CLINICAL STUDY PROTOCOL

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 Doses (Prime + Boost) Schedule, and Compared to a Boostrix™ Prime Dose With or Without a BPZE1 Boost Dose

IB-200P

Sponsor:	ILiAD Biotechnologies 4581 Weston Road, Suite 260 Weston, FL 33331
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CONFIDENTIAL

The study will be conducted according to the International Council for Harmonisation harmonised tripartite guideline E6(R2): Good Clinical Practice.

Table of Contents

List of Tables

Protocol Synopsis

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Sponsor:	ILiAD Biotechnologies 4581 Weston Road, Suite 260 Weston, FL 33331
Study Phase:	2b
Study Sites:	3 to 5 clinical sites in the United States
Indication:	Pertussis in adults and adolescents
Rationale:	The availability of a cost-effective pertussis vaccine that provides improved efficacy and prolonged protection with the potential to reduce or eliminate transmission would present a breakthrough in the prevention of colonizing pertussis infections. This novel approach may not only protect BPZE1-vaccinated individuals from *Bordetella 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 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 (type 1 T helper cells, type 2 T helper cells, and nasal mucosal immune responses). The study population, healthy adults, has been chosen to maximize the quality of immunogenicity data while minimizing risk and potential safety signals. 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. Immunity will be measured in enzyme-linked immunosorbent assays (ELISA) specific for pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae (FIM) 2/3, and a broader pertussis ELISA developed with BPZE1 whole cell extract. 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.

Objectives:

Primary objectives

Immunogenicity:

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

Safety

- 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.

Secondary Objectives

Immunogenicity:

- To assess the systemic immune response (immunoglobulin G [IgG], immunoglobulin A [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.

Colonization:

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

Safety:

- To describe (severity and clinical significance) of vaccine-related adverse events (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 serious AEs during the study.

Exploratory Objective

- To examine cell-mediated (eg, B cell, CD4 T lymphocytes + T cell, CD8T lymphocytes + T cell) responses in a subset of no more than 60 subjects using peripheral blood mononuclear cells 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 immunological responses across time, relative to baseline status and relative to vaccination response, with the current assays and with any future assays developed for BPZE1.

Primary Endpoints:

Immunogenicity:

- **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, 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 (eg, IgA). To be further defined during assay development and within the statistical analysis plan.

Mucosal baseline samples will be taken at the screening visit.

Safety:

- Solicited AEs (local, nasal/respiratory, 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 intramuscular (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.

Secondary Endpoints:

Systemic Immunogenicity (serum sampling) expressed separately for IgG, IgA, and IgG or IgA ELISA when possible:

- 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, or 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 detection). Both IgG and IgA ELISA will measure antibodies against the following pertussis-specific antigens of PT, FHA, PRN, FIM 2/3, and IgG ELISA will measure antibodies against broader pertussis-specific antigens in BPZE1 whole cell extract.

  Serum baseline samples will be 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 (aP) antigens PT, FHA, and PRN over baseline:
  - On **either** Day 29 (prime) or 113 (boost)
  - On **both** Days 29 (prime) or 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 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 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.

Mucosal Immunogenicity S-IgA ELISA (nasal mucosal secretion sampling):

- 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 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 Geometric Mean Fold Rise 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 Geometric Mean Titer 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.

Colonization:

- Proportion of subjects with positive *B. pertussis* by bacterial culture of nasal sample on each 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).

Safety:

- Unsolicited AEs (eg, treatment-emergent AEs, serious AEs, and suspected unexpected serious adverse reactions) collected for 28 days following each vaccination by Medical Dictionary for Regulatory Activities (MedDRA) classification and severity score.
- Unsolicited AEs related to vaccination through Day 113 by MedDRA classification and severity score.
- Serious AEs through 6 months following the last vaccination (or until resolved or stable) by MedDRA classification, relatedness, and severity score.
- Vital sign measurements with severity scoring immediately following vaccination.

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 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 second analyses, additional exploratory endpoints may be tested for systemic or nasal mucosal immunogenicity (IgG or IgA) responses at any time point collected and not already performed in the primary or secondary analysis sets.
- The Geometric Mean Titer, expressed for serum IgG ELISA against tetanus and diphtheria on Days 29 and 113.

Study Population:

Inclusion Criteria

Each subject must meet all the following criteria to be enrolled in this study:

1. Is a male or nonpregnant female 18 to 50 years of age, inclusive, on Day 1 (primary vaccination).
2. Is capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with protocol requirements.
3. Female subjects must be nonpregnant and nonlactating and meet 1 of the following criteria:
   a) Postmenopausal (defined as 12 consecutive months with no

menses without an alternative medical cause or documented plasma follicle-stimulating hormone level in the postmenopausal range);

b) Surgically sterile (ie, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy).

NOTE: These procedures and laboratory test results must be confirmed by physical examination, or by subject recall of specific date and hospital/facility of procedure, or by medical documentation of said procedure.

c) Is of childbearing potential (defined as any female who has experienced menarche and who is NOT permanently sterile or postmenopausal), agrees to be heterosexually inactive from at least 21 days prior to enrollment and through 3 months after the boosting vaccination or agrees to consistently use any of the following methods of contraception from at least 21 days prior to enrollment and through 3 months after the boosting vaccination:
   i. Condoms (male or female) with spermicide
   ii. Diaphragm with spermicide
   iii. Cervical cap with spermicide
   iv. Intrauterine device
   v. Oral or patch contraceptives
   vi. Norplant®, Depo-Provera®, or other FDA-approved contraceptive method that is designed to protect against pregnancy.

NOTE: Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

4. Has a stable health status as assessed by the investigator, as established by physical examination, vital sign measurements, and medical history.
5. Has access to a consistent and reliable means of telephone contact, which may be in the home, workplace, or by personal mobile electronic device.
6. Is able to understand and comply with planned study procedures.
7. Lives a reasonable distance from the clinical site to be able to travel to and from the clinical site for follow-up visits and agrees to go to the clinical site for evaluation (or provide medical record access if evaluated elsewhere) in the event of an AE.
8. Agrees to stay in contact with the clinical site for the duration of the

study, has no current plans to move from the study area, and provides updated contact information as necessary.

Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. History of being vaccinated in the past 5 years against pertussis (subject recall).
2. Any significant past reaction to any component of Boostrix (at the discretion of the investigator).
3. Subject reported diagnosis of pertussis in the past 10 years (must be laboratory confirmed or physician diagnosed from medical records).
4. Vital signs by FDA toxicity scoring >1 (may be repeated once during the screening period to allow for inclusion and the most recent measurement taken at baseline).
5. Chronic illness being treated actively and with evidence of recent intervention for worsening or fluctuating symptoms (at the discretion of the investigator).
6. The subject has a history of active cancer (malignancy) in the last 10 years (exception is subjects with adequately treated non-melanomatous skin carcinoma, who may participate in the study).
7. Current use of any smoking products and unwillingness to refrain from the use of any smoking products from screening through 28 days after the boosting vaccination.
8. Use of narcotic drugs, evidenced by urine toxicology screen or a history of drug/alcohol abuse within the past 2 years.
9. Has donated blood or suffered from blood loss of more than 450 mL (1 unit of blood) within 60 days prior to screening or donated plasma within 14 days prior to screening.
10. Receipt of immunoglobulin, blood-derived products, systemic corticosteroids, or other immunosuppressant drugs within 90 days prior to Day 1.
11. Asthma*, obstructive nasal canal, recurrent or acute sinusitis or other chronic respiratory problems inclusive of the diagnosis of any significant pulmonary disease. *Asthma as diagnosed with spirometry showing reversibility of disease and must meet at least Step 1 classification with current prescription/use of medications to control symptoms (EPR-3 2007).
12. History of nasal surgery or Bell’s palsy.
13. Use of repeated nasal sprays, Neti pot, routine nasal washing within

the past 1 month (more than 2 times per week). Subjects must agree to refrain from use of any of these modalities through Day 113.

14. A temporary exclusion to vaccinate if acute respiratory tract infection or rhinorrhea or temperature >100.4°F (no symptoms for 3 days prior to vaccination day). Subjects may be vaccinated if they stay within the vaccination window (screening [30 days] or at the time of the booster [10 days]).

NOTE: If a subject exceeds the screening window, they must be reconsented and screening must be reinitiated.

15. Use of corticosteroids in the respiratory tract (eg, nasal steroids, inhaled steroids) within 30 days prior to Day 1.
16. Receipt of a licensed vaccine within the last 30 days prior to Day 1 or planned vaccination during the active study conduct through Day 113. In the case of seasonal influenza, vaccination should not be withheld and is not contraindicated for subject participation. However, vaccination should be planned outside of a 30-day pre- and 30-day post-vaccination window whenever possible.
17. Known hypersensitivity to any component of the study vaccines.
18. Participation in any other clinical trial for the testing of an unlicensed product during the previous 6 months or planned during the study conduct.

NOTE: “Testing” is intended to mean receipt an investigational product. Subjects in long term safety follow-up studies where vaccination has been completed greater than 6 months prior to this study enrollment are allowed.

19. Inability to adhere to the protocol, including plans to move from the area.
20. Personal history or family (first degree) history of congenital or hereditary immunodeficiency.
21. Past or present infection with human immunodeficiency virus, hepatitis B, or hepatitis C by screening test.
22. Any autoimmune or immunodeficiency disease/condition (inherited or iatrogenic).

NOTE: Stable endocrine disorders that have a confirmed autoimmune etiology (eg, thyroid, pancreatic) are allowed.

23. Any neurological disease or history of significant neurological disorder (eg, meningitis, seizures, multiple sclerosis, vasculitis, migraines*, Guillain-Barré syndrome [genetic/congenital or acquired]). *Significant neurological migraine includes frequent migraine (2 times a month or greater), migraine with aura or

migraine with complications (status migraninosus, persist aura without infarction, infarction or aura triggered seizure defined by International Classification of Headache Disorders-3 [ICHD-3 2018).

24. Any medical condition that, in the opinion of the investigator, might interfere with the evaluation of the study objectives or might affect the safety of the individual, (eg, major depression or history of suicidal attempt).
25. Toxicity grading >1 for screening laboratory test results for specific kidney, hepatic, and hematologic values for all subjects and in screening laboratory values for the safety cohort as indicated in Table 13-2 (may be repeated once during the screening period to allow for inclusion and the most recent measurement taken at baseline). See Table 13-2 for specifically designated parameters.
26. Body mass index <17 kg/m^2 or >40 kg/m^2.
27. Frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc.) or residence in the same household as persons with known immunodeficiency including persons on immunosuppressant therapy.
28. Study team member or first-degree relative of study team member.

Study Design: 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, 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 informed consent 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.

