered by the VaxINator™ conical-shape atomization device connected to a 1-mL syringe. This study will assess the new lyophilized formulation of the BPZE1 vaccine to be evaluated in US adults and will provide an opportunity to characterize the immunological response of 2 different dosages delivered by nasal immunization. For this Phase 2b study, the BPZE1 experimental vaccine will be supplied in a lyophilized vial for reconstitution prior to intranasal administration via the VaxINator atomization device. Two lyophilized BPZE1 experimental vaccine doses, 10^7 CFU and 10^9 CFU, will be evaluated in the safety lead-in cohort of the 2b study with subjects continuing with the same intranasal dose level through the booster (10^7 CFU or 10^9 CFU). The dose targeted for the remainder of the subjects in the Phase 2b study will be 10^9 CFU unless a safety concern arises and the Safety Monitoring Committee (SMC) recommends proceeding with the 10^7 CFU dose only.

This novel approach may not only protect BPZE1-vaccinated individuals from *B. pertussis* infection but may also reduce the *B. pertussis* reservoir in the adult population. The ability to prevent colonization by wild type *B. pertussis* that enable transmission may facilitate substantial reduction in the incidence of pertussis in infants.

The intranasally administered BPZE1 vaccine provides an opportunity to generate a locally effective nasal mucosal antibody response at the site of potential exposure, and thereby mimics the route of entry of the wild type pathogen and results in a broader immune response. We hypothesize that the BPZE1 vaccine will be safe and induce nasal mucosal immunity beyond that observed with standard vaccination with Boostrix™ and that BPZE1 will also induce systemic

immunity. In addition, we hypothesize that a priming dose of intranasal BPZE1 vaccine will result in fewer subjects colonized (eg, lower colony counts) following a boosting dose of BPZE1, whereas a priming dose of Boostrix will not reduce colonization following a boosting dose of BPZE1, ie, Boostrix will generate less nasal mucosal immunity to prevent colonization by the BPZE1 live attenuated B. pertussis strain. Lastly, given that the majority of the population has received pertussis vaccination on multiple occasions, this study will assess the safety and immune response of BPZE1 as booster delivered after a prime dose of Boostrix in an adult population. The study population, healthy adults, has been chosen to maximize the quality of immunogenicity data while minimizing risk and potential safety signals.

This study is focused on the safety and immunogenicity after initial (prime) and second (boost) vaccination of intranasal administered BPZE1 (10^9 CFU) in healthy adults. The Statistical Analysis Plan (SAP) provides details of the analyses and data presentations regarding the endpoints identified in the protocol.

2. Objectives

2.1. Primary Objectives

2.1.1. Primary Immunogenicity Objective

- To assess nasal mucosal secretion immune response (secretory IgA [S-IgA]) following intranasal vaccination with BPZE1 (10^9 CFU) when used as a single (eg, prime) or 2-dose (eg, prime + boost) series.

2.1.2. Primary Safety Objective

- To assess reactogenicity (all) and specific safety laboratory parameters (safety lead-in cohort only) following intranasal vaccination with BPZE1 either 10^7 CFU (safety lead-in cohort only) or 10^9 CFU (safety lead-in cohort and full cohort), in healthy adults.

2.2 Secondary Objectives

2.2.1 Secondary Immunogenicity Objectives

- To assess the systemic immune response (IgG, IgA) following intranasal vaccination with BPZE1 when used as a single (eg, prime) or 2-dose (eg, prime + boost) series.
- To assess nasal mucosal secretion (S-IgA) and systemic (IgG, IgA) immune response following intranasal vaccination with BPZE1 boost dose proceeded by Boostrix or BPZE1 prime dose.
- To assess nasal mucosal secretion (S-IgA) and systemic (IgG, IgA) immune response through 9 months after a single (eg, prime) dose and 6 months after a 2-dose series (eg, prime + boost), where the prime dose is BPZE1 or Boostrix, and BPZE1 (or placebo) is the boost dose.
- To assess nasal mucosal secretion (S-IgA) and systemic (IgG, IgA) immune response following immunization with BPZE1 or Boostrix prime dose, with or without a BPZE1 boost dose, in relation to baseline immunity status (positive [Yes/No]) of pertussis antibodies PT, PRN, FHA, and FIM 2/3.

2.2.2 Secondary Colonization Objectives

- To assess nasopharyngeal colonization or clearance of BPZE1 in either a prime or prime + boost strategy, and in relationship to vaccination strategies with Boostrix.

2.2.3 Secondary Safety Objectives

- To describe (severity and clinical significance) of vaccine-related AEs following a single or 2-series intranasal vaccination (prime or prime + boost) with BPZE1 or Boostrix prime dose with or without a BPZE1 boost dose.
- To describe all SAEs during the study.

2.3 Exploratory Objective

- To examine cell-mediated (eg B cell, CD4 + T cell, CD8 + T cell) responses in a subset of no more than 60 subjects using peripheral blood mononuclear cells (PBMCs) to be collected at baseline and 8 days post vaccination (prime and boost). This subset will be from the randomized cohort population of 10^9 CFU BPZE1 only.
- To further characterize nasal mucosal secretion and serum immunologica1 responses across time, relative to baseline status and relative to vaccination response, with the current assays and with any future assays developed for BPZE1.

3. Investigational Plan

3.1. Overall Study Design and Plan

This is a multi-center, randomized, placebo-controlled, and observer-blinded trial with a 6-month safety follow-up after the last vaccination. After signing the informed consent form (ICF), subjects will be enrolled in the trial and screened over a window of 30 days; screening will include obtaining a nasal sample for mucosal pertussis immune status. Subjects will also be asked to provide a signed ICF for the use of samples for further pertussis-specific testing or assay development. On Day 1, eligible subjects will be randomized to 1 of 4 treatment arms and receive BPZE1 intranasal vaccine or formulation buffer for injection (placebo) (via the VaxINator atomization device) and a licensed aP comparator vaccine (ie, Boostrix) or sterile normal saline (placebo), given by IM injection. To maintain the blind throughout the trial period, placebo vaccination via intranasal and IM routes, using formulation buffer and normal saline, respectively will be included, and unblinded pharmacy staff will manage vaccine logistics, preparation, and (if needed) administration but will not be involved in study-related assessments or have subject contact for data collection after study vaccine administration. Approximately 300 subjects will be randomly assigned 2:1 for the first (prime) vaccination with 200 subjects assigned to BPZE1 vaccination and 100 subjects assigned to Boostrix (aP vaccine) vaccination. During the second (boosting) vaccination, half of each of the treatment groups will be further randomly assigned to receive BPZE1 or placebo resulting in a 2:2:1:1 randomization scheme (Table 3-1).

Table 3-1 Dosing Scheme

Timing	Day 1				Day 85	
Treatment Arm	Nasal Vaccination		IM Vaccination		Nasal Vaccination	
	BPZE1	Placebo[a]	Boostrix	Placebo[b]	BPZE1	Placebo[a]
A (N = 100)	X	-	-	X	X	-
B (N = 100)	X	-	-	X	-	X
C (N = 50)	-	X	X	-	X	-
D (N = 50)	-	X	X	-	-	X

Abbreviation: IM, intramuscular.

[a] Intranasal application of 2×0.4 mL (0.4 mL per nostril) placebo (formulation buffer).

[b] IM injection of 0.5 mL placebo (normal saline) to the deltoid region.

As part of the screening procedures, all subjects will provide a nasal sample for determination of mucosal (S-IgA) pertussis antibody status, with this sample collected at least 6 days prior to randomization. Day 1 vaccination (primary) will be delivered by intranasal application (BPZE1 or placebo) and by IM injection into the deltoid (Boostrix [aP vaccine] or placebo), with subjects randomly assigned to receive 1 of the 4 final treatment regimens. Nasal secretion sampling for mucosal antibody response (S-IgA ELISA) to pertussis-specific antigens will occur at baseline (screening) and on Days 29 and 78 post-primary vaccination. Serum sampling for antibody response (IgA and IgA ELISA) will occur at baseline (Day 1 and prior to primary vaccination) and on Days 29 and 85 (prior to boosting vaccination). Subjects will be tested for BPZE1 clearance (colonization) from the nasal tract via a standard nasal secretion sampling on Day 78 and used for pertussis culture and colony count. There needs to be at least 6 days between the nasal secretion sampling, which will occur at Visit 4 (Day 78), and the boosting intranasal vaccination. The boosting vaccination visit (Day 85 [+7 days]) will consist of all subjects receiving intranasal administration (BPZE1 or placebo) with half of the subjects who received BPZE1 for the primary vaccination receiving a second dose of BPZE1 and the other half of subjects receiving placebo (Table 3-1). Similarly, half of the subjects receiving Boostrix will receive BPZE1 and half will receive placebo. Colonization following the boosting vaccination will be measured on Days 92, 96, and 113 by standard nasal secretion sampling. Any subject who tested positive by pertussis culture on Day 113 will be retested on Day 254 or at the time of the end-of-study visit. Chronic carriage of BPZE1 has not been reported (ie, the 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 254 will be provided a short course of azithromycin (or an appropriate antibiotic if the subject is allergic to azithromycin), which is clinically used to eradicate *B. pertussis* from the nasopharynx. Should a subject be allergic to azithromycin, an appropriate antibiotic will be substituted that has effectiveness against B. pertussis. Mucosal (S-IgA ELISA) and serum response (IgG and IgA ELISA) to pertussis-specific antigens after boosting will be tested on Days 113, 169, and 254.

