CLINICAL STUDY PROTOCOL

A Phase 2b, Multi-Center, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy School-Age Children to Assess the Immunological Response and Safety Profile of a Single Dose BPZE1 With and Without Co-Administration of Tetanus, Diphtheria, and Acellular Pertussis (Boostrix™)

IB-201P

Sponsor:	ILiAD Biotechnologies 4581 Weston Road, Suite 260 Weston, FL 33331
Sponsor Contacts:	[redacted]
Medical Monitor:	
Date of Amendment 6 (v7.0)	30 March 2023
Date of Previous Versions:	03 February 2021 (Version 1.0) 02 April 2021 (Version 2.0) 09 June 2021 (Version 3.0) 07 July 2021 (Version 4.0) 16 August 2021 (Version 5.0) 30 May 2022 (Version 6.0)

CONFIDENTIAL

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

Protocol Approval – Sponsor Signatory

Study Title	A Phase 2b, Multi-Center, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy School-Age Children to Assess the Immunological Response and Safety Profile of a Single Dose BPZE1 With and Without Co-Administration of Tetanus, Diphtheria, and Acellular Pertussis (Boostrix™)
Protocol Number	IB-201P
Protocol Date and Version	30 March 2023 (Amendment 6, v7.0)

Protocol accepted and approved by:

[redacted]

Chief Medical Officer

ILiAD Biotechnologies
4581 Weston Road, Suite 260
Weston, FL 33331

[redacted]

Protocol Approval – Principal/Coordinating Investigator

Study Title	A Phase 2b, Multi-Center, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy School-Age Children to Assess the Immunological Response and Safety Profile of a Single Dose BPZE1 With and Without Co-Administration of Tetanus, Diphtheria, and Acellular Pertussis (Boostrix™)
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Protocol accepted and approved by:

Principal/Coordinating Investigator

[redacted]

Protocol Approval – Lead Statistician

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Protocol accepted and approved by:

Lead Statistician

[redacted]

Declaration of Investigator

I have read and understood all sections of the protocol entitled "A Phase 2b, Multi-Center, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy School-Age Children to Assess the Immunological Response and Safety Profile of a Single Dose BPZE1 With and Without Co-Administration of Tetanus, Diphtheria, and Acellular Pertussis (Boostrix™)" and the accompanying investigator's brochure.

I agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the Original Protocol, the International Council for Harmonisation harmonised tripartite guideline E6 (current version): Good Clinical Practice and all applicable government regulations. I will not make changes to the protocol before consulting with ILiAD Biotechnologies or implement protocol changes without independent ethics committee approval except to eliminate an immediate risk to subjects. I agree to administer study treatment only to subjects under my personal supervision or the supervision of a sub-investigator.

I will not supply the investigational drug to any person not authorized to receive it. Confidentiality will be protected. Subject identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorization from ILiAD Biotechnologies.

Signature of Principal Investigator	Date

Printed Name of Principal Investigator

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Protocol Synopsis

Protocol Number:	IB-201P
Title:	A Phase 2b, Multi-Center, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy School-Age Children to Assess the Immunological Response and Safety Profile of a Single Dose BPZE1 With and Without Co-Administration of Tetanus, Diphtheria, and Acellular Pertussis (Boostrix™)
Sponsor:	ILiAD Biotechnologies 4581 Weston Road, Suite 260 Weston, FL 33331
Study Phase:	2b
Study Sites:	Approximately 30 centres in the United Kingdom, European/Commonwealth countries, and/or Costa Rica
Indication:	Pertussis booster in adults and school-age children
Rationale:	Acellular pertussis (aP) vaccines were introduced into most developed countries in the late 1990's and early 2000's, and for 2 decades, children in these countries have received only aP vaccinations. The resurgence of *Bordetella pertussis* (*B. pertussis*) in these countries, despite high vaccination rates, is hypothesized to be linked to the sole use of aP vaccines, which do not alter *B. pertussis* acquisition and have limited durability. 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 *B. pertussis* infections. The current pertussis vaccine strategies do not provide mucosal immunity or alter *B. pertussis* acquisition and therefore have not been successful in halting human-to-human transmission. As humans are the only known reservoir for *B. pertussis*, targeting colonization would provide a novel approach through mucosal-induced immunity to reduce the *B. pertussis* reservoir in the population and thereby reduce transmission. The timing of aP vaccine introduction has resulted in a school-age population who could benefit from a broader immune response achievable with a live attenuated pertussis vaccine such as BPZE1. The intranasally administered BPZE1 live attenuated vaccine provides an opportunity to generate a locally effective mucosal antibody response at the site of potential exposure, mimic the route of entry of the wild-type pathogen, and induce a broader immune response as measured by cellular, mucosal, and serum indices. Inducing mucosal