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 randomized 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 Days 29 and 78 post-primary vaccination. Serum sampling for antibody response (IgG and IgA ELISA) will occur at baseline (Day 1, 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. 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 (formulation buffer for injection). 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 Day 113 will be retested at 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 for pertussis at Day 254 will be provided a short course of 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 products 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 monitor for activation of any halting rule. Following accumulation of all safety data through Day 8 post-vaccination and without a safety pause being triggered, 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 also 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. Subjects will continue with the booster using the same intranasal dose level (10^7 CFU or 10^9 CFU) assigned for the first dose. The medical monitor has the authority to request a review by the Safety Monitoring Committee should a halting rule be initiated or there are any other safety concerns. The Safety Monitoring Committee 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 Safety Monitoring Committee will be authorized to allow the remainder of the subjects to be randomly assigned. The randomization scheme for the safety lead-in will follow the same scheme as the full cohort for second vaccination 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 1 Dosing and Safety Lead-In Cohort

BPZE 1 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	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 examination for reactogenicity (local, nasal/respiratory, and systemic) with 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 subsequent 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 subsequent 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 and AEs of special interest (if newly identified) will be monitored to the end of the study. The primary database lock will occur at Day 113, and all data collected through Day 113 will be included in the clinical study report 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 clinical study report. Subjects will return to the clinical site on Day 254 (±15 days) for end-of-study procedures. A subset of no more than 60 subjects (randomly assigned to the 10^9 CFU dose) will opt in to provide blood samples for peripheral blood mononuclear cell harvesting on Days 1, 8, and 92.
Estimated Study Duration:	Study duration is approximately 15 months; subject participation duration is approximately 10 months.
Immunogenicity Assessments:	Immune measurements (ELISA) will be conducted on serum (IgG and IgA) and nasal secretion (S-IgA) for the common pertussis antigens of PT, FHA, PRN, FIM 2/3 and on serum (IgG) and nasal secretions (S-IgA) for BPZE1 whole cell extract. Testing of additional antigens specific to *B. pertussis* may be performed at a later date as BPZE1 induces broader immunity more similar to whole cell pertussis and does not contain the purified antigen levels of PT, FHA, and PRN found in Boostrix. Subjects will be asked to provide a signed informed consent for the use of samples for further pertussis-specific testing or assay development. Aliquots of collected samples from this study may be retained for additional testing of antigens specific to *B. pertussis* for a maximum of 10 years (starting from the date at which the last subject had the last study visit), unless local rules, regulations, or guidelines require different timeframes or different procedures, in accord with subject consent. Testing for colonization using *B. pertussis* culture following the primary vaccination and post-boost will also occur. Colony counts as well as culture positivity (yes/no) will be included in the colonization assessments post-boosting.
Safety Assessments:	Safety assessments will include the following: • Vital sign measurements (oral temperature, pulse rate, and blood pressure) • Physical examination • Clinical safety laboratory tests (hematology, chemistry, coagulation at baseline and in the safety lead-in cohort following vaccination) and urine pregnancy test in women of childbearing potential • Solicited local, nasal/respiratory, and systemic reactogenicity symptoms during the 7 days after each vaccination (Days 1 through 8 and 85 through 92). Local reactogenicity will only be monitored following the IM vaccination. • Adverse events through 28 days after the primary and boosting vaccination

	• Adverse events associated with vaccination through Day 113 (unless resolved or stable at an earlier date) • Serious AEs and any AEs of special interest from the time of the primary vaccination through the last visit
Study Drug, Dosage, and Route of Administration:	Live attenuated *B. pertussis* BPZE1 vaccine, 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) and a dilution of the same stock to achieve 10^7 CFU in the same total volume for the first cohort lead-in (n = 24 subjects). See the pharmacy manual for specific dilution requirements. Boostrix IM injection, 0.5 mL to the deltoid region (aP vaccine, manufactured by GlaxoSmithKline) Placebo (BPZE1 formulation buffer for injection) 2 × 0.4 mL (0.4 mL per nostril) Placebo IM injections of 0.5 mL sterile normal saline to the deltoid region
Sample Size:	The sample size is based on clinical considerations but not a statistical power analysis as the study does not test any formal null hypothesis. A total of 300 subjects will be globally assigned at 2:2:1:1 allocation ratio and receive BPZE1 intranasal administration with placebo IM or Boostrix IM with placebo intranasal administration on Day 1 and then BPZE1 or placebo intranasal administration on Day 85.
Statistical Methods:	The objectives of this study are to assess the nasal mucosal and systemic immunogenicity, reactogenicity, and general safety parameters of lyophilized BPZE1 at 10^7 and 10^9 CFU administered intranasally via VaxINator atomization device to either a single (prime) or 2 dose (prime + boost) schedule. Secondly, to compare responses to a licensed aP comparator vaccine (ie, Boostrix) given by IM injection, in healthy volunteers 18 to 50 years of age, as well as to assess safety and immune response to BPZE1 boost after Boostrix priming. Statistical analysis will be performed using SAS software Version 9.3 or later. 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 Clopper-Pearson method. Where applicable, data analyses will be conducted for the prime period (prior to boosting) and boost period (at or after boosting).
Version and Date of Protocol:	Version 6.0; 20 November 2019

List of Abbreviations

Abbreviation	Definition
AE	adverse event
aP	acellular pertussis
CDROM	compact disc read-only memory
CFR	Code of Federal Regulations
CFU	colony-forming units
CRO	contract research organization
CSR	clinical study report
DNT	dermonecrotic toxin
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FHA	filamentous hemagglutinin
FIM	fimbriae
GCP	Good Clinical Practice
GMFR	Geometric Mean Fold Rise
GMT	Geometric Mean Titer
ICF	informed consent form
ICH	International Council for Harmonisation
IgA	immunoglobulin A
IgG	immunoglobulin G
IM	intramuscular
IRB	institutional review board
MedDRA	Medical Dictionary for Regulatory Activities
PBMC	peripheral blood mononuclear cell
PRN	pertactin
PT	pertussis toxin
S-IgA	secretory immunoglobulin A
SAE	serious adverse event
SMC	Safety Monitoring Committee
SUSAR	suspected unexpected serious adverse reaction

Abbreviation	Definition
TCT	tracheal cytotoxin
WFI	water for injection

1 Introduction

1.1 Background Information

Currently registered acellular pertussis (aP) vaccines protect against respiratory disease but not against colonizing *Bordetella pertussis* infection and transmission. *B. pertussis* is a gram-negative bacterium and a causative agent of pertussis, more commonly known as whooping cough. In a nonhuman primate model, convalescence from a natural *B. pertussis* infection protected against colonizing infection and transmission caused by a subsequent virulent *B. pertussis* challenge (Warfel et al 2014), ie, a natural infection confers sterilizing immunity. Similarly, 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). ILiAD has licensed BPZE1 from the Institut Pasteur de Lille and Inserm with worldwide rights and the company is conducting further clinical and regulatory development.

Despite the dramatic decline in whooping cough cases and deaths in industrialized nations during the 20th century due to public health vaccine initiatives, recent decades have witnessed a sharp increase in cases and it is generally agreed that there is a critical need for a new and more effective vaccine targeting *B. pertussis*. 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). Infants are not fully protected and require a series of vaccinations against pertussis. 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. The safety of liquid BPZE1 has been assessed in immunocompetent and immunosuppressed animals (Feunou et al 2008; Meilcarek et al 2006; Meilcarek et al 2010; Skerry et al 2009). These results contributed to downgrading BPZE1 from a biosafety level 2 organism to biosafety level 1 in France and other European countries. Good Laboratory Practice-compliant toxicological studies have confirmed the safety of BPZE1 in 2 animal models (mouse and rabbit) (ILiAD Biotechnologies 2017).

BPZE1 in a liquid formulation has been studied at various doses from 10^3 colony-forming units (CFU) to 10^9 CFU in 2 Phase 1 clinical studies in Sweden with no vaccine-related serious adverse events (SAEs) and comparable general adverse events (AE) to placebo controls. A total of 78 healthy subjects have now received intranasally administered BPZE1 in a liquid formulation. The most recent Phase 1b study has demonstrated the ability for a 0.4 mL (400 µL) per nostril dose of 10^7, 10^8, and 10^9 CFU BPZE1 to achieve transient nasopharyngeal colonization in >80% of subjects. Furthermore, 92% of the subjects in the 10^7 and 10^8 CFU/dose groups and 100% of the 10^9 CFU group had a positive serological response (immunoglobulin G [IgG] or immunoglobulin A [IgA]) to any of the 4 *B. pertussis* antigens tested, and 100% of the 10^9 CFU subjects had a positive serological response (IgG or IgA) to 2 or more of the antigens tested. The 10^9 CFU dosage appears to be the most promising to elicit the needed immune response in the majority of adults. None of the placebo subjects had a positive serological response (IgG or IgA) to 2 or more antigens tested (ILiAD Biotechnologies 2017).

A separately ongoing Phase 2a clinical study is comparing 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. The 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. The main advantage of the lyophilized BPZE1 formulation is long-term stability and storage at refrigeration temperature instead of storage at a very low freezing temperature. Mouse potency and adherence assays have demonstrated similar results for the lyophilized and liquid formulations. Prior to vaccination, the lyophilized product will be reconstituted, and vaccine will be delivered as a liquid formulation using a nasal applicator to improve consistent delivery.

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. The 10^9 CFU is described throughout this protocol as the default dose level for the full cohort for purposes of consistent presentation.

The vaccine will be shipped from ILiAD's European contract manufacturing organization to the contract research organization's (CRO's) repository and distribution center and should be stored at –20°C or below prior to shipping to the clinical sites, where the vaccine can be stored at –10°C or below, and is expected to have a 1-year shelf-life that can be extended with periodic ongoing retests conducted every 4 months by ILiAD's European contract manufacturing organization. The lyophilized BPZE1 is reconstituted in 1 mL of sterile water for injection (WFI), with 0.8 mL per dose removed from the vial for use. The reconstituted vaccine is recommended to remain at room temperature for 60 minutes or less prior to vaccination and therefore attention to the timing of pharmacy reconstitution and vaccination of individuals will be critical. After correct dilution (to obtain 10^7 or 10^9 CFU per the pharmacy manual) the VaxINator will be attached to the syringe filled with reconstituted BPZE1, with half of the dose (0.4 mL) administered into each nostril. For the placebo control nasal vaccination, the formulation buffer used for BPZE will be similarly diluted with sterile water (WFI) and used with the vaccinator at the same dose (0.4 mL per nostril). Only clinical staff trained on use of the VaxINator will be allowed to provide intranasal vaccinations and the study blind will be maintained by use of an opaque syringe (or other similar blinding technique). Unblinded study drug personnel will deliver the intramuscular (IM) vaccination unless the syringes can be masked to ensure no unblinding will occur for clinical staff involved in subject assessments.