The first 48 subjects randomly assigned will be designated the safety lead-in cohort and will be sequentially enrolled by escalating dose. The first 24 subjects will be randomly assigned as noted above in a ratio of 2:1 to receive active product of either an intranasal dose of 10^7 CFU of BPZE1 or IM Boostrix. These subjects will be followed through Day 8 with safety laboratory tests, reactogenicity, and AE assessments with daily review by the medical monitory for activation of any halting rule. Following accumulation of all safety data through Day 8 post-vaccination, the 24 additional subjects will be randomly assigned 2:1 to receive active products of either an intranasal dose of 10^9 CFU of BPZE1 or IM Boostrix. To maintain the blind for intranasal and IM administered products, all subjects will receive appropriate placebo vaccinations. All safety lead-in subjects will have safety laboratory testing at baseline (screening), and on Days 8, 85 (prior to vaccination), and 92. The medical monitor has the authority to request a review by the SMC should a halting rule be initiated or there are any other safety concerns. The SMC will convene after the subjects in the entire safety lead-in cohort (both dose levels) have completed Day 8 (Visit 2) and will review all safety data through Day 8 (reactogenicity and safety laboratory results) and any AEs which have occurred since study initiation. The SMC will be authorized to allow the remainder of the subjects to be randomly assigned (see Table 3-2). The randomization scheme for the safety lead-in will follow the same scheme as the full cohort for the second vaccination (keeping at either 10^7 CFU or 10^9 CFU based on initial dosing assignment) such that half the BPZE1 and half the Boostrix vaccinated subjects will receive intranasal BPZE1 vaccination on Day 85 and the other half will receive placebo.

Table 3-2 Dosing and Safety Lead-In Cohort

BPZE1 Dose	BPZE1	Boostrix	Trigger to advance	Review Process	Advancing to
10^7	N = 16	N = 8	Day 8 safety on 10^7 dosing cohort	Pause rules; daily medical monitor reviews	10^9 safety lead-in
10^9	N = 16	N = 8	Day 8 safety on full safety lead-in cohort; all adverse events to date	Safety Monitoring Committee review	Full cohort

All subjects will be monitored for 60 minutes after vaccine administration on Days 1 and 85. After the subjects have been monitored for 60 minutes post vaccination, vital signs will be collected and a post vaccination injection site examination for reactogenicity (local, nasal/respiratory, and systemic) and toxicity grade will be completed.

Subjects will receive a daily subject diary on Days 1 and 85 after each vaccination. All subjects will record reactogenicity in the daily subject diary starting the same day of the primary (Day 1) and boosting (Day 85) vaccinations and for 7 additional days (not counting vaccination day). For primary vaccination site-specific local (arm), nasal/respiratory, general systemic reactogenicity reactions, and other unsolicited symptoms/complaints (including start and stop dates) will be recorded and standard toxicity grading will be applied by the investigator at the next visit (Visit 2). For the boost vaccination, nasal/respiratory, general systemic reactogenicity reactions, and other unsolicited symptoms/complaints (including start and stop dates) will be recorded and

standard toxicity grading will be applied by the investigator at the next visit (Visit 6). The clinical staff will review the information from the subject diaries with the subjects on Days 8 and 92 (Visits 2 and 6). Should any reactogenicity event extend beyond 7 days post vaccination and be clinically significant by toxicity grade 1 or greater, then it will be entered as an AE with the same start date as the reactogenicity event and followed to resolution.

All AEs will be monitored through 28 days after the primary and boosting vaccinations. Unsolicited AEs related to vaccination will be monitored through Day 113 (unless resolved or felt to be stable at an earlier date). Serious AEs will be monitored to the end of the study. The primary database lock will occur on Day 113, and all data collected through Day 113 will be included in the clinical study report (CSR) and submitted to regulatory authorities. A subsequent longer-term safety follow-up, including longer term persistence of immune responses, will occur through Day 254. These data will be provided, following a second database lock, in an addendum to the CSR. Subjects will return to the clinical site on Day 254 (±15 days) for end-of-study procedures. The clinical site study team directly participating in subject contact/care will remain blinded to the treatment assignment during this extended safety follow-up period. All unblinded data analyses prior to final database lock on Day 113 will be handled by the unblinded team of statisticians and programmers. A strict firewall between the blinded and unblinded teams will be maintained during study conduct.

A subset of no more than 60 subjects (randomly assigned to the 10^9 CFU dose) will opt in to provide blood samples for PBMCs harvested on Days 1, 8, and 92.

3.2. Study Endpoints

3.2.1. Primary Endpoints

3.2.1.1 Primary Immunogenicity Endpoints

- **Mucosal seroconversion (nasal mucosal secretion sampling):** Proportion of subjects who achieve seroconversion against at least 1 pertussis antigen (PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract) in nasal secretions on Day 29 or 113 (prime or prime + boost).

  Mucosal seroconversion is defined as a 2-fold increase over the baseline value (collected during screening) or a 4-fold increase over the minimal limit of assay detection (whenever the baseline value falls below the limits of assay detection) for any of the pertussis-specific antibodies (S-IgA ELISA): PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract. Seroconversion will be calculated based on absolute titer response over baseline **and** by standardizing pertussis specific ELISA responses relative to non-pertussis specific total mucosal secretion (Total IgA).

  Mucosal baseline samples will be taken at the screening visit.

3.2.1.2. Primary Safety Endpoints

The primary safety endpoints of this study are:

- Solicited AEs (local, nasal mucosal secretion, and systemic reactogenicity events) for 7 days following each vaccination by severity score, duration, and peak intensity. Local reactogenicity will only be monitored following the IM vaccination.
- Safety laboratory results (serum chemistry, hematology, coagulation) by US Food and Drug Administration (FDA) toxicity score (change from baseline or absolute toxicity score) in the safety lead-in cohort at Day 8 following each vaccination. In the case of no toxicity classification the score of 0 will be assigned.

3.2.2. Secondary Endpoints

3.2.2.1. Secondary Systemic Immunogenicity Endpoints

The secondary systemic immunogenicity (serum sampling [expressed separately for IgG, IgA, and IgG or IgA ELISA when possible]) endpoints of this study are:

- Proportion of subjects who achieve seroconversion against pertussis antigen (PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract) over baseline for:
  - At least 1 antigen **on each** of the Days 29, 85, 113, 169, or 254
  - At least 1 antigen **on any** of the Days 29, 85, 113, 169, or 254
  - At least **any 1** antigen **on all** Days 29, 85, 113, 169, and 254

  Systemic seroconversion is defined as a 2-fold increase over the baseline value or a 4-fold increase over the minimal limit of assay detection (whenever the baseline value falls below the limits of assay detections). Both IgG and IgA ELISA will measure antibodies against the following pertussis-specific antigens of PT, FHA, PRN, and FIM 2/3, and IgG ELISA will measure antibodies against broader pertussis-specific antigens in BPZE1 whole cell extract.

  Serum baseline samples taken at Day 1 prior to vaccination.
- Proportion of subjects who achieve seroconversion (IgG ELISA only) against BPZE1 whole cell extract over baseline:
  - On **either** Day 29 (prime) or 113 (boost)
  - On **both** Days 29 (prime) and 113 (boost).
- Proportion of subjects who achieve seroconversion against the acellular pertussis antigens PT, FHA, and PRN over baseline:
  - On **either** Day 29 (prime) or 113 (boost)
  - On **both** Days 29 (prime) and 113 (boost).
- Proportion of subjects who achieve seroconversion against 2 or more pertussis antigens (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract) over baseline:
  - On **each** of the Days 29, 85, 113, 169, or 254
  - On **any** of the Days 29, 85, 113, 169, or 254

- At least the same **2 antigens** on **all** Days 29, 85, 113, 169, and 254.

- Proportion of subjects who demonstrate boosting for each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract) on Day 113. Boost is defined as at least a 2-fold increase from the pre-boost sample taken on Day 85.
- The Geometric Mean Fold rise (GMFR) against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract):
  - On Days 29, 85, 113, 169, and 254 **over baseline** (Day 1)
  - On Days 113, 169, and 254 **over pre-boost** (Day 85)
  - The maximum **over baseline** on either Day 29 or 85 (post-priming response)
  - The maximum **over pre-boost** (Day 85) on any of the Days 113, 169, or 254 (post-boost response)
  - The maximum **during** the study.
- The Geometric Mean Titer (GMT) against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract):
  - On Days 29, 85, 113, 169, and 254
  - The maximum on Day 29 or 85 (**after priming** dose)
  - The maximum after Days 113, 169, or 254 (**after boosting** dose)
  - The maximum **during** the study.

3.2.2.2. Secondary Mucosal Immunogenicity Endpoints

The secondary mucosal immunogenicity S-IgA ELISA endpoints (nasal mucosal secretion sampling) of this study are:

- Proportion of subjects who achieve seroconversion against any pertussis-specific antigen (PT, PRN, FHA, FIM 2/3, or BPZE1 whole cell extract) over baseline:
  - At least 1 antigen **on each** of the Days 29, 78, 113, 169, or 254
  - At least 1 antigen **on any** of the Days 29, 78, 113, 169, or 254
  - At least **any** 1 antigen **on all** Days 29, 78, 113, 169, and 254.
- Proportion of subjects who achieve seroconversion against BPZE1 whole cell extract over baseline:
  - On **either** Day 29 (prime) or 113 (boost)
  - On **both** Days 29 (prime) and 113 (boost).
- Proportion of subjects who achieve seroconversion against acellular pertussis (aP) antigens PT, FHA, and PRN over baseline:
  - On **either** Days 29 (prime) or 113 (boost)
  - On **both** Days 29 (prime) and 113 (boost).