immunity with an attenuated live vaccine delivered through intranasal vaccination could halt progression from upper airway to lower airway disease in BPZE1-vaccinated individuals as well as strengthen herd immunity by reducing upper airway person-to-person transmission. The overall effect would be improved protection of even the most vulnerable from *B. pertussis* through a robust booster vaccination strategy with the best potential to disrupt *B. pertussis* epidemics.

Acellular pertussis vaccines are currently recommended as both primary and booster vaccinations and usually given in a combination vaccine containing diphtheria (D/d), tetanus (T), and pertussis (P/p) with the level of antigen content dictated by standard nomenclature of DTaP (higher antigen content overall) or Tdap (lower antigen content for diphtheria and pertussis). The current recommendation in the United Kingdom includes the childhood booster of Tdap at 3 years of age with no adolescent booster of aP-containing vaccine, whereas some other European/Commonwealth countries and the US have a similar early childhood DTaP vaccination schedule but have added a Tdap booster during the school-age period (up to and including adolescence). This study will investigate BPZE1-induced immune responses in a background of DTaP or Tdap, with the last pertussis-containing booster occurring more than 3 years prior. BPZE1 alone as well as coadministration with Boostrix will be investigated to test safety and whether augmenting/broadening mucosal immune responses are possible in an aP primed-only population. Furthermore, the testing of interference of BPZE1 with all components found in Boostrix will be investigated as a key secondary objective. In addition, demonstrating protection against subsequent colonization using the attenuated challenge model (re-vaccination/attenuated challenge with BPZE1) will be investigated at 3 months following initial vaccination (optional substudy) as a secondary objective. As this population has never received a whole cell pertussis (wP) vaccination, this will be the first time that a broader immunization against *B. pertussis* is provided; therefore, additional hypotheses include that a single intranasal vaccination with BPZE1 will be sufficient to induce a differential immune response compared to Boostrix (eg mucosal immunity induction and reduction in *B. pertussis* acquisition), and a differential cellular-mediated response will be investigated following vaccination with either BPZE1, BPZE1 + Boostrix, or Boostrix.

Objectives and Endpoints

Immunogenicity	
Primary objectives	**Primary endpoints**
• To demonstrate BPZE1 induction of broad pertussis mucosal secretory immunoglobulin A (S-IgA) immunity is measurable at Day 29 by geometric mean fold rise (GMFR) by antibodies to whole cell extract (WCE) (BPZE1, BPZE1 + Boostrix).	• GMFR of BPZE1 induction of mucosal S-IgA against WCE at Day 29 by treatment group (BPZE1, BPZE1 + Boostrix).
Safety	
Primary objective	**Primary endpoint**
• To assess reactogenicity by toxicity scoring through 7 days following first study vaccination with any combination of pertussis-containing vaccines (BPZE1, BPZE1 + Boostrix, Boostrix).	• Occurrence of solicited adverse events (AEs) including local, nasal/respiratory, and systemic reactogenicity events through 7 days following first study vaccination with any combination of pertussis-containing vaccines.
Immunogenicity	
Key secondary objectives	**Key secondary endpoints**
• To demonstrate non-interference of immunoglobulin G (IgG) serum responses for all Boostrix-containing antigens (diphtheria, tetanus, pertussis toxin [PT], filamentous hemagglutinin [FHA], pertactin [PRN]) at Day 29 when BPZE1 is co-administered with Boostrix (BPZE1 + Boostrix vs Boostrix).	• BPZE1 + Boostrix induction of serum IgG against diphtheria, tetanus, and all aP antigens (PT, FHA, PRN) compared with Boostrix at Day 29 (criteria to be assessed for diphtheria and tetanus responses per WHO guidelines).