1.2 Rationale

The availability of a cost-effective pertussis vaccine that provides improved efficacy and prolonged protection with the potential to reduce or eliminate transmission would present a breakthrough in the prevention of colonizing pertussis infections. 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 (type 1 T helper cells, type 2 T helper cells, and nasal mucosal immune responses) (Wearing and Rohani 2009). The study population, healthy adults, has been chosen to maximize the quality of immunogenicity data while minimizing risk and potential safety signals.

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. Immunity will be measured in enzyme-linked immunosorbent assays (ELISA) specific for PT, filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae (FIM) 2/3, and a broader pertussis ELISA developed with BPZE1 whole cell extract. 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.

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

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

1.3.2 Risks of Study Participation

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

1.3.3 BPZE1 Risks to Study Subjects

The risks of BPZE1 administration are expected to be minimal and clinically manageable. *B. pertussis* colonization is strictly limited to respiratory epithelium without dissemination of the bacteria outside the respiratory tract, which also excludes systemic bacteremia of the BPZE1 strain.

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

B. pertussis has not been shown to be allergenic in any preclinical or clinical studies to date, nor to have any of the excipients in the lyophilized formulation. BPZE1 has been shown to protect against airway inflammation induced by allergens or viral infections in a murine model (Althouse and Scarpino 2015). BPZE1 has also been shown to protect against wild type *B. pertussis* infection 3 hours after immunization in a murine model (Mielcarek et al 2006). However, there remains a theoretical risk of allergic reaction, as is present with any vaccine product.

BPZE1 vaccine or the placebo will be administered nasally via the VaxINator atomization device attached to a syringe to healthy adult volunteers under strictly controlled conditions. The VaxINator atomizes the liquid vaccine as it exits the syringe. There are no additional risks to study subjects based on the use of the VaxINator device.

To minimize the risk of transmission, the subjects will stay at the study center for at least 60 minutes after administration on Days 1 and 85. In addition, subjects with frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc.) or subjects who live in the same household as individuals with known immunodeficiency or individuals on

immunosuppressant therapy will be excluded from participation in the study. The attenuated BPZE1 bacteria colonize the upper respiratory tract similarly to wild-type *B. pertussis*. Colonization of the live organism will be assessed from nasopharyngeal sampling performed on Days 78 (after a mucosal pertussis nasal antibody collection is completed), 92, 96, and 113 (after a mucosal pertussis nasal antibody collection is completed). Any subject who tested positive by pertussis culture at Day 113 will also have a nasal collection taken for pertussis culture on Day 254. Any subject who remains positive at Day 254 will be provided a short course of 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*.

Furthermore, in animal studies, when female mice were vaccinated with BPZE1 shortly before mating, no negative effect on either the pregnancy or their offspring was observed. The offspring were protected against *B. pertussis* challenge (Feunou et al 2016).

Previous clinical trials completed in subjects who received 10^7, 10^8, or 10^9 CFU/subject reported AEs. None of the subjects had spasmodic cough of grade 2 or more or any other AE related or possibly related to vaccination of grade 3 or higher. Among 54 subjects (42 vaccinated with an active vaccine and 12 with placebo), the most frequently solicited AEs during the first 28 days were sneezing (n = 61 in 29 subjects), fatigue (n = 51 in 28 subjects), headache (n = 42 in 27 subjects), rhinorrhea (n = 41 in 26 subjects), and nasal congestion (n = 39 in 25 subjects). The reported symptoms were mostly mild or moderate. There were no consistent signals for differences in AE occurrence between placebo and the different dose groups.

In summary, the risk assessment for this study shows a very low potential risk for the study subjects and impact associated with administering BPZE1.

1.3.4 Risks to the Environment or Potential for Interaction With Wild-Type *B. Pertussis* Strains

To avoid accidental exposure actions should be taken to minimize generation of aerosols, since the bacterium is strictly a respiratory tract organism. The subjects and clinical staff members should wear eye-protective glasses and masks during the vaccination. Persons handling the BPZE1 bacteria should wear gloves and must wash their hands with a suitable

disinfecting soap before touching their skin and eyes. Effective antibiotic treatment with azithromycin (or an appropriate antibiotic if the subject is allergic to azithromycin) should be given in case of accidental transmission to other humans.

The attenuated strain of *B. pertussis* (BPZE1) was engineered by genetically altering or removing 3 *B. pertussis* toxins: PT, TCT, and DNT. The genetic modifications (replacement of the *ampG* gene, deletion of the DNT, and the mutations of the PT) are not expected to alter the host range of *B. pertussis* BPZE1 compared to the wild-type *B. pertussis*.

Due to the robust preclinical safety data, BPZE1 has been classified as a Biosafety Level 1 organism by French authorities Republique Francaise Ministere De L'enseignement Superieur Et De La Recherche (French Ministry of Higher Education and Research). Germany, Belgium, Spain, and Sweden have accepted the French Authority's Biosafety Level 1 rating for the purpose of manufacturing and clinical studies.

The genetic modifications in BPZE1 strongly increase the in vivo and in vitro safety:

- The double nucleotide mutation in the substrate binding and the active site of the PT results in a strong reduction of the enzyme activity.
- The replacement of the *B. pertussis ampG* gene by the *Escherichia coli ampG* gene results in an over 95% reduction in release of the TCT in the medium.
- The DNT is not expressed in the BPZE1 strain.
- BPZE1 is not invasive and has no selective advantage in the environment. The potential for exchange of genetic material is virtually nonexistent since *B. pertussis* does not harbor plasmids or conjugative transposons. In addition, *B. pertussis* Tohama I (origin of BPZE1) does not harbor intact prophage genomes and is therefore incapable of producing functional phage particles.

Chronic carriage of *B. pertussis* has not been reported and is therefore not expected. No cross-contamination between the subjects was observed in the previous Phase 1 clinical trials of BPZE1, nor was any risk to the family members of study subjects observed. In case of transmission to other humans, accidentally exposed, an efficient treatment against *B. pertussis* is commercially available and is based on administering erythromycin. BPZE1 has been shown to be sensitive to erythromycin.

In summary, the preliminary risk assessment for this study suggests there is an extremely low risk for potential environmental impact associated with administering the BPZE1 to study subjects.

1.3.5 Boostrix Risks to Study Subjects

The risks of Boostrix administration are expected to be minimal and clinically manageable. Local adverse reactions include pain, redness, swelling, and injected arm circumference increase. General adverse reactions include headache, fatigue, gastrointestinal symptoms, and fever.

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk of Guillain-Barré syndrome may be increased following a subsequent dose of tetanus toxoid-containing vaccine, including Boostrix.

Syncope can occur in association with administration of injectable vaccines, including Boostrix. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures for administration should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

Progressive or unstable neurologic conditions (eg, cerebrovascular events and acute encephalopathic conditions) are reasons to defer vaccination with a pertussis-containing vaccine, including Boostrix. It is not known whether administration of Boostrix to subjects with an unstable or progressive neurologic disorder might hasten manifestations of the disorder or affect the prognosis. Administration of Boostrix to subjects with an unstable or progressive neurologic disorder may result in diagnostic confusion between manifestations of the underlying illness and possible adverse effects of vaccination.

Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine usually have a high serum tetanus antitoxin level and should not receive Boostrix or other tetanus toxoid-containing vaccines unless at least 10 years have elapsed since the last dose of tetanus toxoid-containing vaccine.

As with any vaccine, if administered to immunosuppressed individuals, including individuals receiving immunosuppressive therapy, the expected immune response may not be obtained.

Prior to administration, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions to allow an assessment of benefits and risks. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

1.3.6 Known Potential Benefits

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

2 Study Objectives and Endpoints

2.1 Primary Objectives

2.1.1 Primary Immunogenicity Objective

- To assess nasal mucosal secretion immune response (secretory Ig A [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 lymphocytes + T cell, CD8 T lymphocytes + 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 immunological responses across time, relative to baseline status and relative to vaccination response, with the current assays and with any future assays developed for BPZE1.

2.4 Primary Endpoints

2.4.1 Primary Immunogenicity Endpoints

The primary immunogenicity endpoints of this study are:

- **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 nasal mucosal secretion (eg, IgA). To be further defined during assay development and within the statistical analysis plan.

Mucosal baseline samples will be taken at the screening visit.

2.4.2 Primary Safety Endpoints

The primary safety endpoints of this study are:

- Solicited AEs (local, nasal/respiratory, 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.

2.5 Secondary Endpoints

2.5.1 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, or 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 detection). 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 will be 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 aP antigens PT, FHA, and PRN over baseline:
  - On **either** Day 29 (prime) or 113 (boost)
  - On **both** Days 29 (prime) or 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.

2.5.2 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 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.

2.5.3 Secondary Colonization Endpoints

- Proportion of subjects with positive *B. pertussis* by bacterial culture of nasal sample collected on each day and on any day (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).

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

2.6 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 PBMC 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 nasal 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 Investigational Plan

3.1 Study Design

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, 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 examination for reactogenicity (local, nasal/respiratory, and systemic) with 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.

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.

Blood or blood cell samples may be stored frozen by ILiAD or companies working for ILiAD for up to 10 years.

3.1.1 Rationale of Study Design

In order to advance BPZE1 into later stage licensure trials, demonstration of an adequate immune response (mucosal and/or serum immunity) is necessary. As BPZE1 is a mucosal immunogen first and foremost, the IgA response is likely to be of key importance. Since regulators are accustomed to seeing the IgG response and IgG is known to be important for long-term protection against disease, this parameter will also be assessed. In addition, demonstrating boosting and persistence of the immune response with BPZE1 is critical. As aP vaccines are given as boosters to the adult population, describing the immune response to BPZE1 following aP vaccination is of interest. Understanding these responses relative to subject's baseline mucosal and serum immunity is of interest. The common pertussis antigens found in acellular products (PT, PRN, FHA, and FIM 2/3) will be measured in serum samples (IgG and IgA) and a specific whole cell pertussis ELISA (based on BPZE1) will be measured in mucosal (S-IgA) and serum (IgG). As the concentration of individual

antigens is vastly reduced in BPZE1 compared to aP vaccines, it is not anticipated that BPZE1 will have the same magnitude of immune response to the antigens found in acellular products. However, demonstration of a measurable response akin to that seen with whole cell pertussis vaccines is anticipated and a measurable response to a whole cell pertussis ELISA provides additional immunological evidence. Development of assays for additional markers of immunity may be needed.