- Proportion of subjects who achieve seroconversion for any 2 or more pertussis antigens (PT, PRN, FHA, or BPZE1 whole cell extract) over baseline:
  - On **each** of Days 29, 78, 113, 169, or 254
  - On **any** of Days 29, 78, 113, 169 or 254
  - At least the **same 2 antigens** on **all** Days 29, 78, 113, 169, and 254.
- Proportion of subjects who demonstrate boosting against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract) on Day 113. Boost is defined as at least a 2-fold increase from pre-boost sample taken at Day 78.
- The GMFR against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract):
  - On Days 29, 78, 113, 169, and 254 **over baseline** (Day 1)
  - On Days 113, 169, and 254 **over pre-boost** (Day 78)
  - The maximum **over baseline** on either Day 29 or 78 (post-priming response)
  - The maximum **over pre-boost** (Day 78) on any of the Days 113, 169, or 254 (post-boost response)
  - The maximum **during** the study.
- The GMT against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract):
  - On Days 29, 78, 113, 169, and 254
  - The maximum on Days 29 or 78 (**after priming** dose)
  - The maximum after Days 113, 169, or 254 (**after boosting** dose)
  - The maximum **during** the study.

3.2.2.3. Secondary Colonization Endpoints

The secondary colonization endpoints are:

- Proportion of subjects with positive *B. pertussis* by bacterial culture of nasal sample collected on each of day and on any of Days 92, 96, and 113.
- *B. pertussis* colony counts on each day (Days 92, 96, and 113).
- Number of subjects who remain culture positive for *B. pertussis* at Days 78 (following priming) and 254 (following boost).

3.2.2.4. Secondary Safety Endpoints

- Unsolicited AEs (eg, treatment-emergent AEs, SAEs, and suspected unexpected serious adverse reactions [SUSARs]) collected for 28 days following each vaccination by Medical Dictionary for Regulatory Activities (MedDRA) (version 22.0 or above) classification and severity score.

- Unsolicited AEs related to vaccination through Day 113 by MedDRA (version 22.0 or above) classification and severity score.
- Serious AEs through 6 months following the last vaccination (or until resolved or stable) by MedDRA (version 22.0 or above) classification, relatedness, and severity score.
- Vital sign measurements with severity scoring immediately following vaccination.

3.2.3. Exploratory Endpoint

- Cell-mediated (eg, B cell, CD4 T lymphocytes + T cell, CD8 T lymphocytes + T cell) responses (eg, cell staining, cytokine production) following stimulation of peripheral blood mononuclear cells (PBMCs) collected at baseline, and 8 days post vaccination (prime and boost) to pertussis-specific antigens. Results expressed both as absolute values and fold over baseline (per specific assay characteristics).
- Following the outcomes of the primary and secondary analyses, additional exploratory endpoints may be tested for systemic or mucosal immunogenicity (IgG or IgA) responses at any time point collected and not already performed in the primary or secondary analysis sets.
- The GMT expressed for serum IgG ELISA against tetanus and diphtheria on Days 29 and 113.

3.2. Treatment

Subjects in Treatment Arm A (N = 100) will receive an intranasal application of 2 × 0.4 mL (0.4 mL per nostril containing half the dose 5×10^8 bacteria to give a total dose of 10^9 CFU) BPZE1 and an IM injection of 0.5 mL placebo (sterile normal saline) to the deltoid region on Day 1 and an intranasal application of 2×0.4 mL (0.4 mL per nostril containing half the dose 5×10^8 bacteria to give a total dose of 10^9 CFU) BPZE1 on Day 85. Note: the first 24 subjects will be assigned to the 10^7 CFU dose.

Subjects in Treatment Arm B (N = 100) will receive an intranasal application of 2×0.4 mL (0.4 mL per nostril containing half the dose 5×10^8 bacteria to give a total dose of 10^9 CFU) BPZE1 and an IM injection of 0.5 mL placebo (sterile normal saline) to the deltoid region on Day 1 and an intranasal application of 2×0.4 mL (0.4 mL per nostril) placebo (formulation buffer) on Day 85. Note: the first 24 subjects will be assigned to the 10^7 CFU dose.

Subjects in Treatment Arm C (N = 50) will receive an intranasal application of 2×0.4 mL (0.4 mL per nostril) placebo (formulation buffer) and an IM injection of 0.5 mL Boostrix (aP vaccine, manufactured by GlaxoSmithKline, Research Triangle Park, North Carolina) to the deltoid region on Day 1 and an intranasal application of 2×0.4 mL (0.4 mL per nostril containing half the dose 5×10^8 bacteria to give a total dose of 10^9 CFU) BPZE1 on Day 85. Note: the first 24 subjects will be assigned to the 10^7 CFU dose.

Subjects in Treatment Arm D (N = 50) will receive an intranasal application of 2×0.4 mL (0.4 mL per nostril) placebo (formulation buffer) and an IM injection of 0.5 mL Boostrix (aP vaccine), to the deltoid region on Day 1 and an intranasal application of 2×0.4 mL (0.4 mL per nostril) placebo (formulation buffer) on Day 85. Note: the first 24 subjects will be assigned to the 10^7 CFU dose.

The dose of BPZE1 active ingredient will be administered to the subject within 60 minutes of removal from the freezer. One milliliter of WFI will be used to reconstitute the 1 mL vial of lyophilized BPZE1, but only 0.8 mL of vaccine will be withdrawn for administration into both nostrils. Following reconstitution, the VaxINator will be attached to the syringe, and 0.4 mL volume will be administered to each nostril. In the case of placebo nasal vaccination, a similar volume of WFI will be reconstituted into a vial containing only lyophilized buffer, followed by 0.4 mL volume administered into each nostril with the vaccinator. The VaxINator provides a uniform, controlled delivery, which allows the vaccinator to accurately deliver 0.4 mL of vaccine to the initial nostril and then administer the remaining 0.4 mL to the opposite nostril.

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

4. General Statistical Considerations

No formal hypothesis will be tested in this study. Statistical analysis will be performed using SAS software Version 9.4. Continuous variables will be summarized using the mean, 2-sided 95% CI of the mean, SD, median, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages, as well as 2-sided 95% CI for proportions computed using the Clopper-Pearson method. Graphics of reverse cumulative distribution of anti-pertussis antibody titers will be generated through Day 113 for the primary database lock and assessing serum and mucosal pertussis antigen response. .

For summary precision, mean and median will have one more decimal place than the reported value, SD will have two more decimal places than the reported value, minimum and maximum will have the same decimal place as the reported value. Percentages and 95% CI will have one decimal place.

Where applicable, data analyses will be conducted for the prime period (prior to boosting) and boost period (at or post boosting). Pre-prime and pre-boost baseline will be used for summaries based on the Immunogenicity Analysis Set.

The 24 subjects in the safety lead-in cohort of the lower dose group (i.e. subjects who were randomized or received BPZE1 10^7 CFU/Boostrix at prime dose and BPZE1 10^7 CFU/Placebo at boost dose) will be presented separately for key safety output (e.g. reactogenicity and laboratory grading) and immune results by visit for each pertussis antigen (e.g. seroconversion, GMT, GMFR).

All data collected will be presented in data listings.

4.1. Sample Size

A total of 300 subjects will be globally assigned at 2:2:1:1 allocation ratio and receive BPZE1 nasal administration with placebo IM or Boostrix IM with placebo nasal administration on Day 1 and BPZE1 or placebo intranasal administration on Day 85. This sample size is based on clinical considerations but not a statistical power analysis as the study does not test any formal null hypothesis. **Table 4-1** presents the width of 95% CI for selected certain sample sizes under response rate assumptions (defined based on nasal mucosal immunogenicity) between 50% and 90%. Calculations were performed using PASS v12.

Table 4-195% Confidence Interval and Width for Selected Response Rate Assumptions for Relevant Treatment Arm Sample Sizes

Response Rate (%)	N = 50		N = 100		N = 200		N = 250	
	CI (%)	Width (%)	CI (%)	Width (%)	CI (%)	Width (%)	CI (%)	Width (%)
50	35.5, 64.5	28.9	39.8, 60.2	20.3	42.9, 57.1	14.3	43.6, 56.4	12.7
60	45.2, 73.6	28.4	49.7, 69.7	19.9	52.9, 66.8	14.0	53.6, 66.1	12.5
70	55.4, 82.1	26.7	60.0, 78.8	18.7	63.1, 76.3	13.1	63.9, 75.6	11.7
80	66.3, 90.0	23.7	70.8, 87.3	16.5	73.8, 85.3	11.5	74.5, 84.8	10.3
90	78.2, 96.7	18.5	82.4, 95.1	12.7	85.0, 93.8	8.8	85.6, 93.4	7.8

Abbreviation: N, sample size.

Note: As described in the study design section, 200 subjects will receive a primary vaccination and 100 out of those 200 subjects will receive a boosting vaccination. In addition, 50 out of 100 active control subjects will receive a BPZE1 boost.

4.2. Randomization and Blinding

Subjects will be randomly assigned to 1 of 4 treatment arms, in a 2:2:1:1 ratio, as presented in Table 3-1. No stratification factors will be used in the randomization. An interactive response technology will be used to administer the randomization schedule centrally. Biostatistics will generate the randomization schedule using SAS® software Version 9.4 (SAS Institute Inc, Cary, North Carolina) for the interactive response technology, which will link sequential subject randomization numbers to treatment codes. The randomization schedule will be created by the dedicated randomization team, stored in a separate project area, and will be blinded to the project team with the exception of the unblinded pharmacy staff who will manage vaccine logistics, preparation, and administration, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

This is an observer-blinded study. To maintain the blind, placebo vaccination via intranasal and IM routes will be included and unblinded pharmacy staff will manage vaccine logistic, preparation, and administration (when necessary) so as to maintain the blind from the remainder of the study personnel and subjects. The pharmacy staff will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

A subject's treatment assignment will not be broken until the end of the study for the clinical site study team unless medical treatment of the subject depends on knowing the study treatment the subject received. In the event that the blind needs to be broken because of a medical emergency, the investigator may unblind an individual subject's treatment allocation.