Colonization (optional substudy only)	
Secondary objectives	**Secondary endpoints**
• To describe colonization in each of the groups: BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control.	• Proportion of subjects with colonization at either Day 92 or 99 (*B. pertussis* culture or polymerase chain reaction [PCR]) with prior BPZE1 immunization following re-vaccination/attenuated challenge at Day 85 (BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control). Expressed as absolute proportion (Yes/No).
Mucosal secretory immunogenicity (S-IgA): WCE, PT, FHA, PRN, and any additional anti-pertussis mucosal antibodies identified during assay development using GMT and geometric mean fold rise (GMFR) unless otherwise stated. **NOTE: To also be tested separately in subjects who participate in the optional substudy as exploratory endpoints, to assess response following re-vaccination/attenuated challenge.**	
Secondary objectives	**Secondary endpoints**
• To demonstrate BPZE1 induction of mucosal S-IgA immunity is **non-inferior** to Boostrix at Day 29 as measured by antibodies to WCE (BPZE1 vs Boostrix).	• BPZE1 induction of mucosal S-IgA against WCE compared with Boostrix at Day 29.
• To demonstrate BPZE1 induction of mucosal S-IgA immunity is greater than baseline at Day 29 (BPZE1, BPZE1 + Boostrix).	• BPZE1 induction of mucosal S-IgA at Day 29 compared to baseline.
• To demonstrate BPZE1 induction of mucosal S-IgA immunity remains greater than baseline over the study period and at Day 169/ end of study (EOS) (BPZE1, BPZE1 + Boostrix).	• BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA compared with baseline at Days 85 and 169/EOS (tested independently).
• To demonstrate BPZE1 + Boostrix induction of mucosal S-IgA immunity is non-inferior to BPZE1 at Day 29 and Day 169/EOS (BPZE1 + Boostrix vs BPZE1).	• BPZE1 + Boostrix induction of mucosal S-IgA compared with BPZE1 at Days 29 and 169/EOS (tested independently).

• To assess if age, time since last aP vaccination (prior to study entry), or other baseline demographics have a differential response to induction of mucosal S-IgA immunity (BPZE1, BPZE1 + Boostrix) at Day 29.	• BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA in covariate analysis by age, time since last aP vaccination (prior to study entry), or other baseline demographics at Day 29.
• To assess if baseline antibody values, by baseline quantile, have variable responses at Day 29 to induction of S-IgA immunity when BPZE1 is given alone or with Boostrix (BPZE1, BPZE1 + Boostrix).	• BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA by baseline quantile at Day 29.
• To assess seroconversion for each of the anti-pertussis S-IgA antibodies at Day 29 (BPZE1, BPZE1 + Boostrix, Boostrix). Additional combinations of antibody response (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) may also be tested.	• Seroconversion rate (2-fold increase over baseline value or a 4-fold increase over the lower limit of assay detection [whenever the baseline value falls below the lower limit of assay detection]) for mucosal S-IgA at Day 29 by treatment group.
• To assess reverse cumulative distribution curves for immune response (S-IgA) at Day 29.	• Reverse cumulative distribution curves of each anti-pertussis mucosal antibodies S-IgA GMT at Day 29 by treatment group.
Serum immunogenicity (IgA and IgG): WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies identified during assay development using GMT and GMFR unless otherwise stated. **NOTE: To also be tested separately in subjects who participate in the optional substudy as exploratory endpoints, to assess response following re-vaccination/attenuated challenge.**	
Secondary objectives	**Secondary endpoints**
• To demonstrate that BPZE1 (BPZE1, BPZE1 + Boostrix) induction of serum immunity is significantly greater than baseline at Day 29.	• BPZE1 and BPZE1 + Boostrix induction of serum immunity (IgA, IgG) at Day 29 compared with baseline (tested independently by treatment group).
• To demonstrate that BPZE1 + Boostrix induction of serum immunity is non-inferior compared to Boostrix at Day 29 (BPZE1 + Boostrix vs Boostrix).	• BPZE1 + Boostrix induction of serum immunity (IgA, IgG) compared with Boostrix at Day 29.