Nasal vaccination with BPZE1 has been shown to induce temporary nasal colonization followed by clearance, which is thought to be the result of development of mucosal immunity. The level of colonization/clearance following a 2-course vaccination series is of interest using this higher dose of BPZE1 and it is anticipated that prior vaccination with BPZE1 will result in rapid mucosal immune response to a second vaccination (boosting) and may avert/reduce colonization at the time of boosting. It is anticipated that prior vaccination with aP will not induce the same protection against colonization as compared to prior vaccination with BPZE1 and therefore a greater proportion of subjects will become colonized following boosting with BPZE1. Prior immunization with aP may result in a boosted serum immune response (IgG) to the pertussis antigens found in Boostrix when subjects then receive BPZE1. Whether prior aP immunization can also enhance the serum IgA response to these pertussis antigens following BPZE1 vaccination is uncertain. Immunization with BPZE1 should result in a measurable whole cell pertussis antibody response (IgG and IgA). A second vaccination with BPZE1 is expected to boost this immunological response. However, it is not anticipated that aP would induce a measurable whole cell pertussis response.

4 Subject Selection and Withdrawal Criteria

4.1 Selection of Study Population

Approximately 300 subjects will be enrolled at 3 to 5 clinical sites in the United States. Subjects will be assigned to study vaccine only if they meet all the inclusion criteria and none of the exclusion criteria.

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

4.1.1 Inclusion Criteria

Each subject must meet all the following criteria to be enrolled in this study:

1. Is a male or nonpregnant female 18 to 50 years of age, inclusive, on Day 1 (primary vaccination).
2. Is capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with protocol requirements.
3. Female subjects must be nonpregnant and nonlactating and meet 1 of the following criteria:
   a) Postmenopausal (defined as 12 consecutive months with no menses without an alternative medical cause or documented plasma follicle-stimulating hormone level in the postmenopausal range);
   b) Surgically sterile (ie, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy).

   NOTE: These procedures and laboratory test results must be confirmed by physical examination, or by subject recall of specific data and hospital/facility of procedure, or by medical documentation of said procedure.

   c) Is of childbearing potential (defined as any female who has experienced menarche and who is NOT permanently sterile or postmenopausal), agrees to be heterosexually inactive from at least 21 days prior to enrollment and through

3 months after the boosting vaccination or agrees to consistently use any of the following methods of contraception from at least 21 days prior to enrollment and through 3 months after the boosting vaccination:

i. Condoms (male or female) with spermicide
ii. Diaphragm with spermicide
iii. Cervical cap with spermicide
iv. Intrauterine device
v. Oral or patch contraceptives
vi. Norplant®, Depo-Provera®, or other FDA-approved contraceptive method that is designed to protect against pregnancy.

NOTE: Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

4. Has a stable health status as assessed by the investigator, as established by physical examination, vital sign measurements, and medical history.
5. Has access to a consistent and reliable means of telephone contact, which may be in the home, workplace, or by personal mobile electronic device.
6. Is able to understand and comply with planned study procedures.
7. Lives a reasonable distance from the clinical site to be able to travel to and from the clinical site for follow-up visits and agrees to go to the clinical site for evaluation (or provide medical record access if evaluated elsewhere) in the event of an AE.
8. Agrees to stay in contact with the clinical site for the duration of the study, has no current plans to move from the study area, and provides updated contact information as necessary.

4.1.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. History of being vaccinated in the past 5 years against pertussis (subject recall).
2. Any significant past reaction to any component of Boostrix (at the discretion of the investigator).

3. Subject reported diagnosis of pertussis in the past 10 years (must be laboratory confirmed or physician diagnosed from medical records).
4. Vital signs by toxicity scoring >1 (may be repeated once during the screening period to allow for inclusion and the most recent measurement taken at baseline).
5. Chronic illness being treated actively and with evidence of recent intervention for worsening or fluctuating symptoms (at the discretion of the investigator).
6. The subject has a history of active cancer (malignancy) in the last 10 years (exception is subjects with adequately treated non-melanomatous skin carcinoma, who may participate in the study).
7. Current use of any smoking products and unwillingness to refrain from the use of any smoking products from screening through 28 days after the boosting vaccination.
8. Use of narcotic drugs, evidenced by urine toxicology screen or a history of drug/alcohol abuse within the past 2 years.
9. Has donated blood or suffered from blood loss of more than 450 mL (1 unit of blood) within 60 days prior to screening, or donated plasma within 14 days prior to screening.
10. Receipt of immunoglobulin, blood-derived products, systemic corticosteroids, or other immunosuppressant drugs within 90 days prior to Day 1.
11. Asthma*, obstructive nasal canal, recurrent or acute sinusitis or other chronic respiratory problems inclusive of the diagnosis of any significant pulmonary disease. *Asthma as diagnosed with spirometry showing reversibility of disease and must meet at least Step 1 classification with current prescription/use of medications to control symptoms (EPR-3 2007).
12. History of nasal surgery or Bell’s palsy.
13. Use of repeated nasal sprays, Neti pot, or nasal washing within the past 1 month (more than 2 times per week). Subjects must agree to refrain from use of any of these modalities through Day 113.

14. A temporary exclusion to vaccinate if acute respiratory tract infection or rhinorrhea or temperature >100.4°F (no symptoms for 3 days prior to Vaccination Day). Subjects may be vaccinated if they stay within the vaccination window (screening [30 days] or at the time of the booster [10 days].

NOTE: If a subject exceeds the screening window, they must be reconsented and screening must be reinitiated.

15. Use of corticosteroids in the respiratory tract (eg, nasal steroids, inhaled steroids) within 30 days prior to Day 1.
16. Receipt of a licensed vaccine within the last 30 days prior to Day 1 or planned vaccination during the active study conduct through Day 113. In the case of seasonal influenza, vaccination should not be withheld and is not contraindicated for subject participation. However, vaccination should be planned outside of a 30-day pre- and 30-day post-vaccination window whenever possible.
17. Known hypersensitivity to any component of the study vaccines.
18. Participation in any other clinical trial for the testing of an unlicensed product during the previous 6 months or planned during the study conduct.

NOTE: “Testing” is intended to mean receipt of an investigational product. Subjects in long term safety follow-up studies where vaccination has been completed greater than 6 months prior to this study enrollment are allowed.

19. Inability to adhere to the protocol, including plans to move from the area.
20. Personal history or family (first degree) history of congenital or hereditary immunodeficiency.
21. Past or present infection with human immunodeficiency virus, hepatitis B, or hepatitis C by screening test.
22. Any autoimmune or immunodeficiency disease/condition (inherited or iatrogenic).

NOTE: Stable endocrine disorders that have a confirmed autoimmune etiology (eg, thyroid, pancreatic) are allowed.

23. Any neurological disease or history of significant neurological disorder (eg, meningitis, seizures, multiple sclerosis, vasculitis, migraines*, Guillain-Barré syndrome [genetic/congenital or acquired]).*Significant neurological migraine includes frequent migraine (2 times a month or greater), migraine with aura or migraine with complications (status migraninosus, persist aura without infarction, infarction or aura triggered seizure defined by International Classification of Headache Disorders-3 [ICHD-3 2018]).
24. Any medical condition that, in the opinion of the investigator, might interfere with the evaluation of the study objectives or might affect the safety of the individual, eg, major depression or history of suicidal attempt.
25. Toxicity grading >1 for screening laboratory test results for specific kidney, hepatic, and hematologic values for all subjects and in screening laboratory values for the safety cohort as indicated in Table 13-2 (may be repeated once during the screening period to allow for inclusion and the most recent measurement taken at baseline). See Table 13-2 for specifically designed parameters.
26. Body mass index <17 kg/m^2 or >40 kg/m^2.
27. Frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc.) or residence in the same household as persons with known immunodeficiency including persons on immunosuppressant therapy.
28. Study team member or first-degree relative of study team member.

4.2 Withdrawal of Subjects From Study Treatment and/or the Study

The duration of the study is defined for each subject as the date signed written informed consent is provided through the last follow-up visit on Day 254.

4.2.1 Reasons for Withdrawal/Discontinuation

Subjects may withdraw or discontinue from the study at any time and for any reason without prejudice to their future medical care by the investigator or at the study center. Every effort should be made to keep subjects in the study. The reasons for subjects not completing the study will be recorded. A subject may be withdrawn from the study or study treatment (ie, no longer receive study vaccine) for any of the following reasons:

1. Failure to continue to meet the protocol inclusion or exclusion criteria.
2. Noncompliance with the protocol.
3. Serious or intolerable AE(s) that in the investigator's opinion requires withdrawal or discontinuation from the study.
4. The subject has laboratory safety results that reveal clinically significant hematological or biochemical changes from the baseline values.
5. The subject has symptoms or an intercurrent illness not consistent with the protocol requirements or that justify withdrawal or discontinuation.
6. The subject is lost to follow-up.
7. Other reasons (eg, development of contraindications of use of study drug, use of disallowed substances).
8. If pregnancy occurs further vaccination will be discontinued.
9. The subject withdraws consent or the investigator or ILiAD Biotechnologies decides to discontinue the subject's participation in the study.

The investigator will also withdraw a subject if ILiAD Biotechnologies terminates the study. Upon occurrence of a serious or intolerable AE, the investigator will confer with the sponsor. If a subject is discontinued because of an AE, the event will be followed until it is resolved. Any subject may withdraw his or her consent at any time.

4.2.2 Handling of Withdrawals

Subjects are free to withdraw from the study or study treatment at any time upon request. Subject participation in the study may be stopped at any time at the discretion of the investigator or at ILiAD Biotechnologies request.

Subjects who discontinue study treatment or active participation in the study will no longer receive study vaccination but will continue to be followed and are not considered withdrawn. When a subject withdraws from the study, the reason(s) for withdrawal shall be recorded by the investigator on the relevant page in the electronic case report form (eCRF).

All subjects who discontinue from study treatment prematurely will follow the same visit schedule as other subjects without further vaccination.

Whenever possible, all subjects who discontinue from the study prematurely will stay in the study for safety reasons and will follow the schedule of events (Table 13-1) if they have received vaccination. Subjects who fail to return for final assessments will be contacted by the clinical site in an attempt to have them comply with the protocol. A subject will not be considered lost to follow-up until every attempt to contact the subject has been made, at minimum 2 (documented) phone calls, followed by a registered letter. Every effort should be made to collect safety data on subjects through the end-of-study visit.

It is vital to obtain follow-up data on any subject withdrawn from the study or study vaccine (ie, no longer receiving study vaccination) because of an AE or serious AE (SAE). In every case, efforts must be made to undertake protocol-specified safety follow-up procedures.

4.2.3 Replacements

Subjects who withdraw, are withdrawn or terminated from this study, or are lost to follow-up after signing the ICF but before receipt of any study vaccine may be replaced. Subjects who receive the study vaccine and subsequently withdraw, are discontinued from receiving further vaccination, are terminated from the study, or are lost to follow-up will not be replaced.

5 Study Treatments

5.1 Method of Assigning Subjects to Treatment Groups

Subjects will be randomly assigned to 1 of 4 treatment arms, in a 2:2:1:1 ratio, as presented in Table 3-1. An interactive response technology will be used to administer the randomization schedule centrally. Biostatistics will generate the randomization schedule using SAS® software Version 9.3 or later (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.

5.2 Treatments Administered

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."