As soon as possible, the investigator should first contact the medical monitor to discuss the medical emergency and the reason for revealing the actual treatment received by that subject. The treatment assignment will be unblinded through interactive response technology. Reasons for treatment unblinding must be clearly explained and justified in the electronic case report form (eCRF). The date on which the code was broken together with the identity of the person responsible must also be documented.

4.3. Analysis Set

4.3.1. Intent-to-Treat (ITT) Analysis Set

The Intent to Treat Analysis Set will consist of all subjects who have been randomized to the study. Subjects will be classified according to the study group randomized. The data of subject disposition, demographics, and medical history will be summarized on this analysis set.

4.3.2. Immunogenicity Analysis Set

The Immunogenicity Analysis Set will include all the Intent to Treat subjects who received the prime dose of the study vaccine(s) and have contributed both pre- and at least 1 post prime vaccination sample (mucosal or serum immunogenicity testing, respectively) for which valid results were reported and who have not received any component of a licensed pertussis vaccine in the 5 years through Day 113 of the study. Subjects will be classified according to the study vaccine received. For analysis of boosting, those subjects who have received all vaccinations and have a pre (Day 78 for mucosal and Day 85 for serum) and a post boost immune sample (Day 113) for which valid results were reported will be utilized. Additional testing for maximum response will be assessed for those subjects who received all vaccinations and have both a baseline sample (screening or Day 1 depending on the sample type) and a sample at Day 113. Immune persistence will be assessed in those subjects who contribute to sampling at Days 169 and 254.

Subjects will be classified according to the study vaccine received and according to humoral and mucosal immunity samples as described. Subjects who discontinued from study will be classified according to randomized treatment arm. Subjects who missed a boost dose, will be classified to no boost treatment arm based on prime arm. Subjects who received wrong vaccination by mistake and discontinued from study will be classified according to actual vaccination, priority higher.

4.3.3. Per Protocol (PP) Analysis Set

The Per Protocol Analysis Set will include all subjects in the Immunogenicity Analysis Set, with the following exclusions:

- Data from all available visits for subjects following the receipt of unsuitable investigational product (either via dispensed/assigned or delivered).
- Data from all available visits for subjects found to be ineligible at baseline per Protocol Version 6.0.
- Data from all visits for subjects following the use of major immune-modulators, immune suppressants, receipt of blood products, or use of forbidden nasal solutions (eg, sprays, washes, neti pot) from 30 days prior to Day 1 through Day 113.
- Data from all visits for subjects following clinically significant protocol deviations that can affect the efficacy (immunogenicity) results.

- Data from any visit that occurs substantially out of window as defined by exceeding 30 days past the visit window or the time point whereby the follow-on visit should have occurred, whichever is most restrictive. Standard visit windows are described in schedule of events (Appendix 13.1).

For analyses using the Per Protocol Analysis Set, subjects will be grouped based on study vaccines actually received.

4.3.4. Safety Analysis Set

The Safety Analysis Set will consist of all subjects who have received at least 1 dose of the study vaccine and have any safety data available. Subjects will be classified according to the vaccine received. The primary safety analysis will be done on this analysis set.

4.3.5. Safety Lead-in Analysis Set

The Safety Lead-in Analysis Set will consist of the first 48 subjects who have been randomized to the study. Subjects will be classified according to the vaccine received and group into BPZE1 prime (10^7 CFU), BPZE1 prime (10^9 CFU) and Boostrix prime arms

4.4. Analysis Periods

Safety data will be summarized by analysis period as outlined below.

- Prime Period:

  This period starts on the day of primary vaccination (prime dose) at Day 1/Visit 1 and ends on either: (1) the day prior to second vaccination (boost dose) on Day 85/Visit 5 for those who received a boosting dose; or (2) the day of early termination visit if the subject discontinued before Day 85/Visit 5; or (3) the last known study day if the subject is lost to follow-up prior to Day 85/Visit 5.

- Boost Period:

  This period starts on the day of boosting vaccination (boost dose) at Day 85/Visit 5 and ends on either: 1) the day of Day 113/Visit 8; or 2) the day of the discontinuation visit if the subject discontinued prior to or at Day 113/Visit 8; or 3) the last known study day if the subject lost to follow-up prior to Day 113/Visit 8.

- Long term safety follow-up:

  This period starts at the next day after Day 113/Visit 8 or discontinuation visit and stops at the last follow-up visit or the last assessment date.

4.5. Baseline and Analysis Window/Visit

Unless otherwise specified, (pre-prime) baseline is defined as the last non-missing value before the prime vaccination on Day 1. Pre-boost baseline is defined as the value collected prior to the boost vaccination (Day 85/Visit 5), with the earliest value being used as Visit 4 (Day 78) and the latest value being the day of boost vaccination (but prior to vaccination being given).

The pre-prime baseline will be used for prime data analysis/summaries and the pre-boost baseline will be used for boost data analysis/summaries, both are based on the Immunogenicity Analysis Set.

No analysis window mapping will be applied. Visit based endpoints will be reported based on study visits as collected for scheduled visits. Any unscheduled visit will be reported as unscheduled visit relative to each study vaccination. (e.g. unscheduled visit after prime dose and unscheduled visit after boost dose)

4.6. Handling of Missing Data

Data will be presented in the listings as reported. For summaries and analysis, the following conventions apply.

Immunogenicity Data

No Imputation methods will be used for missing immunogenicity data and all analyses will be based on complete records only.

Any pertussis antigen specific antibody titer (PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract) from either serum or nasal mucosal sample that is below the minimal limit of assay detection will be imputed with a value equal to half of the minimal limit of assay detection of the antigen specific antibody titer.

Adverse Event Data

Missing date will be imputed as described in Appendix 13.4. Missing information regarding 'relationship to study vaccine' or 'severity' will be imputed as related to study vaccine and severe, as the worst-case scenario.

Prior/Concomitant Medication Data

Missing date will be imputed as described in Appendix 13.4.

5. Subject Disposition

5.1. Disposition

The number of subjects who were randomized and not randomized, the eligibility criteria met/failed will be summarized based on all screened subjects. The number and percentage of screen failure subjects will be summarized for each exclusion criteria met and/or inclusion criteria failed.

Disposition of all randomized subjects (ITT Analysis Set) will be summarized by treatment arms including:

- Number of subjects never vaccinated
- Safety Set
- Immunogenicity Analysis Set
- Per Protocol Set
- Number of subjects who completed study
- Number of subjects who prematurely discontinued study
- Primary reason for premature discontinuation of study

Primary reasons for study discontinuation collected on the Study Termination eCRF will be summarized with the following categories:

- Adverse Event
- Death
- Clinically Significant Hematological or Biochemical Changes
- Failure to Meet Continuation Criteria
- Symptoms or an Intercurrent Illness not Consistent with Protocol
- Lost to Follow-Up
- Physician Decision
- Pregnancy
- Study Terminated by Sponsor
- Withdrawal by Subject
- Other

Screen failure data will be listed by subject as collected based on screen failure subjects. Disposition data will be listed by subjects as collected based on ITT Analysis Set

5.2. Protocol Deviations

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Significant protocol deviations will be summarized using the ITT Analysis Set during the study for the first primary database lock on Day 113 and final database lock on end of study.

A by subject listing will be provided based on the ITT Analysis Set including both the significant and non-significant protocol deviations. A separate listing will be provided on protocol deviations based on Immunogenicity Analysis Set but excluded from the PP Immunogenicity Analysis Set.

6. Demographics and Baseline Characteristics

6.1. Demographics

Demographic and baseline characteristics data to be analyzed will include age, sex, race, ethnicity, weight, height, and body mass index on the ITT Analysis set. Age will be calculated as (Date of randomization - Date of birth)/365.25.

The following characteristics will be summarized and presented as continuous variables:

- Age (years)
- Weight (kg)
- Height (cm)
- Body Mass Index (BMI) (kg/m^2)

The following characteristics will be summarized and presented as categorical variables:

- Sex (Male, Female)
- Race (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Multiple, Other)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino)

6.2. Baseline Disease Characteristics and Substance Usage

History of tobacco usage (yes/no), Past pertussis vaccination (yes/no), and any past surgeries/significant procedures for medical conditions (yes/no) will be summarized and presented as categorical variables on the ITT Analysis Set.

A by-subject listing will be provided on the ITT Analysis set.

6.3. Medical History

Medical history will be summarized based on the ITT Analysis Set. Medical history will be coded according to Medical Drug Regulatory Activities (MedDRA) version 22.0 or above and will be summarized by system organ class (SOC) and preferred term (PT), with SOCs sorted alphabetically and PTs within each SOC in descending order of frequency. A by-subject listing on medical history will be provided based on the ITT analysis set.

7. Treatments and Medications

7.1. Prior and Concomitant Medications

Partial missing dates will be imputed as described in Appendix 13.4 for medications analysis. The Anatomical Therapeutic Chemical (ATC) coding scheme of the latest World Health Organization Drug Dictionary (WHODD) will be used to group medications into relevant categories.

7.1.1. Prior Medications

Prior medications are defined as non-study medications with a start date before the date of prime dose. Prior medications that continue on and after the date of prime dose will be reported as both prior and concomitant medications. Prior medications will be summarized based on the ITT Analysis Set by ATC2 level and preferred medication names, with ATC2 level sorted alphabetically and preferred names within each ATC2 level in descending order of frequency.

A by-subject listing will be provided for prior medications based on the ITT Analysis Set.

7.1.2. Concomitant Medications

Concomitant medications are defined as non-study medications (including herbal supplements, multivitamins, and over-the-counter medications) taken by the subject from the time of prime vaccination through 28 days after the boosting vaccination (or through early termination visit if prior to that time).

Concomitant medications will be summarized by ATC2 level and standardized medication names, with ATC2 level sorted alphabetically and standardized names within each ATC2 level in descending order of frequency. Summaries will be provided based on the Safety Analysis Set.