• To demonstrate that BPZE1 (BPZE1, BPZE1 + Boostrix) induction of serum immunity remains significantly greater than baseline over the course of the study and at Day 169/EOS and to assess antibody decay curves for BPZE1, BPZE1 + Boostrix and Boostrix.	• BPZE1 and BPZE1 + Boostrix induction of serum immunity (IgA, IgG) at Day 85 and Day 169/EOS compared with baseline (tested independently by treatment group and day). • Antibody decays (IgA, IgG) over the course of the study (tested independently by treatment group).
• To demonstrate that BPZE1 induction of serum immunoglobulin A (IgA) is non-inferior to Boostrix at Day 169/EOS (BPZE1 vs Boostrix; BPZE1 + Boostrix vs Boostrix).	• BPZE1 induction of serum IgA compared with Boostrix at Day 169/EOS. • BPZE1 + Boostrix induction of serum IgA compared with Boostrix at Day 169/EOS.
• To assess if age, time since last aP vaccination (prior to study entry), or other baseline demographics have a differential response to BPZE1 or Boostrix at Day 29 (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) by age, time since last aP vaccination (prior to study entry), or other baseline demographics at Day 29 (tested independently by treatment group).
• To assess if baseline antibody values, by baseline quantile, have variable serum immunity responses at Day 29 when BPZE1 is given alone or with Boostrix or with Boostrix alone (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix and Boostrix induction of serum immunity (IgA, IgG) at Day 29 by baseline quantile (tested independently by treatment group).
• To demonstrate that BPZE1 (BPZE1, BPZE1 + Boostrix) induces a measurable functional antibody response (subset of subjects) at Day 29; and to assess whether there is similar magnitude as that seen with Boostrix (BPZE1 vs BPZE1 + Boostrix vs Boostrix).	• BPZE1 and BPZE1 + Boostrix induction of measurable functional antibody (subset of subjects) compared with Boostrix at Day 29 (tested independently by treatment group).

• To assess for seroconversion at Days 29 and 85 (BPZE1, BPZE1 + Boostrix, Boostrix). Additional combinations of antibody response (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) may also be tested.	• Seroconversion rate for serum immunity (IgA, IgG) defined as a 2-fold increase over baseline value; or a 4-fold increase over the lower limit of assay detection (whenever the baseline value falls below the lower limit of assay detection) at Day 29 and Day 85 (tested independently by treatment group and day).
• To assess reverse cumulative distribution curves for immune responses (IgA and IgG) at Day 29.	• Reverse cumulative distribution curves of GMT at Day 29 by treatment group.
Safety **NOTE: Subjects participating in the substudy will be evaluated for safety endpoints in a separate evaluation after Day 85 re-vaccination/attenuated challenge.**	
Secondary objectives	**Secondary endpoints**
• To describe reactogenicity events during the 7 days following any study vaccination. Primary and re-vaccination/attenuated challenge (substudy subjects) to be described separately, by treatment group. Reactogenicity will be classified by local, nasal/respiratory, and systemic.	• Maximum daily severity during the 7 days following study vaccination (by treatment group). • Incidence by toxicity grade during the 7 days following study vaccination (by treatment group). • Mean response (for any measurements) and duration during the 7 days following study vaccination (by treatment group).
• To describe (severity and relationship to study vaccination) all AEs through 28 days following any study vaccination. Primary and re-vaccination/attenuated challenge (substudy subjects) to be described separately and by treatment group.	• All AEs through 28 days following study vaccination by severity and relationship to study vaccination (by treatment group).