5.3 Identity of Investigational Product

The BPZE1 investigational vaccine is for intranasal administration and is an off-white lyophilized cake that contains genetically modified, live *B. pertussis* strain BPZE1 bacteria in lyophilization buffer.

ILiAD Biotechnologies will provide the CRO with the investigational vaccine and lyophilized buffer and the CRO will ensure proper labelling and distribution to the clinical sites.

The following supplies will be used for vaccination in the study:

Product	Dosage and Route of Administration:
BPZE1	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) administered intranasally by syringe + VaxINator
aP (Boostrix vaccine; manufactured by GlaxoSmithKline)	0.5 mL administered by IM injection to the deltoid region as active control
Intranasal placebo	2 × 0.4 mL (0.4 mL per nostril) reconstituted lyophilized buffer administered intranasally by syringe + VaxINator
IM placebo	0.5 mL sterile normal saline USP administered by IM injection to the deltoid region

Abbreviations: aP, acellular pertussis; CFU, colony-forming units; IM, intramuscular; USP, United States Pharmacopeia.

The intranasal placebo consists of lyophilized buffer diluted with sterile WFI which, is the same constituents in the same quantities as the BPZE1 investigational vaccine, absent the attenuated *B. pertussis* cells. The IM placebo is sterile normal saline (eg, normal saline).

Each vaccination with BPZE1 or Boostrix will utilize a single vial/syringe of product for each single use (1:1 assignment from study drug accountability). See pharmacy manual for specifics on product preparation.

5.3.1 Study Drug Packaging and Storage

The primary packaging for BPZE1 is a sterile glass 2R DIN vial with a chlorobutyl lyophilization stopper. The vial closure system is a combination of the stopper and the aluminum cap. The vials are crimped directly after the lyophilization process using the automated Fill & Finish unit that filled the vials with 1.0 mL suspension. The intranasal placebo dose contains the lyophilized buffer (ie, the vaccine formulation without the BPZE1 attenuated bacteria) and packaged in similar fashion.

Sufficient supplies of 1 vial for each subject in the clinical trial will be provided, with approximately 30% spare vials, packaged in a box of 49 vials for the BPZE1 drug product and 41 vials per box for the placebo. The vials and the boxes will be labeled with the required information applicable to investigational vaccine or placebo product used in clinical trials. The boxes either contain the vaccine or the placebo (lyophilized buffer only).

The 18G (or larger) needles, WFI, and syringes capable of holding 1 mL WFI used to reconstitute the investigational vaccine or placebo (formulation buffer) vials will be stored separately from the investigational vaccine vials at ambient temperature.

Boostrix will be procured from commercial stock and supplied as either single dose vials or prefilled syringes containing a 0.5 mL suspension for injection. The IM placebo is sterile normal saline United States Pharmacopeia (from a commercial supplier) and is supplied in a similar fashion.

Vaccines and placebos must be stored in a secure area (eg, locked room or locked refrigerator), protected from light and moisture as required by manufacturer. Vaccine will be shipped from ILiAD's European contract manufacturing organization to the CRO's repository and distribution center and should be stored at –20°C or below prior to shipping to the clinical sites, where the vaccine will be stored at –10°C or below. Clinical sites will store the vaccine in order to maintain viability of BPZE1 prior to reconstitution with WFI. The exposure of reconstituted BPZE1 to room temperature prior to vaccination should not exceed

60 minutes. A detailed description regarding the storage, reconstitution, and handling the investigational vaccines will be in the Pharmacy Manual.

Boostrix should be stored at 2°C to 8°C.

5.3.2 Investigational Product Accountability

The investigator will maintain accurate records of receipt of all study vaccine and placebo vials, including dates of receipt. In addition, accurate records will be kept regarding when and how much study vaccine is dispensed and used by each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. At the completion of the study, to satisfy regulatory requirements regarding drug accountability, all study vaccine will be reconciled and retained or destroyed according to applicable regulations. No study vaccine will be destroyed until authorized in writing by the sponsor.

5.3.3 Other Supplies

Other clinical supplies that will be provided to the clinical sites for distribution to subjects include subject diaries, a measuring tool for measuring injection site erythema and swelling, and a thermometer for measuring body temperature.

A single VaxINator kit (sterile pouch containing a VaxINator atomization devise, a 1-mL single-use syringe, and other accessories not used in this protocol) is provided for each intranasal vaccination. The VaxINator boxes will be labeled with the required information applicable to a medical device accessory, for clinical trial investigational use only. The VaxINator is manufactured by Teleflex Medical. Teleflex has DMF #025388 for the VaxINator. The VaxINator is a conical shape plastic component that Luer-locks onto a 1-mL syringe. The VaxINator atomizes the liquid vaccine as it exits the syringe. The conical shape forms a plug in the nostril and high applied-pressure atomizes the liquid into a fine mist.

Kits for serum immune collection, mucosal immune collection, PBMCs and mucosal pertussis culture will all be provided and in sufficient quantity to allow for each subject sample collection. Bar coding will be applied to allow for sample tracking at the time of collection and throughout storage, transport, and testing.

Adequate supplies for immunization of individuals including, 18G (or larger) needles for injection, syringes, normal saline, nasal vaccinators, and blinding tape will be supplied. The

WFI will be provided for reconstituting the investigational vaccine and placebo vials for nasal immunization and will be stored separately from the investigational vaccine vials, at ambient temperature in a secure area.

5.4 Overdose Management

An overdose is any dose of study treatment given to a subject or taken by a subject that exceeds the dose described in the protocol. Any overdose, with or without associated AEs, must be promptly reported to the CRO's pharmacovigilance reporting center. In case of any AEs associated with the overdose, these should be reported on relevant AE/SAE sections in the eCRF. Any overdose should be recorded as a protocol violation and promptly reported to the sponsor. No overdoses are expected as the vaccine and adjuvant will be administered by clinical staff.

5.4.1 Treatment of Overdose

Treatment of overdose would include supportive and symptomatic care, according to the standards of care at the site. The investigator should promptly notify the medical monitor about the overdose and seek his/her input on the medical management, as needed.

5.5 Blinding

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.

The full database lock will occur on Day 113 and all data collected through Day 113 will be included in the 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 in an addendum to the CSR. 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 full 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.

5.5.1 Breaking the Blind Due to Medical Emergency

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 eCRF. The date on which the code was broken together with the identity of the person responsible must also be documented.

5.6 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. Any deviations from the dosing schedule outside the defined visit windows (Table 13-1) will be flagged in the clinical database.

5.7 Prior Vaccinations and Concomitant Therapy

Administration of medications, therapies, or vaccines will be recorded in the eCRF. Concomitant medications will include all medications (including vaccines) taken by the subject from the time of signing the ICF through 28 days after the boosting vaccination (or through the early termination visit if prior to that time). Intake will include whether there was knowledge of past pertussis vaccination and (when available) the year of receipt. Prescription and over-the-counter drugs will be included, as well as herbals, vitamins, and supplements. In addition, receipt of nonstudy vaccines will be solicited through approximately 28 days after the boosting vaccination and reported in the eCRF.

Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition. Medications that might interfere with the evaluation of the study vaccines should not be used during the study-reporting period unless clinically indicated as part of the subject's health care. New medications may indicate a new onset medical condition. As an example, the use of a bronchodilator medication due to wheezing (new prescription) will be used along with signs and symptoms to define a medically significant wheezing AE.

6 Study Assessments and Procedures

Before performing any study-related procedures, the investigator will explain the study to potential subjects and all potential subjects will be given an ICF to read and sign. Subjects will have the opportunity to have any questions answered before signing the ICF. The investigator must address all questions raised by the subject. The investigator or designee will also sign the ICF and a copy of the ICF will be given to the subjects. In addition, subjects will be asked to provide a signed informed consent for the use of samples for further pertussis-specific testing or assay development. The subjects will also be informed that a urine drug screen may be performed, at the discretion of the investigator, on any subject who experiences an AE. The schedule of events by study visit is shown in Table 13-1.

6.1 Demographic and Baseline Data

Demographic and baseline data such as date of birth (day, month, and year), sex, race, ethnicity, weight, height, and body mass index should be collected at screening and recorded in the subject's eCRF.

6.2 Medical History

A complete medical history (including prior and concomitant medical conditions, surgeries/significant procedures for medical conditions [eg, endoscopy, uterine ablation] history of past tobacco use) will be collected at screening. If the year of past pertussis vaccination is known by the subject this will be entered as part of the medical history (eg, Tdap booster).

6.2.1 Concomitant Medication

Concomitant medications include all known medications (including herbal supplements, multivitamins and over-the-counter medications) 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). Concomitant medications received associated with an SAE will be collected throughout the study period.

6.3 Safety Assessments

Safety assessments for all subjects will include the following: vital sign measurements, targeted symptom directed physical examination, clinical safety laboratory tests (screening for all subjects, safety cohort to have additional testing), reactogenicity (post-vaccination), AEs and the occurrence of SAEs. These assessments are detailed in Sections 6.3.1 through 6.3.6.

The first 48 subjects randomly assigned will be designated the safety lead-in cohort and will have additional safety laboratory testing performed on Days 8, 85 (prior to vaccination), and 92.

6.3.1 Vital Sign Measurements

Vital sign measurements will include oral temperature, pulse rate, and diastolic and systolic blood pressure (after subject is seated for at least 5 minutes). Subjects must not eat or drink anything hot or cold within 10 minutes prior to taking oral temperature. Vital signs will be toxicity graded per the FDA toxicity (Table 13-3).

Vital signs will be collected at screening and on Days 1 (before vaccine administration and 60 minutes [±15 minutes] after vaccine administration [before subject is discharged]), 8, 29, 85 (before vaccine administration and 60 minutes [±15 minutes] after vaccine administration [before subject is discharged]), 92, 96, and 113.

6.3.2 Post-vaccination Evaluation

On Days 1 and 85, subjects will be monitored for 60 minutes after vaccine administration. Details regarding collecting and reporting site-specific local and nasal/respiratory and systemic reactogenicity symptoms are presented in Section 6.3.5.3. Subject diaries will be distributed according to Table 13-1 and will be used to record site-specific local, nasal/respiratory, and systemic reactions. Solicited local, nasal/respiratory, and systemic reactogenicity will be graded by the investigator at the return visit (Days 8 and 92). 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.

In addition, a measuring tool for measuring injection site erythema and swelling and a thermometer for measuring body temperature will also be distributed.

After the subject has been monitored for 60 minutes after vaccination, the following evaluations will be performed:

1. Obtain vital sign measurements (oral temperature, pulse rate, and diastolic and systolic blood pressure) at 60 minutes (±15 minutes) after vaccination. Vital signs will be toxicity graded per the FDA toxicity (Table 13-3).
2. Complete an injection site examination for reactogenicity (local, nasal/respiratory, and systemic) and toxicity grade (see Section 6.3.5.3) at 60 minutes (±15 minutes) after vaccination.