A by-subject listing will be provided for concomitant medications based on the Safety Analysis set.

7.2. Study Treatments

7.2.1. Treatment Compliance

The study vaccination will be administered to maintain the blind to site personnel conducting subject assessments. The location (in case of IM administration, right or left arm), date, and timing of all product administrations will be recorded in the subjects' eCRF. Compliance will be determined by the number and percentage of subjects who receive study vaccination.

Compliance rate will be summarized by counts and percentages for subjects receiving the prime vaccination, boost vaccination, and for those receiving both vaccinations (including a separate group of subjects who received different investigational products (IPs) at the prime and boost vaccination, if any) based on the ITT Analysis Set and Safety Analysis Set. For compliance rate of prime vaccination, subjects will be grouped into BPZE1 prime (A, B, A+B) and Boostrix prime (C, D, C+D) arms. For boost vaccination, subjects will be grouped into BPZE1 boost (A, C, A+C) and Placebo boost (B, D, B+D). For both vaccinations, subjects will be grouped into BPZE1 prime with BPZE1 boost (A), BPZE1 prime with no boost (B), Total Post BPZE1 prime (A+B), Boostrix prime with BPZE1 boost (C) and Boostrix prime with no boost (D), Total Post Boostrix prime (C+D), Total Post Booster BPZE1 (A+C).

Any deviations from the dosing schedule outside the defined visit windows (Appendix 13.1) will be flagged in the clinical database.

A by-subject listing will also be provided based on the Safety Analysis Set.

8. Immunogenicity Analyses

8.1. Primary Immunogenicity Analyses

The primary immunogenicity endpoint of this study is mucosal seroconversion (nasal mucosal secretion sampling) defined as the proportion of subjects who achieve seroconversion against at least 1 pertussis antigen (PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract) in nasal secretions on Day 29 for the prime vaccination or Day 113 for the prime + boost vaccination.

Mucosal seroconversion is defined as a 2-fold increase over the baseline value (collected during screening) or a 4-fold increase over the minimal limit of assay detection (whenever the baseline value falls below the limits of assay detection) for any of the pertussis-specific antibodies (S-IgA ELISA): PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract.

Seroconversion will be calculated based on absolute titer response (based on IgA) over baseline **and** by standardizing pertussis specific ELISA responses relative to non-pertussis specific total mucosal secretion (Total IgA). Overall mucosal seroconversion defined as either seroconversion based on absolute titer response (based on IgA) or seroconversion based on standardizing pertussis specific ELISA responses relative to non-pertussis specific total mucosal secretion (Total IgA).

Any pertussis antigen specific antibody titer (PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract) from nasal mucosal sample that is below the minimal limit of assay detection will be imputed with a value equal to half of the minimal limit of assay detection of the antigen specific antibody titer.

The proportion of subjects who achieved mucosal seroconversion on Day 29/Visit 3 or Day 113/Visit 8 will be summarized based on the Immunogenicity Analysis Set. Baseline is defined as the last non-missing value collected before the prime vaccination.

Data will be summarized using frequency counts and percentages.

A by-subject listing will be provided for mucosal immunogenicity data as collected based on Immunogenicity Analysis Set.

8.2. Secondary Immunogenicity Analyses

8.2.1 Secondary Systemic Immunogenicity Analyses

All secondary systemic immunogenicity endpoints are as described in section 3.2.2.1.

Systemic seroconversion is defined as a 2-fold increase over the baseline value or a 4-fold increase over the minimal limit of assay detection (whenever the baseline value falls below the limits of assay detections).

Seroconversion, GMT and GMFR will be derived using titer results based on IgA, IgG and IgA or IgG for all the acellular pertussis antigens (PT, FHA, PRN, FIM2/3). For BPZE1 whole cell extract, titer results based on IgG will be used to derived seroconversion, GMT and GMFR.

The secondary systemic immunogenicity endpoints are:

8.2.1.1. Proportion of subjects who achieve seroconversion against pertussis antigen (PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract) over (pre-prime) baseline based on Immunogenicity Analysis Set for the following:

- At least 1 antigen **on each** of the Days 29, 85, 113, 169, or 254
- At least 1 antigen **on any** of the Days 29, 85, 113, 169, or 254
- At least **any 1** antigen **on all** Days 29, 85, 113, 169, and 254

8.2.1.2. Proportion of subjects who achieve seroconversion (IgG ELISA only) against BPZE1 whole cell extract over (pre-prime) baseline based on Immunogenicity Analysis Set for the following:

- On **either** Day 29 (prime) or 113 (boost)
- On **both** Days 29 (prime) and 113 (boost).

8.2.1.3. Proportion of subjects who achieve seroconversion against all acellular pertussis antigens PT, FHA, and PRN over (pre-prime) baseline based on Immunogenicity Analysis Set for the following:

- On **either** Day 29 (prime) or 113 (boost)
- On **both** Days 29 (prime) or 113 (boost).

8.2.1.4. Proportion of subjects who achieve seroconversion against 2 or more pertussis antigens (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract) over (pre-prime) baseline based on Immunogenicity Analysis Set for the following:

- On **each** of the Days 29, 85, 113, 169, or 254
- On **any** of the Days 29, 85, 113, 169, or 254
- At least the same **2 antigens** on **all** Days 29, 85, 113, 169, and 254.

8.2.1.5. Proportion of subjects who demonstrate boosting for each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract) on Day 113 based on Immunogenicity Analysis Set. Boost is defined as at least a 2-fold increase from the pre-boost baseline.

8.2.1.6. The Geometric Mean Fold rise (GMFR) against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract):

- On Days 29, 85, 113, 169, and 254 **over (pre-prime) baseline** based on Immunogenicity Analysis Set.
- On Days 113, 169, and 254 **over pre-boost** (Day 85) based on Immunogenicity Analysis Set.
- The maximum **over (pre-prime) baseline** on either Day 29 or 85 (post-priming response) based on Immunogenicity Analysis Set.
- The maximum **over pre-boost** (Day 85) on any of the Days 113, 169, or 254 (post-boost response) based on Immunogenicity Analysis Set.
- The maximum **during** the study based on Immunogenicity Analysis Set using (pre-prime) baseline.

8.2.1.7. The Geometric Mean Titer (GMT) against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract):

- On Days 29, 85, 113, 169, and 254 based on Immunogenicity Analysis Set
- The maximum on Day 29 or 85 (after priming dose) based on Immunogenicity Analysis Set.
- The maximum after Days 113, 169, or 254 (after boosting dose) based on Immunogenicity Analysis Set.
- The maximum **during** the study based on Immunogenicity Analysis Set.

For systemic immunogenicity endpoints, unless otherwise specified, baseline will refer to the pre-prime baseline (defined as the last non-missing serum sample taken at Day 1/ Visit 1 prior to prime vaccination) and will be used for summaries based on Immunogenicity Analysis Set, as applicable. Pre-boost baseline is defined as the value collected on either Day 78/Visit 4 or Day 85/Visit 5 prior to boost vaccination and will be used for summaries based on Immunogenicity Analysis Set, as applicable. Data will be summarized based on the endpoint type as described in Section 3.2. Analyses will include the GMTs along with corresponding 95% CIs and GMFR at each time point and maximum titer or ratio at any time point.

For all systemic immunogenicity endpoints, summaries against each of the following Pertussis antigen specific antibodies will be provided based on results from IgA and IgG (PT, FHA, PRN, FIM2/3) separately and together. Summaries against BPZE1 whole cell extract will be provided based on results from IgG.

Listings will be provided for systemic immunogenicity data as collected based on the Immunogenicity Analysis Set.

For GMT, descriptive statistics will be provided for each acellular pertussis antigen as well as BPZE1 whole cell extract (the number of subjects with non-missing assessments, geometric means with 95% CI, geometric SD, median, minimum, maximum) For each antigen, if IgA and IgG results are reported as lower than the minimal limit of assay detection, a value equal to half of the minimal limit of assay detection will be imputed in the calculation. The minimal limit of assay detection for each of the following antibody titers PT, FHA, PRN, FIM 2, FIM3, and BPZE1 whole cell will be established following assay validation and prior to database lock.

GMT will be calculated as anti-logarithm of ∑ (log-transformed titer/number of subjects with titer information). The 95% CI for GMT will be calculated as the anti-log transformation of upper and lower limits for a 2-sided CI of the mean of the log-transformed titers.

GMFR will be calculated as anti-logarithm of ∑ [log-transformed titer ratio of Yi/Bi)/number of subjects with titer information], where Yi is the post-dose titer and Bi is the baseline titer for subject i. The same data summaries will be provided as GMT. For any antibody titer below the minimal limit of assay detection, the value to be imputed in the calculation will follow the same as GMT.

8.2.2. Secondary Mucosal Immunogenicity Analyses

Secondary mucosal immunogenicity endpoints are as described in Section 3.2.2.2.

Summaries and analyses will be the same as secondary systemic immunogenicity endpoints with the following exceptions:

1. the pre-boost baseline is defined as the value collected on Day 78/Visit 4 prior to boost vaccination. If the visit is missed on Day 78 but the subject arrives for boost vaccination, then a sample obtained prior to the vaccination will be used as the pre-boost value.
2. All mucosal immunogenicity endpoints will be calculated and summarized based on absolute titer response over baseline and by standardizing pertussis specific ELISA responses relative to non-pertussis specific total mucosal secretion of total IgA .

8.2.3. Secondary Colonization Analyses

Secondary colonization endpoints are as described in section 3.2.2.3. Analyses on these endpoints are listed below.

- Proportion of subjects with positive *B. pertussis* by bacterial culture of nasal sample collected on each of day and on any of Days 92, 96, and 113 will be summarized based on Immunogenicity Analysis Set.
- *B. pertussis* colony counts on each day (Days 92, 96, and 113) will be summarized based on Immunogenicity Analysis Set.
- Number of subjects who remain culture positive for *B. pertussis* at Days 78 (following priming) and 254 (following boost) will be summarized based on Immunogenicity Analysis Set.

Data will be summarized for each endpoint as described in Section 4.

Listings will be provided for colonization data as collected.

8.3. Sensitivity and Subgroup Analyses

Sensitivity analysis for primary endpoint will be performed based on the Per Protocol Analysis Set.

Subgroup analysis will be performed for following mucosal and systemic endpoints by positive baseline status (yes/no) of pertussis antibodies against PT, PRN, FHA, FIM 2/3, BPZE1 whole cell extract, and at least one antigen (PT, or FHA, or PRN).

- Proportion of subjects achieving seroconversion on Day 29 or 113.

In addition, subgroup analysis will be performed for following mucosal and systemic endpoints by positive baseline status (yes/no) of pertussis antibodies against PT, PRN, FHA, FIM 2/3, and BPZE1 whole cell extract.

- The Geometric Mean Fold rise (GMFR) against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract):
- The Geometric Mean Titer (GMT) against each pertussis antigen (PT, FHA, PRN, FIM 2/3, and BPZE1 whole cell extract)

8.4. Exploratory Analysis

- The GMT expressed for serum IgG ELISA against tetanus and diphtheria on Days 29 and 113.

The GMT will be summarized using descriptive statistics (mean, median, standard deviation, min, max) along with 95% CI, by each pertussis antigen on Days 29 and 113 based on Immunogenicity Analysis Set.

- T-cell responsiveness utilizing PBMC stimulated cells. Responsiveness (day 8 and 92) will be analyzed relative to baseline as well as absolute value.

The exploratory analyses will be completed in the future with subject treatment assignment maintaining the blind for the laboratory personnel conducting the testing.

9. Safety Analysis

Unless otherwise specified, safety analysis will be performed based on the Safety Analysis Set. All summaries and analyses will be presented over all subjects and by treatment arms for the prime (Day 1 vaccination) and boost (Day 85 vaccination) periods. Summaries of the prime period will be presented based on treatment arms of BPZE1 prime (A, B, A+B), Boostrix prime (C, D, C+D) and Total (A+B+C+D). Summaries of the boost period will be presented based on treatment arms of BPZE1 prime with BPZE1 boost (A), BPZE1 prime with no boost (B), Boostrix prime with BPZE1 boost (C) and Boostrix prime with no boost (D), Total Post Booster BPZE1 (A+C), and Total Post Placebo Boost (B+D), Total Any BPZE1 Exposure (A+B+C) and Total (A+B+C+D).

9.1. Solicited Adverse Events

One of the primary safety endpoints of the study are solicited adverse events (local, nasal/respiratory, and systemic reactogenicity events) for 7 days following vaccination by severity score, duration, and peak intensity. Local reactogenicity are only collected after Day 1 vaccination, while nasal/respiratory and systemic reactogenicity are collected after both Day 1 and Day 85 vaccination. Solicited adverse events (AEs) are collected at 60 minutes (± 15 minutes) after each vaccination at the site (in-clinic assessment). In addition, subjects will record reactogenicity in the daily subject diary starting post-vaccination for 7 additional days (after each vaccination). For each vaccination, specific local (arm), nasal/respiratory, and general systemic reactogenicity reactions will be graded by the investigator using standard toxicology grading at the subsequent visit following vaccination (see Table 9-1).

Solicited reactogenicity adverse events (AEs) will be summarized for local reactogenicity, nasal/respiratory reactogenicity, and systemic reactogenicity adverse events separately based on the Safety Analysis Set and relative to the most recent vaccination given. For prime vaccination the results of greatest interest are A+B (Total Post BPZE1 prime) and C+D (Total Post Boostrix prime). For the boost vaccination the results of greatest interest are A+C (Total Post Booster BPZE1) and B+D (Total Post Placebo boost). In the case of the Safety Lead-in Analysis set subjects assigned to 10^7 and 10^9 BPZE1 will be tabulated separately.

Frequency counts will be provided by severity grades for each symptom (Table 9-1) for the following:

- On the day of vaccination (Day 1 and Day 85) within 60 mins (± 15 minutes) after each vaccination at the site (in-clinic assessment)
- Highest score in each past 24 hours (with the first score recorded on Day 1 post vaccination) through Day 7.
- Maximum severity grade over 7 days.

Additionally, maximum severity will be dichotomized into a binary variable (mild, moderate or severe vs none) and to be summarized as proportion with 2-sided 95% CI using Clopper Pearson methods. Grade 0 (none) will be the classification if the observation is less than a Grade 1.

Fever – oral, Erythema/redness, and Induration/swelling for 7 days following each vaccination will be summarized using descriptive statistics (mean, standard deviation, median, min, max).

Duration through 7 days and Duration through resolution will be summarized using descriptive statistics (mean, standard deviation, median, min, max). Duration through 7 days is defined as days with the reactogenicity event onset through 7 days following vaccination per patient reported daily diary. Duration beyond 7 days is defined as days with the reactogenicity event continuing beyond 7 days as captured in adverse events. Duration Through Resolution is defined as days with the reactogenicity event onset until resolution, which includes duration through 7 days and duration beyond 7 days. Duration beyond 7 days will be listed in adverse event listing.

Listings will be provided for each type of reactogenicity based on the Safety Analysis Set.

Table 9-1

Local Reactogenicity Grading

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of nonnarcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room visit or hospitalization
Erythema/redness[a]	2.5 to 5 cm	5.1 to 10 cm	>10 cm	Necrosis or exfoliative dermatitis
Induration/swelling[b]	2.5 to 5 cm and does not interfere with activity	5.1 to 10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis

Nasal/Respiratory 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)
Fever[c] - oral[d]	100.4°F – 101.1°F	101.2°F – 102.0°F	>102.0°F
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[e]	Pruritus OR local rash	Diffuse rash	Diffuse rash with blisters or mouth ulcerations, anaphylaxis, or angioedema

Note: Grade 0 will be the classification if the observation is less than a Grade 1.

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[b] Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

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

[d] Subjects must not eat or drink anything hot or cold within 10 minutes prior to taking oral temperature. Oral temperature assessed on Day 1 prior to the first study vaccination will be considered as baseline.

[e] Adapted from the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 2. 2014.

Source: DHHS 2007

9.2. Unsolicited Adverse Events

Unsolicited Adverse events will be coded according to Medical Dictionary for Regulatory Activities (MedDRA) version 22.0 or above. Unless otherwise specified, unsolicited AEs will be summarized by System Organ Class (SOC) and Preferred Term (PT), with SOCs sorted in the alphabetical order and PTs within each SOC in descending order of subject incidence. Partial missing unsolicited adverse event start dates will be imputed based on Appendix 13.4.

TEAE is defined as an AE that starts or an existing AE that worsens on or after the date of prime vaccination. AE will be summarized by period, including Day 1 to Day 84, Day 85 to Day 113, and Day 114 to End of Study (EOS). From Day 1 to Day 29, all AE regardless causality will be recorded and summarized. From Day 30 to Day 84, all AE related to vaccination and SAE will be recorded and summarized. From Day 85 to Day 113, all AE regardless causality will be recorded and summarized. From Day 114 through end of study (EOS), all SAE will be recorded and summarized.

9.2.1. Incidence of Adverse Events

An overview summary of the following TEAE categories for the prime and boost analysis period will be provided separately:

- Any TEAE

- Any TEAE related to vaccination
- Any severe TEAE
- Any serious TEAE
- Any serious TEAE related to vaccination
- Any TEAE Leading to Withholding of Day 85 Vaccination
- Any TEAE leading to study discontinuation
- Any TEAE leading to death

9.2.2. Severity of Adverse Event

TEAEs will be rated as Mild, Moderate, or Severe based on the following criteria:

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

If a subject reports multiple occurrence of a specific event, the subject will be counted only once by the maximum severity. If the severity is missing for one or more of the occurrences, the maximum severity of the remaining occurrences will be used.

9.2.3. Relationship of Adverse Events to Study Vaccination

Study treatment related TEAEs will be summarized by SOC and PT as well as maximum severity grade. Study vaccination related AEs are those with a relationship to study treatment of 'Related' based on the eCRF page. AEs assessed as 'Not Related' will be considered unrelated for reporting purpose. Missing relationship will be counted as related to study vaccination.

9.2.4. Serious Adverse Events

Serious AEs are those identified on the eCRF as meeting the protocol defined serious criteria. Serious TEAEs will be summarized by SOC and PT and severity for each period.

A by-subject listing will also be provided.

9.2.5. Suspected Unexpected Serious Adverse Reaction

Suspected Unexpected Serious Adverse Reaction (SUSAR) will be summarized by SOC and PT, and maximum severity for each period. For this study any SAE related to vaccination will be a SUSAR by definition and therefore these two terms will be provided in a single output.

9.2.6. Adverse Events Leading to Withholding of Day 85 Vaccination

TEAEs leading to Withholding of Day 85 Vaccination will be summarized by SOC and PT for periods Day 1 to Day 84.

A by-subject listing will also be provided.

9.2.7. Adverse Events Leading to Study Discontinuation

TEAEs leading to study discontinuation will be summarized by SOC and PT for periods (for periods (Day 1- Day 84, Day 85-Day 113 and Day 114 through EOS).

A by-subject listing will also be provided.

9.2.8. Death

TEAEs leading to death will be summarized by SOC and PT for periods (Day 1 to Day 113 and Day 114-EOS).

A by-subject listing will also be provided for all adverse events leading to death.