6.3.3 Complete Physical Examination

A full physical examination will be completed at screening and will include the following: an assessment of 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 targeted or symptom-directed physical examination will be performed as mentioned in the schedule of events for all subjects on Days 1, 8, 29, 85, 92, 96, and 113 (Table 13-1). On Days 1 and 85, this physical examination is done before vaccination. For unscheduled visits, targeted symptom directed physical examinations will be performed at the discretion of the investigator, if necessary, to evaluate AEs or clinical laboratory abnormalities.

6.3.4 Clinical Safety Laboratory Tests

Clinical safety laboratory tests will include the following and will be collected as mentioned in the schedule of events for all subjects at screening and for the safety lead-in cohort at screening, and Days 8, 85 (prior to vaccination), and 92 (Table 13-1):

- Hematology: hemoglobin, white blood cell count and differential, and platelet count.
- Chemistry panel: sodium, potassium, glucose (random), blood urea nitrogen, creatinine, calcium, albumin, total protein, bilirubin, alanine aminotransferase, and aspartate aminotransferase

- Coagulation: Prothrombin time/international normalized ratio and partial thromboplastin time

For the full cohort, toxicity scoring of >1 for hemoglobin, white blood count, platelets, creatinine, alanine aminotransferase, aspartate aminotransferase, bilirubin, prothrombin time and partial thromboplastin time will be used as exclusionary criteria (Table 13-2). For the safety cohort all laboratory tests noted in Table 13-2 with toxicity score >1 will be used as exclusionary criteria. For the safety cohort being followed with additional scheduled safety labs, 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. In the case of an alternative explanation for the abnormal laboratory (eg, urinary tract infection with an elevated WBC) the illness should be the AE classification. Investigators should determine if any laboratory values resulting in a grade 2 toxicity score should be entered as an AE, with repeat testing based on clinical judgement and the laboratory value of concern.

6.3.4.1 Serology

Serology testing will be collected at screening and will include hepatitis B, hepatitis C, and human immunodeficiency virus.

6.3.4.2 Urine Drug Screen

A urine drug screen will be performed at screening and will include the following non-prescription drugs of abuse: opiates, cocaine, phencyclidine, amphetamines, benzodiazepines, and methadone.

At the discretion of the investigator a urine drug screen may be performed on any subject who experiences an AE.

6.3.4.3 Urine Pregnancy Test

For female subjects of childbearing potential, a urine pregnancy test will be performed at screening and prior to vaccination on Days 1 and 85.

6.3.5 Adverse Events

6.3.5.1 Definitions of Adverse Events

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study vaccination or their clinical significance.

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to study vaccination. Subjects will be instructed to contact the investigator at any time after randomization if any symptoms develop.

6.3.5.2 Serious Adverse Events

An SAE is defined as any event that

- results in death
- is immediately life threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect

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

At the discretion of the investigator, a urine drug screen may be performed on any subject who experiences an SAE.

6.3.5.3 Site-Specific and General Systemic Reactogenicity Symptoms

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 (after each

vaccination, not counting vaccination day). Reactogenicity events are common and known to occur following administration of these types of vaccinations. 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 the investigator will apply a standard toxicology grading at the subsequent visit (Table 6-1). For the boosting vaccination, nasal/respiratory, general systemic reactogenicity reactions, and other unsolicited symptoms/complaints, including start and stop dates will be recorded and then toxicity graded by the investigator (Table 6-1). The clinical staff will review the information from the subject diary with the subjects on Days 8 and 92. The subject diary will be collected from the subjects and stored as a source document. 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. The following toxicity grading scales will be used to grade solicited local, nasal/respiratory, and systemic reactions:

Table 6-1 Toxicity Grading Scales

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

Nasal/Respiratory Reactogenicity Grading			
Local Reaction	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
			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

6.3.5.4 Assessing and Documenting Adverse Events

Safety will be assessed by the frequency and severity of:

1. Serious AEs occurring from the time of vaccination through the end of the study.

2. Solicited reactogenicity – reactogenicity events occurring from the time of each study vaccination through 7 days after each study vaccination:

    a. Nasal/respiratory reactions including runny nose, stuffy nose/congestion, nasal pain/irritation, epistaxis, sneezing, sinus pressure/pain, sore/irritated throat, cough, and shortness of breath/wheezing.

    b. Systemic reactions including fever, fatigue, malaise, myalgia, arthralgia, headache, and rash/hypersensitivity.

    c. Local reactions – pain, tenderness, erythema/redness, induration/swelling

3. Unsolicited AEs – all AEs regardless of causality, will be collected for 28 days after each vaccination. Adverse events related to vaccination will be collected through Day 113. Between screening (signing of ICF) and Day 1, AEs will be collected if classifies as serious or if related to study procedure or study involvement. Any AE that results in vaccination delay (eg, temporary exclusions due to acute illness or fever, or investigator determined medical condition) during screening or during study conduct will also be recorded as an AE. Any newly identified potential AE of special interest will be followed through Day 254.

4. Any scheduled safety laboratory value that results in a Grade 3 toxicity score in the safety cohort will be recorded as an AE. Recording will be appropriate for either the

variable itself (eg, elevated creatine) or the condition which is diagnosed (eg, dehydration or chronic renal insufficiency).

At every study visit, subjects will be asked a standard nonleading question to elicit any medically related changes in their well-being. They will also be asked if they have been hospitalized, had any accidents, used any new medications, or changed concomitant medication regimens (both prescription and over-the-counter medications).

In addition to subject observations, AEs identified from any study data (eg, laboratory values, physical examination findings) or identified from review of other documents (eg, subject diaries) that are relevant to subject safety will be documented on the AE page in the eCRF.

6.3.5.5 Reporting Adverse Events

All AEs reported or observed during the study will be recorded on the AE page in the eCRF.

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

Adverse events not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited nonserious AEs includes event description, date of onset, licensed study physician's assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness, and outcome. Adverse events occurring during the collection and reporting period will be documented appropriately regardless of relationship (as defined in Section 6.3.5.4).

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

Adverse events must be graded for severity and assessed for relationship to study product. Adverse events characterized as intermittent require documentation of onset and duration of

each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution. The MedDRA will be used to code all AEs.

6.3.5.6 Reporting Serious Adverse Events

Any AE that meets SAE criteria (Section 6.3.5.2) must be reported to the CRO immediately (ie, within 24 hours) after the time site personnel first learn about the event. The contact information to be used for SAE reporting will be provided to the CRO and the CRO will oversee the SAE reporting.

6.3.5.7 Suspected Unexpected Serious Adverse Reactions and Nonserious Adverse Events of Special Interest

The sponsor will promptly evaluate all SUSARs and nonserious AEs of special interest against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, institutional review boards (IRBs), and applicable health authorities based on applicable legislation.

To determine reporting requirements for single AE cases, the sponsor will assess the expectedness of these events using the following reference documents:

- study vaccination investigator's brochure

The sponsor will compare the severity of each SUSAR and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the sponsor as needed.

6.3.5.8 Assessment of Severity

The severity, or intensity, of an AE refers to the extent to which an AE affects the subject's daily activities. The intensity of the AE will be rated as mild, moderate, or severe using the following criteria:

Mild (Grade 1):	These events require minimal or no treatment and do not interfere with the subject's daily activities.
Moderate (Grade 2):	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 (Grade 3):	These events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

6.3.5.9 Assessment of Causality

The investigator's assessment of an AE's relationship to study vaccination is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

The relationship or association of the test article in causing or contributing to the AE will be characterized using the following classification and criteria:

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

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

6.3.5.10 Follow-up of Subjects Reporting Adverse Events

All AEs must be reported in detail on the appropriate page in the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, or until the subject is considered to be stable. Adverse events will be collected, assessed, and followed through resolution based on Section 6.3.5.4. Serious AEs will be collected, assessed, and followed through end of study or until satisfactory resolution or clinically stable.

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

If the investigator becomes aware of an acute febrile illness and the investigator decides to bring the subject in for an evaluation to determine etiology, then the investigator, at their own discretion, can determine the specific testing that should be performed. Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

6.3.6 Immunogenicity Assessments

At least 6 days prior to vaccination (during screening and on Day 78), and on Days 29, 113, 169, and 254 a mucosal nasal secretion collection will be taken using a nasal secretion collection device for testing of mucosal antibodies (S-IgA) to pertussis antigens. The mucosal nasal secretion collected at screening will only be tested if the subject is randomized into the study unless consent is provided to use the sample for assay development needs specific to this vaccine program.

On Days 78 (after a mucosal collection is completed), 92, 96, and 113 (after a mucosal collection is completed) a nasal secretion sample will be taken for pertussis culture. Any subject who tested positive for pertussis culture at Day 113 will also have a nasal secretion sample taken for pertussis culture on Day 254.

Blood samples will be taken on Days 1 (prior to primary vaccination), 29, 85 (prior to boosting vaccination), 113, 169, and 254 for testing of serum pertussis antibodies (IgG and IgA ELISA) to pertussis antigens.

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

The details on the handling, processing, and shipping of immunogenicity samples will be provided in the Laboratory Manual.

6.4 Halting Rules

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

- Any subject experiences ulceration, abscess, or necrosis in the nose that is considered to be related to study product administration, through 28 days following the boosting vaccination.
- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study vaccination that is considered to be related to study vaccination.
- Three or more subjects experience generalized urticaria grade 3 or higher within 3 days after administration of study vaccination that is considered to be related to study vaccination.
- Any subject experiences a study product-related SAE from the time of the primary vaccination through the subject's last study visit on Day 254.
- Any subject experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of Guillain-Barré syndrome) after administration of study vaccination, through 28 days following the boosting vaccination.

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

- 5% of subjects experience the same severe study product-related subjective systemic, local, or mucosal reaction, for which the severity (grade) is corroborated by study personnel.

Grading scales for solicited reactions are included in Section 6.3.5.3.

If any of the halting rules are met following any subject receipt of study vaccination, then this study will not continue with the remaining enrollments or future vaccination without a review by and recommendation from the SMC to proceed. Vaccination windows will be adjusted for any such pause to allow subjects to remain within the expected schedule of events. All events that classify for halting rules will be entered as adverse events.

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

6.5 Pregnancy

Pregnancy is not regarded as an AE unless there is a suspicion that an investigational vaccine may have interfered with the effectiveness of a contraceptive medication. Any pregnancy that occurs during study participation must be reported using a clinical study pregnancy form. To ensure subject safety, each pregnancy must be reported to the sponsor, ILiAD Biotechnologies, within 2 weeks of learning of its occurrence. If pregnancy occurs further vaccination will be discontinued. The pregnancy must be followed-up to determine outcome (including spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) and status of mother and child, even if the subject was discontinued from the study. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous miscarriages must be reported as an SAE.