9.3. Clinical Laboratory Evaluations

For those subjects in the Safety lead-in analysis set, laboratory results will be summarized for serum chemistry, hematology and coagulation as observed values and change from baseline on Day 8/Visit 2 following prime vaccination and Day 92/Visit 6 following boost vaccination using descriptive statistics (mean, median, standard deviation, min, max). Laboratory results will also be classified by toxicity score based on the US Food and Drug Administration (FDA) toxicity scoring system (Appendix 13.2). Counts and percentages of subjects will be summarized by toxicity grade on Day 8 and 92. In the case of no toxicity classification the score of 0 will be assigned.

For those subjects in the safety set, baseline value will be summarized for serum chemistry, hematology and coagulation using descriptive statistics (mean, median, standard deviation, min, max).

Summaries will be presented based on treatment arms of BPZE1 prime with BPZE1 boost (A), BPZE1 prime with no boost (B), Total Post BPZE1 (A+B), Boostrix prime with BPZE1 boost (C) and Boostrix prime with no boost (D), Boostrix prime (C+D), Total Post Booster BPZE1 (A+C), and Total Post Placebo Boost (B+D), Total Any BPZE1 Exposure (A+B+C) and Total (A+B+C+D).

9.3.1. Hematology

Hematology will be collected at screening for all screened subjects, and at Visit 2 Day 8, Visit 5 Day 85 prior to vaccination, and Visit 6 Day 92 for the Safety Lead-In Analysis Set. Hematology tests will include hemoglobin, white blood cell count and differential, and platelet count.

A by-subject listing will also be provided.

9.3.2. Serum Chemistry

Serum chemistry will be collected at the same scheduled visit and timepoints as hematology for the same analysis set. The serum chemistry panel will include sodium, potassium, glucose (random), blood urea nitrogen, creatinine, calcium, albumin, total protein, bilirubin, alanine aminotransferase, and aspartate aminotransferase.

A by-subject listing will also be provided.

9.3.3. Coagulation

Coagulation will be collected at the same scheduled visit and timepoints as hematology for the same analysis set. Coagulation tests will include prothrombin time/international normalized ratio and partial thromboplastin time.

A by-subject listing will also be provided.

9.3.4. Urine Drug Test, Urine Pregnancy Test and Serology

Urine pregnancy tests will be performed at screening, on Day 1/Visit 1 and Day 85/Visit 5 prior to vaccination for female subjects with child bearing potentials. Urine drug test will be performed at screening (exclusion criteria) and may be performed, at the discretion of the investigator, on any subject who experiences an adverse reaction. Serology will be performed at screening only and include hepatitis B, Hepatitis C, and HIV and if positive are a reason for exclusion.

Only a by-subject listing will be provided for urine pregnancy test.

9.4. Vital Sign Measurements

Vital sign measurements are one of the secondary safety endpoints. Vital sign measurements include oral temperature, pulse rate, and diastolic and systolic blood pressure (after subject is seated for at least 5 minutes). Vital signs will be collected at screening and on Days 1/Visit 1 (before vaccine administration and 60 minutes [±15 minutes] after vaccine administration [before subject is discharged]), Day 8/Visit 2, Day 29/Visit 3, Day 85/Visit 5 (before vaccine administration and 60 minutes [±15 minutes] after vaccine administration [before subject is discharged]), Day 92/Visit 6, Day 96/Visit 7, and Day 113/Visit 8.

Vital sign measurements will be summarized using descriptive statistics (mean, standard deviation, median, min, max) for reported values and change from baseline at each scheduled visit according to Appendix 13.1. Vital sign measurements will also be summarized as counts and percentages by severity grade (Appendix 13.3).

Summaries will be presented based on treatment arms of BPZE1 prime with BPZE1 boost (A), BPZE1 prime with no boost (B), Total Post BPZE1 prime (A+B), Boostrix prime with BPZE1 boost (C) and Boostrix prime with no boost (D), Boostrix prime (C+D), Total Post Booster BPZE1 (A+C), and Total Post Placebo Boost (B+D), Total Any BPZE1 Exposure (A+B+C) and Total (A+B+C+D).

9.5. Physical Examination

A full physical examination will be completed at screening and will include the following: skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, musculoskeletal system/extremities, and neurological system. Height and weight will be measured at the screening visit only.

A symptom-directed (targeted) physical examination will be performed as mentioned in the schedule of events for all subjects on Day 1/Visit 1 (pre-vaccination), Day 8/Visit 2, Day 29/Visit 3, Day 85/Visit 5 (rep-vaccination), Day 92/Visit 6, Day 96/Visit 7, and Day 113/Visit 8.

A by-subject listing will be provided for physical exam.

10. Interim Analysis

The interim analysis of this study will be purely for monitoring for safety.

A SMC will be convened by ILiAD Biotechnologies to review study progress and participant, clinical, safety, and reactogenicity data, at the following time points:

- After the subjects in the entire safety lead-in cohort (both dose levels) have completed Day 8 (Visit 2). The SMC will review all safety data through Day 8 (reactogenicity and safety laboratory results) and any AEs which have occurred since study initiation.
- Ad hoc when a halting rule is met, for immediate concerns regarding observations

The SMC will review grouped and unblinded data in the closed session only. The SMC will meet and review these data at scheduled time points or ad hoc as needed during this study as defined in the SMC charter. As an outcome of any meeting (after the safety lead-in cohort or with a safety pause), 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.

For analysis on the Safety Lead-In Analysis Set for the Interim Analysis, safety endpoints include

- Solicited local, nasal/respiratory, and subjective systemic reactogenicity adverse event by toxicity grade at post-prime vaccination on Day 1, and on each of the 8 days following vaccination.
- Treatment-emergent adverse events (TEAEs) occurred since study initiation, and their relatedness to vaccination. severity, and serious TEAE.
- Laboratory and vital sign tests and toxicity grade at each scheduled visit.

Tables will be provided based on the safety lead-in analysis set by treatment arms of BPZE1 10^7 CFU, BPZE1 10^9 CFU, and Boostrix. Tables will be provided for

- subject disposition,
- demographics,
- Solicited local, nasal/respiratory, subjective systemic reactogenicity on each day (Appendix 13.1) by toxicity grade based on FDA standard (Table 9-1).
- Overview of TEAE, TEAE by SOC/PT, TEAE by severity grade, SAE, and SAE related to study vaccination.
- Change from baseline of laboratory test (hematology and serum chemistry) and vital sign tests on Day 2 (Visit 2)
- Summary of laboratory and vital sign tests by toxicity grade on Day 8 (Visit 2) (Appendix 13.2, 13.3)
- By-subject listing will also be provided to support the above tables. In addition, a by-subject listing of IP administration will also be provided.

11.Changes in the Planned Analysis

The SAP contains no changes in the planned analyses described in the protocol.

12.References

Department of Health and Human Services (DHHS), Food and Drug Administration, Center for Biologics Evaluation and Research (US). Guidance for industry: Toxicity grading for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. September 2007 [cited 2017 Nov 29]. Available from: https://www.gpo.gov/fdsys/pkg/FR-2007-09-27/pdf/E7-19155.pdf

ILiAD Biotechnologies. BPZE1. Investigator's brochure, 0.42 ed. Weston (FL); 2017. 44 p.

Yeung KHT, Duclos P, Nelson EAS, et al. An update of the global burden of pertussis in children younger than 5 years: a modelling study. Lancet Infect Dis. 2017;17(9):974-80.

13. Appendices

13.1. Schedule of Study Procedures

Table 13-1

Procedure	Screening	Treatment Period									
Study visit	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10[a]**
Days relative to most recent vaccination	**–30 to 0**	**1**	**8**	**29**	**78**	**85/1**	**8**	**12**	**29**	**85**	**169**
Days relative to first vaccination[b]	**–30 to 0**	**1**	**8**	**29**	**78**	**85**	**92**	**96**	**113**	**169**	**254**
Window allowance	**30**	**0**	**+3**	**+7**	**-7**	**+10**	**+2**	**+/-2**	**+7**	**-7**	**±15**
Informed consent	X										
Inclusion/exclusion criteria	X	X[c]				X[c]					
Demographic and baseline data[d]	X										
Medical history[e]	X	X[c]				X[c]					
Medication[f]	X	X	X	X	X	X	X	X	X	X[g]	X[g]
Serology[h]	X										
Urine drug screen	X										
Physical examination[i]	X	X[j]	X	X		X[j]	X	X	X		
Vital sign measurements	X	X[k]	X	X		X[k]	X	X	X		
Safety laboratory tests for enrollment[l]	X										
Safety laboratory tests for safety cohort[m]	X		X			X[n]	X				
Urine pregnancy test (female subjects of childbearing potential)	X	X[n]				X[n]					
Randomization		X									
Vaccination[o]		X				X					
Subject diary dispensing[p]		X				X					
Subject diary reviewed[q]			X				X				
Reactogenicity - post vaccination		X[r]	X[s]			X[r]	X[s]				
Immunogenicity laboratory tests for serum pertussis IgG and IgA ELISA		X[n]		X		X[n]			X	X	X
Mucosal pertussis nasal antibody testing (S-IgA ELISA) – nasal absorption device[t]	X[u]			X	X				X	X	X
Nasal collection for *B. pertussis* colonization					X[v]		X	X	X[v]		X[w]
PBMC for cellular immunity (subset of no more than 60 subjects)		X	X				X				

Collection of serious adverse events and adverse events of special interest only x										X	X
Adverse events x y		X	X	X	X	X	X	X	X		
End-of-study form completion											X

Abbreviations: ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; PBMC, peripheral blood mononuclear cell; S-IgA, secretory immunoglobulin A.

[a] End-of-study Visit.
[b] Days relative to vaccination are only estimates as the window allowance is not inclusive. Should a study pause occur than vis its/windows will be adjusted to allow for subjects to continue without protocol deviation. Visit schedule following the boosting vaccination is calculated relative to the day the boosting vaccination was received.
[c] Inclusion/exclusion criteria and medical history will be reviewed and updated on Day 1 and Day 85 with specific vaccination e xclusions applied.
[d] Including date of birth (day, month, and year), sex, race, ethnicity, weight, height, and body mass index (derived).
[e] Including prior and concomitant medical conditions, surgeries significant procedures.
[f] Concomitant medications include all medications (including vaccines) taken by the subject from the time of signing the informed consent through 28 days after the boosting vaccination (or through early termination visit if prior to that time).
[g] Only medications associated with serious adverse events.
[h] Serology testing will include hepatitis B, hepatitis C, and human immunodeficiency virus.
[i] Full physical examination at screening; symptom-directed (targeted) physical examination at all other scheduled time points. Interim physical examinations will be performed at the discretion of the investigator, if necessary. Height and weight will be measured at screening only.
[j] On Day 1 (Visit 1) and 85 (Visit 5), targeted symptom-directed physical examination will be performed before vaccine administration.
[k] On Day 1 (Visit 1) and 85 (Visit 5), vital sign measurements will be collected once before vaccine administration and at 60 minutes (±15 minutes) after vaccine administration (before subjects are discharged).
[l] Safety laboratory testing for safety cohort include (Table 13-2) hemoglobin, white blood count with differential, platelet count, sodium, potassium, random glucose, blood urea nitrogen, creatinine, calcium, albumin, total protein, bilirubin, alanine aminotransferase, aspartate aminotransferase, prothrombin time, and partial thromboplastin time. See Table 13-2 for specific laboratory tests to utilize for exclusion for the full cohort (kidney, hepatic, and hematology/coagulation only). Laboratory testing may be repeated once during the 30-day screening period if specific values used for exclusion criteria exceed toxicity Grade 1, with the last value being the value of record.
[m] The first 48 subjects randomly assigned will be designated the safety lead-in cohort and safety laboratory testing will be performed in this subset beyond the screening visit. Should any laboratory value result in a grade 3 or greater toxicity score this lab must be entered as an adverse event and followed with retesting and observing for resolution or new clinical baseline
[n] Performed prior to vaccination.
Subjects will be randomly assigned as per Table.
[p] All subjects will record reactogenicity in the daily subject diary starting on the same day of the p rime (Day 1) and boosting (Day 85) vaccinations and for additional days (not counting vaccination day).
[q] The clinical staff will review the information from the subject diary with the subjects on Days 8 and 92. Toxicity grading wi ll be applied by investigator for all subject recorded reactogenicity on days 1-8.
[r] Reactogenicity will be collected on Day 1 (Visit 1) and 85 (Visit 5) at 60 minutes (±15 minutes) after dose administration (before subjects are discharged).
[s] To be collected from day of vaccination through additional 7 days post-vaccination by subject.

[t] Must occur at least 6 days prior to intranasal vaccination.
[u] Collected but only tested within the trial if the subject is randomly assigned into the study. Samples may be used per subject consenting for additional product development needs.
[v] To be collected after mucosal pertussis nasal antibody collection.
[w] Only in subjects who tested positive by pertussis culture at Day 113. Any subject who remains positive on Day 254 will be provided a short course of azithromycin.
[x] At the discretion of the investigator a urine drug screen may be performed on any subject who experiences an adverse event.
[y] All adverse events will be collected 28 days after each vaccination. Adverse events related to vaccination will be collected through Day 113

13.2. FDA Table for Laboratory Grading

Table 12-2 Laboratory Abnormalities

Serum[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[b]
Sodium – hyponatremia (mEq/L)	132-134	130-131	125-129	< 125
Sodium – hypernatremia (mEq/L)	144-145	146-147	148-150	> 150
Potassium – hyperkalemia (mEq/L)	5.1-5.2	5.3-5.4	5.5-5.6	> 5.6
Potassium – hypokalemia (mEq/L)	3.5-3.6	3.3-3.4	3.1-3.2	< 3.1
Glucose – hyperglycemia Random (mg/dL)	110-125	126-200	> 200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen (mg/dL)	23-26	27-31	> 31	Requires dialysis
Creatinine (mg/dL)*	1.5-1.7	1.8-2.0	2.1-2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia (mg/dL)	8.0-8.4	7.5-7.9	7.0-7.4	< 7.0
Calcium – hypercalcemia (mg/dL)	10.5-11.0	11.1-11.5	11.6-12.0	> 12.0
Albumin – hypoalbuminemia (g/dL)	2.8-3.1	2.5-2.7	< 2.5	--
Total Protein – hypoproteinemia (g/dL)	5.5-6.0	5.0-5.4	<5.0	--
Liver Function Tests – ALT, AST increase by factor*	1.1-2.5 × ULN	2.6-5.0 × ULN	5.1-10 × ULN	> 10 × ULN
Bilirubin – when accompanied by any increase in liver function test increase by factor*	1.1-1.25 × ULN	1.26-1.5 × ULN	1.51-1.75 × ULN	> 1.75 × ULN
Bilirubin – when liver function test is normal; increase by factor*	1.1-1.5 × ULN	1.6-2.0 × ULN	2.0-3.0 × ULN	> 3.0 × ULN
Hematology[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[b]
Hemoglobin (Female) (gm/dL)*	11.0-12.0	9.5-10.9	8.0-9.4	< 8.0
Hemoglobin (Female) change from baseline value (gm/dL)	Any decrease - 1.5	1.6-2.0	2.1-5.0	> 5.0
Hemoglobin (Male) (gm/dL)*	12.5-13.5	10.5-12.4	8.5-10.4	< 8.5
Hemoglobin (Male) change from baseline value (gm/dL)	Any decrease - 1.5	1.6-2.0	2.1-5.0	> 5.0
WBC Increase (cell/mm^3)*	10,800-15,000	15,001-20,000	20,001-25,000	> 25,000
WBC Decrease (cell/mm^3)*	2,500-3,500	1,500-2,499	1,000-1,499	< 1,000
Platelets Decreased - cell/mm^3*	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
Prothrombin time – increase by factor*	1.0-1.10 × ULN	1.11-1.20 × ULN	1.21-1.25 × ULN	> 1.25 × ULN

partial thromboplastin time – increase by factor *	1.0-1.2 × ULN	1.21-1.4 × ULN	1.41-1.5 × ULN	> 1.5 × ULN

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal; WBC, white blood cell.

*Screening labs with toxicity scores greater than 1 are exclusion criteria for the full cohort only (greyed line items).

All safety labs listed in Table 13-2 with a toxicity score greater than 1 are exclusion criteria for the safety cohort.

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[b] The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

Source: DHHS 2007

13.3. FDA Table for Vital Sign Grading – Vital Sign Abnormality

Table 12-3 Vital Sign Abnormality

Vital Signs[0]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°F)b	100.4 – 101.1	101.2 – 102.0	102.1 – 104	>104
Tachycardia (beats per minute)	101 – 115	116 – 130	>130	Emergency room visit or hospitalization for arrhythmia
Bradycardia (beats per minute)[c]	50 – 54	45 – 49	<45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) (mm Hg)	141 – 150	151 – 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) (mm Hg)	91 – 95	96 – 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) (mm Hg)	85 – 89	80 – 84	<80	ER visit or hospitalization for hypotensive shock

Note: Grade 0 will be the classification if the observation is less than a Grade 1. Respiratory rate was removed from the FDA table for vital sign grading as this is not a parameter being measured in this study.

a. Subject should be at rest for all vital sign measurements.

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

c. When resting heart rate is between 60 to 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Source: DHHS 2007.

13.4. Guideline of Missing Date Imputation for Safety Analysis

Impute Missing Adverse Events/ Prior or Concomitant Medications

A. Incomplete Start Date:

If the stop date is not missing, and the imputed start date is after the stop date, the start date will be imputed by the stop date.

Missing day and month

- If the year is the **same** as the year of the first dosing date, then the day and month of the first dosing date will be assigned to the missing fields.
- If the year is **prior to** the year of first dosing date, then December 31 will be assigned to the missing fields.
- If the year is **after** the year of first dosing, then January 1 will be assigned to the missing fields.

Missing day only

- If the month and year are the **same** as the year and month of first dosing date, then the first doing date will be assigned to the missing day.
- If either the year of the partial date is **before** the year of the first dosing date or the years of the partial date and the first dosing date are the same but the month of partial date is **before** the month of the first dosing date, then the last day of the month will be assigned to the missing day.
- If either the year of the partial date is **after** the year of the first dosing date or the years of the partial date and the first dose date are the same but the month of partial date is **after** the month of the first dosing date, then the first day of the month will be assigned to the missing day.
- If the stop date is not missing, and the imputed start date is after the stop date, the start date will be imputed by the stop date, i.e. set to the stop date.

Missing day, month, and year

- No imputation is needed; the corresponding AE will be included as TEAE provided the end date of the AE is after the first dose date or the end date is also missing.

B. Incomplete End Date:

If the imputed stop date is before the start date, then the imputed stop date will be equal to the start date.

Missing day and month

- If the year of the incomplete stop date is the **same** as the year of the last dosing date, then the day and month of the last dosing date will be assigned to the missing fields.
- If the year of the incomplete stop date is **prior to** the year of the last dosing date, then December 31 will be assigned to the missing fields.

- If the year of the incomplete stop date is **prior to** the year of the last dosing date but is the same as the year of the first dosing date, then the first dosing date will be assigned to the missing date.
- If the year of the incomplete stop date is **after** the year of the last dosing date, then January 1 will be assigned to the missing fields.

Missing day only

- If the month and year of the incomplete stop date are the **same** as the month and year of the last dosing date, then the day of the last dosing date will be assigned to the missing day.
- If either the year of the partial date is **not equal to** the year of the last dosing date or the years of the partial date and the last dosing date are the same but the month of partial date is **not equal to** the month of the last dosing date, then the last day of the month will be assigned to the missing day.