Any pregnancy, brought to the investigator's attention after the subject has completed the study, but occurring while the subject was in the study, must be promptly reported to ILiAD Biotechnologies.

6.6 Laboratory Analyses

Any abnormal laboratory test results (hematology, chemistry, or coagulation) resulting in a grade 3 or greater toxicity score which is associated with the scheduled safety cohort follow up testing must be entered as an adverse event and followed with retesting and observing for resolution. If an underlying disease or new condition is identified that accounts for the elevation than this should be documented as the adverse event.

Any clinically significant safety assessments that are associated with the underlying or new onset condition for which laboratory testing occurs during the course of the study will not be

entered as adverse events per se, rather the new onset or worsening condition should be entered as judged by the investigator.

6.7 Sample Collections

Procedures for the handling and processing of biological samples are provided in the Laboratory Manual or the Site Manual.

The hematology, coagulation, and chemistry laboratory analyses will be performed at a central laboratory. Laboratory data will be graded according to the FDA Toxicity Grading Scale (Table 13-2) and will be adjusted according to local laboratory reference ranges.

The samples collected for immunogenicity (B and T cell) testing and pertussis culture will be stored in appropriate conditions as specified in the Laboratory Manual until shipped for testing at a facility designated by the sponsor.

Subjects will be asked to provide a signed informed consent for the use of samples for further pertussis-specific testing or assay development. Aliquots of collected samples from this study may be retained for additional testing of antigens specific to *B. pertussis* for a maximum of 10 years (starting from the date at which the last subject had the last study visit), unless local rules, regulations, or guidelines require different timeframes or different procedures, in accord with subject consent. Testing for colonization using *B. pertussis* culture following the primary vaccination and post-boosting vaccination will also occur.

7 Statistical and Analytical Plan

The primary objectives of this study are to assess nasal mucosal immune response S-IgA following intranasal vaccination with BPZE1 (10^9 CFU) when used as a single (eg, prime) or 2-dose (eg, prime + boost) series and reactogenicity (all) and specific safety laboratory parameters (safety lead-in cohort only) following intranasal vaccination with BPZE1 (10^9 CFU), in healthy volunteers 18 to 50 years of age. This study is designed as a randomized, placebo-controlled, observer-blinded clinical trial evaluating the safety and immunogenicity of 1 or 2 doses of 10^9 CFU of BPZE1 delivered by intranasal VaxINator device or Boostrix delivered by IM injection. To maintain the blind, placebo vaccination via IM and intranasal routes will be included.

The primary database lock will occur on Day 113 and all data collected through Day 113 will be included in the 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 in an addendum to the CSR. 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 primary database lock at 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.

This Phase 2b study is not designed to test a formal null hypothesis. Rather, it is intended to obtain preliminary assessment of the safety and immunogenicity induced by the BPZE1 lyophilized vaccine in healthy adults.

7.1 Primary Immunogenicity and Safety Endpoints

Immunogenicity:

- **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 nasal mucosal secretion (eg, IgA). To be further defined during assay development and within the statistical analysis plan.

Mucosal baseline samples will be taken at the screening visit.

Safety:

- Solicited AEs (local, nasal/respiratory, 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 FDA toxicity score (change from baseline or absolute toxicity score as defined in Table 13-2) 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.

7.2 Secondary Immunogenicity and Safety Endpoints

Systemic Immunogenicity (serum sampling [expressed separately for IgG, IgA, and IgG or IgA ELISA when possible):

- 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, or 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 detection). 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 will be 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 aP antigens PT, FHA, and PRN over baseline:
  - On **either** Day 29 (prime) or 113 (boost)
  - On **both** Days 29 (prime) or 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 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 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.

Mucosal Immunogenicity S-IgA ELISA (nasal mucosal secretion sampling):

- 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 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.

Colonization:

- Proportion of subjects with positive *B. pertussis* by bacterial culture of nasal sample collected on each day and on any day (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).

Safety:

- Unsolicited AEs (eg, treatment-emergent AEs, SAEs, and SUSARs) collected for 28 days following each vaccination by MedDRA classification and severity score.

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

7.3 Exploratory Endpoints

- Cell-mediated (eg, B cell, CD4 T lymphocytes + T cell, CD8 T lymphocytes + T cell) responses (eg, cell staining, cytokine production) following stimulation of 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 nasal 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.

7.4 Sample Size Calculations

A total of 300 subjects will be globally assigned at 2:2:1:1 allocation ratio and receive BPZE1 intranasal administration with placebo IM or Boostrix IM with placebo intranasal administration on Day 1 and BPZE1 or placebo intranasal administration on Day 85 (See Section 5.2). 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 7-1 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 7-1 95% 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.

7.5 Analysis Sets

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 actually received. The primary safety analysis will be done on this 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.

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 subject 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.

The Per Protocol Analysis Set will include all subjects in the Prime/Boost 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.

7.6 Description of Subgroups to be Analyzed

Immunogenicity endpoints (seroconversion, GMT, and GMFR) will be summarized by baseline negative and positive status of various pertussis antibodies (eg, any subject positive for PT, PRN, FHA, or FIM 2/3). Other subgroup analyses may be performed for selected factors, such as pertussis vaccination history, smoking status, or nasal spray use at study baseline or during the study.

7.7 Statistical Analysis Methodology

Statistical analysis will be performed using SAS software Version 9.3 or later. 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 Clopper-Pearson method. Graphics of reverse cumulative distribution of anti-pertussis antibody titers will be generated for Days 29 and 113.

Where applicable, data analyses will be conducted for the prime period (prior to boosting) and boost period (at or post-boosting).

Data will be listed in data listings.

Further details of the planned statistical analyses, methods, and data conventions are described in the statistical analysis plan.

No formal hypothesis will be tested in this study.

7.7.1 Analysis of Primary Immunogenicity Endpoint

Summaries and analysis of immunogenicity data will be presented for the Immunogenicity and Per Protocol Analysis Set. For immunogenicity data in the prime period, subjects in the Prime Immunogenicity Analysis Set will be grouped into BPZE1 prime (A+B) and Boostrix prime (C+D) arms. For immunogenicity data in the boost period, subjects in the Boost Immunogenicity Analysis Set will be grouped to 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). In addition, as multiple antigens are being tested across vaccine combinations, all possible treatment group combinations will be displayed. For priming data analysis, the primary endpoint will be based on nasal mucosal immunogenicity change from screening, and for boost data analysis that will be based on screening and Day 78 prior to boost vaccination. The Immunogenicity and Per Protocol Analysis Sets will be used for sensitivity analysis.

The proportion and 95% CI of subjects who achieve seroconversion at Day 29 for prime vaccination and Day 113 for boost vaccination will be analyzed. Seroconversion is defined as a 2-fold increase over the baseline value or a 4-fold increase over the minimal limit of assay detection in S-IgA ELISA from nasal secretions for any of the pertussis-specific antibodies (S-IgA ELISA): PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract). Baseline is defined as the value at screening.

7.7.2 Analysis of Primary Safety Endpoint

All summaries and analyses will be presented over all subjects and by treatment arm for prime and boost periods whenever possible. The treatment arms are BPZE1 versus Boostrix in prime period. For boost period, treatment arms are 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). The combination of A + C (received BPZE1 on Day 85) and B + D (did not receive BPZE1 on Day 85) will be combined when assessing short term reactogenicity and AEs through Day 28 post-boost.

Solicited AEs will be summarized by severity for each day after each study vaccination for 7 days and as the maximum severity over all 7 days after each vaccination. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) to summarize the proportion (with exact 95% CIs) of subjects reporting each symptom, any local, mucosal, or general systemic symptom. Summaries of solicited AEs will be presented separately by treatment arm for the prime period and the boost period when applicable as mentioned in the previous paragraph. Should any reactogenicity event extend beyond 7 day post-vaccination and be clinically significant by toxicity grade of 1 or greater, than the event will be entered as an AE with the same start date as the reactogenicity event and followed to resolution.

Clinical safety laboratory test results for the safety lead-in cohort will be classified per FDA toxicity scoring system (Table 13-2) based on test value and change from baseline when applicable (as defined in Table 13-2). Counts and percentages of subjects by toxicity classifications and classification shift from baseline will be presented in tabulations by treatment arm. The full cohort will have baseline safety values expressed as mean/median with standard error per treatment group(s).

7.7.3 Analysis of Secondary Immunogenicity Endpoint

The secondary nasal **mucosal** immunogenicity endpoints will be summarized by treatment arm. Analyses will include the GMTs along with corresponding 95% CIs and GMFRs from baseline at each time point and maximum titer or ratio at any time point, as well as the proportion of subjects with seroconversion, defined as a 2-fold increase in S-IgA over baseline or 4-fold over the minimal limit of assay detection at any time point and at each time point. The baseline refers to screening and Day 78 prior to boost vaccination.

Systemic immune responses in terms of seroconversion of IgG and IgA antibody titers (PT, FHA, PRN, FIM 2/3, or BPZE1 whole cell extract for IgG only) will be summarized by treatment arm. 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, as well as the proportion and 95% CI of subjects achieving seroconversion (defined as 2-fold increase over baseline or 4-fold increase over the minimum limit of detection) at any time point and at each time point.

Colonization data will be summarized by treatment arm at available time points. Analysis will include dichotomized results (Yes/No) for *B. pertussis* culture along with colony counts from Days 92 to 113 following vaccination with BPZE1 or placebo. The effect of recent and post vaccination with BPZE1 on absolute colony counts and clearance of colonization will be assessed for this period. The analyses will include proportion of subjects with positive *B. pertussis* at Day 78 (prior to boosting vaccination) as well as Day 254 (end-of-study).

7.7.4 Analyses of Efficacy Endpoint

No clinical efficacy endpoint will be collected in this study other than immunological response.

7.7.5 Analysis of Secondary Safety Endpoints

Adverse events (eg, treatment-emergent AEs, SAEs, and SUSARs) will be coded by MedDRA for preferred term and system organ class.

Serious AEs will be reported by detailed listings showing the event description, MedDRA preferred term and system organ class, relevant dates (study vaccinations and AEs), severity, relatedness, and outcome for each event. Any SAE attributed to vaccination will be classified as a SUSAR. Nonserious unsolicited AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA preferred term and system organ class, and cross tabulated by severity and relationship to study product.

Measurements of vital signs will be statistically described, and categorical severity scores of vital signs will be summarized in counts and percentages of subjects by treatment arm. Change from baseline will be expressed where applicable.

7.7.6 Other Analyses

Summary statistics will be provided for exploratory endpoints, demographics, medical history, study dose compliance, physical examination, social history, and risk factor variables at baseline. Exploratory analysis will include testing of T-cell responses using stimulated PBMCs for the subset of subjects at a later time point. Tetanus and diphtheria testing (per validated ELISA) may occur at a later date and may include only selected subsets. Future assays developed for BPZE1 may also utilize samples from this study for either development or testing at a future time.

7.7.7 Interim Analyses

A SMC will be convened by ILiAD Biotechnologies to review study progress and participant, clinical, safety, and reactogenicity data, as described after all doses in the safety lead-in cohort complete Day 8 following first (prime) vaccination to allow the remainder of the subjects to be randomized. The SMC safety review must include at minimum demographic information, clinical laboratory values (with toxicity grading), dosing compliance, reactogenicity (with toxicity grading), and AE/SAEs (graded and attributed). Additional data may be requested by the SMC, and an interim statistical report may be generated as deemed necessary and appropriate by ILiAD Biotechnologies. The SMC may receive data in aggregate and presented by treatment arm, including expected and observed rates of the expected AEs. The SMC will review grouped data in the closed session only. The SMC will meet and review these data at scheduled time points or ad hoc as needed during this trial 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. The SMC will be provided with an executive summary of safety and immunogenicity results (graphic and written) for the overall study.

8 Data Quality Assurance

The sponsor will perform quality control and assurance checks on all clinical studies that it sponsors. Before enrolling any subjects in this study, sponsor personnel and the investigator will review the protocol, the package inserts, investigator brochures, the eCRFs and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs. A qualified representative of the sponsor will monitor the conduct of the study by visiting the site and by contacting the site by telephone and e-mail. During these site visits, information recorded in the eCRFs will be verified against source documents.

8.1 Data Management

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include laboratory reports and subject diaries for reactogenicity reporting.

Study site staff will enter subject data into a FDA-approved and auditable data management system. The analysis data populations will be a combination of these data and data from other sources (eg, laboratory data).

Clinical data management will be performed in accordance with applicable PPD and/or ILiAD standards and data cleaning procedures to ensure the integrity of the data, eg, removing errors and inconsistencies in the data. Adverse event terms (including SAEs) will be coded using the MedDRA, an internal validated medical dictionary, and concomitant medications will be coded using the WHO Drug Dictionary.

After final database lock, each clinical site will receive a compact disc read-only memory (CDROM) containing all of their site-specific eCRF data as entered into an FDA-approved and auditable data management system for the study, including full discrepancy and audit history. Additionally, a CDROM copy of all of the study site’s data from the study will be created and sent to the sponsor for storage. The CRO will maintain a duplicate CDROM copy for their records. In all cases, subject initials will not be collected or transmitted to the sponsor.

9 Ethics

9.1 Institutional Review Board

Federal regulations and the International Council for Harmonisation (ICH) guidelines require that approval be obtained from an IRB before participation of human subjects in research studies. Before study onset, the protocol, informed consent, advertisements to be used for the recruitment of study subjects, and any other written information regarding this study to be provided to the subject or the subject's legal guardian must be approved by the IRB. Documentation of all IRB approvals and of the IRB compliance with ICH harmonised tripartite guideline E6(R2): Good Clinical Practice (GCP) will be maintained by the site and will be available for review by the sponsor or its designee.

All IRB approvals should be signed by the IRB chairman or designee and must identify the IRB name and address, the clinical protocol by title or protocol number or both, and the date approval or a favorable opinion was granted.

The investigator is responsible for providing written summaries of the progress and status of the study at intervals not exceeding 1 year or otherwise specified by the IRB. The investigator must promptly supply the sponsor or its designee, the IRB, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to subjects.

9.2 Ethical Conduct of the Study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki, ICH GCP, the protocol, and all applicable regulations.

9.3 Subject Information and Consent

A written informed consent in compliance with regulatory authority regulations US Title 21 Code of Federal Regulations (CFR) Part 50 shall be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An informed consent template may be provided by the sponsor to investigative sites. If any institution-specific modifications to study-related procedures are proposed or made by the site, the consent should be reviewed by the sponsor or its designee or both before IRB submission. Once reviewed, the consent will be submitted by the investigator to

his or her IRB for review and approval before the start of the study. If the ICF is revised during the course of the study, all active participating subjects must sign the revised form.

Before recruitment and enrollment, each prospective subject or his or her legal guardian will be given a full explanation of the study and be allowed to read the approved ICF. Once the investigator is assured that the subject/legal guardian understands the implications of participating in the study, the subject/legal guardian will be asked to give consent to participate in the study by signing the ICF.

The investigator shall retain the signed original ICF and give a copy of the signed original form to the subject or legal guardian.

Subjects will also be asked to provide a signed informed consent for the use of samples for further pertussis-specific testing or assay development.

10 Investigator's Obligations

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes will be reported to the IRB but will not result in protocol amendments.

10.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject (or the subject's legal guardian), except as necessary for monitoring and auditing by the sponsor, its designee, the FDA, or the IRB.

The investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

10.2 Financial Disclosure and Obligations

Investigators and subinvestigators are required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigator and subinvestigator must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Neither the sponsor nor the CRO is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, neither the sponsor nor the CRO is financially responsible for further treatment of the subject's disease.

10.3 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ICH E6(R2) 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- IRB approval
- Original investigator-signed investigator agreement page of the protocol
- Form FDA 1572, fully executed, and all updates on a new fully executed Form FDA 1572
- Current (within 2 years) curriculum vitae for the investigator and each subinvestigator listed on Form FDA 1572
- Financial disclosure information to allow the sponsor to submit complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigators must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.
- Executed clinical study agreement
- Documentation of Federal Wide Assurance number and expiration date
- IRB-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject or legal guardian
- Site delegation log
- Documentation of human subjects' protection training by all staff listed on the site delegation log
- Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with 42 CFR 493
- Centers for Laboratory Improvement Amendments waived on site urine pregnancy test

10.4 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH E6(R2) and 21 CFR 312. The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

10.5 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6(R2) and 21 CFR 312 and all applicable guidelines and regulations.

10.6 Adverse Events and Study Report Requirements

By participating in this study, the investigator agrees to submit reports of SAEs according to the time line and method outlined in the protocol. In addition, the investigator agrees to submit annual reports to the study site IRB as appropriate.

10.7 Investigator's Final Report

Upon completion of the study, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB with a summary of the study's outcome and the sponsor and regulatory authority(ies) with any reports required.

10.8 Records Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product, as described in 21 CFR 312.62. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10.9 Publications

After completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the sponsor will be responsible for these activities to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues. The sponsor has final approval authority over all such issues.

Data are the property of the sponsor and cannot be published without prior authorization from the sponsor, but data and publication thereof will not be unduly withheld.

11 Study Management

The administrative structure will include an unblinded biostatistics team (statisticians and programmers).

11.1 Monitoring

11.1.1 External Data Monitoring Committee

11.1.1.1 Safety Monitoring Committee

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

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

- 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 during this study, or as needed.

The SMC will operate under the rules of an ILiAD Biotechnologies-approved charter that will be approved at the organizational meeting of the SMC. At this time, each data element that the SMC needs to assess will be clearly defined. Procedures for SMC reviews/meetings will be defined in the charter. The SMC will review applicable data to include, but not limited to, study progress and participant, clinical, safety, and reactogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, and solicited and unsolicited AE/SAEs. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by ILiAD Biotechnologies. The SMC may receive data in

aggregate and presented by treatment arm. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion and may request the treatment assignment be unblinded for an individual subject if required for safety assessment.

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

ILiAD Biotechnologies or the SMC chair may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. The ILiAD Biotechnologies medical monitor is empowered to stop enrollment and study vaccinations if AEs that meet the halting criteria are reported. The ILiAD Biotechnologies/CRO assigned medical monitor will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during the study.

11.1.2 Monitoring of the Study

The clinical monitor, as a representative of the sponsor, has the obligation to follow the study closely. In doing so, the monitor will visit the investigator and study site at periodic intervals, in addition to maintaining necessary telephone and letter contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and personnel.

All aspects of the study will be carefully monitored, by the sponsor or its designee, for compliance with applicable government regulation with respect to current GCP and current standard operating procedures.

11.1.3 Inspection of Records

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspections by providing direct access to all study records.

In the event of an audit, the investigator agrees to allow the sponsor, representatives of the sponsor, or a regulatory agency (eg, FDA) access to all study records.

The investigator should promptly notify the sponsor and the CRO of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the sponsor.

11.2 Management of Protocol Amendments and Deviations

11.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the subject, must be reviewed and approved by the sponsor or its designee. Amendments to the protocol must be approved by the IRB before subjects can be enrolled into an amended protocol. Letters of amendment or clarification do not need to be approved by the IRB.

11.2.2 Protocol Deviations

The investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study subjects without prior IRB approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB for review and approval, to the sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the sponsor and the IRB and agreed to by the investigator. A significant deviation occurs when there is nonadherence to the protocol by the subject or investigator that results in a significant, additional risk to the subject. Significant deviations can include nonadherence to inclusion or exclusion criteria, or nonadherence to FDA regulations or ICH GCP guidelines, and will lead to the subject being withdrawn from the study (Section 4.2).

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Investigators will be notified in writing by the monitor of deviations. The

IRB should be notified of all significant protocol deviations in a timely manner, as defined by the IRB.

11.3 Study Termination

Although ILiAD Biotechnologies has every intention of completing the study, ILiAD Biotechnologies reserves the right to discontinue the study at any time for clinical or administrative reasons.

The end of the study is defined as the date on which the last subject completes Visit 9 (end-of-study visit) (Table 13-1).

11.4 Final Report

Whether the study is completed or prematurely terminated, the sponsor will ensure that the CSRs are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor will also ensure that the CSRs in marketing applications meet the standards of the ICH harmonised tripartite guideline E3: Structure and content of CSRs.

The primary database lock will occur at Day 113, and all data collected through Day 113 will be included in the 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.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the CSR. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results.

Upon completion of the CSR, the sponsor will provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate. The study results will be posted on publicly available clinical trial registers.

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13 Appendices

13.1 Appendix: Schedule of Events

Table 13-1 Schedule of Events

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]				

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**
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 visits/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 exclusions 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, and 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 3-1.

[p] All subjects will record reactogenicity in the daily subject diary starting on the same day of the prime (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 will be applied by investigator for all subject recorded reactogenicity on Days 1 through 8. 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.

[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. If a subject fails to return for Day 78 (Visit 4) than nasal sampling for colonization should occur on Day 85 (Visit 5) AFTER mucosal sampling has been performed but PRIOR to nasal vaccination (and there must be a 60 minute window between nasal aspiration and 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 to pertussis on Day 254 will be provided a short course of azithromycin. Should a subject be allergic to azithromycin, an appropriate antibiotic will be substituted that has effectiveness against *B. pertussis*.

[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 Appendix: FDA Table for Laboratory Grading

Table 13-2 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 Appendix: FDA Table for Vital Sign Grading

Table 13-3 Vital Sign Abnormality

Vital Signs[a]	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.