• To describe (severity and relationship to study vaccination) any medically attended AE (MAAE) through 84 days following any study vaccination. Primary and re-vaccination/attenuated challenge to be described separately and by treatment group.	• All MAAEs through 84 days following study vaccination by severity and relationship to study vaccination, and by time since study vaccination (by treatment group).
• To describe any AE of special interest (AESI) and serious AE (SAE) through Day 169/EOS including relationship to study vaccination.	• All AESIs and SAEs through Day 169/EOS by severity and relationship to study vaccination, and by time since study vaccination (by treatment group).
• To describe any severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or AESI associated with COVID-19 disease if the COVID-19 pandemic remains ongoing in the region or if there are mass vaccinations using a COVID-19 vaccine during study conduct.	• Incidence of SARS-CoV-2 infections or AESIs associated with COVID-19 disease by severity, and by time since study vaccination (by treatment group).
• To assess proportion of subjects (subset of subjects) cleared from colonization (present/absent) through Day 85.	• *B. pertussis* clearance following study vaccination (subset of subjects at varying time points through Day 85).
Mucosal secretory immunogenicity (S-IgA) (optional substudy only) **Note that S-IgA objectives and endpoints for the overall study (as noted above) will also be analysed for the optional substudy and will be considered exploratory.**	
Exploratory objectives	**Exploratory endpoints**
• To evaluate mucosal immunity of individual and combinations of pertussis antibodies (eg, WCE, PT, FHA and PRN and other known anti-pertussis mucosal antibodies) are relational to protection against colonization (BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control).	• Induction of mucosal S-IgA at Day 29 or 85 (tested independently) compared by protection against colonization (Colonizers/Non-colonizers) by treatment group (BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control).

• To measure BPZE1 induction of mucosal immunity (S-IgA) at Day 113 by treatment group; and to assess if induction of a boost response (relative to Day 85 response) is observed for those subjects with prior BPZE1 vaccination (BPZE1, BPZE1 + Boostrix).	• Induction of mucosal S-IgA (WCE, PT, FHA, PRN and any additional anti-pertussis mucosal antibodies identified during assay development) at Day 113 compared with Day 85 following BPZE1 attenuated challenge by treatment group (BPZE1, BPZE1 + Boostrix).
• To assess if Day 113 response and longer-term durability through Day 169/EOS (absolute GMT) is altered with re-vaccination/attenuated challenge when compared to those subjects who did not participate in the substudy, relative to each randomized treatment group (BPZE1, BPZE1 + Boostrix, Boostrix).	• Induction of mucosal S-IgA at Days 113 and 169/EOS (tested independently) in subjects who participated in the substudy compared with subjects who did not participate in the substudy by treatment group.
Serum immunogenicity (IgA and IgG) (optional substudy only): WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies identified during assay development using GMT and GMFR unless otherwise stated. **Note that serum IgA and IgG objectives and endpoints for the overall study (as noted above) will also be analysed for the optional substudy and will be considered exploratory.**	
Exploratory objectives	**Exploratory endpoints**
• To measure BPZE1 induction of serum immunity at Day 113; and to assess if induction of a boost response (relative to Day 85 response) is observed for those subjects with prior BPZE1 vaccination (BPZE1, BPZE1 + Boostrix).	• BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) at Day 113 compared with baseline (by treatment group). • BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) at Day 113 compared with Day 85 (by treatment group).

• To assess if Day 113 response and longer-term immune durability through Day 169/EOS is altered with re-vaccination/attenuated challenge when compared to those subjects who did not participate in the substudy, relative to each randomized treatment group (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) at Day 113 and Day 169/EOS in subjects who participated in the substudy compared with subjects who did not participate in the substudy (tested independently by treatment group and day).
• To assess if serum immunity of individual and combinations of pertussis antibodies (eg, WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) at Day 29 or 85 is relational to protection against colonization by treatment group and overall (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix induction of serum immunity (IgA, IgG) at Day 29 or Day 85 compared by protection against colonization (Colonized/Non-colonized) (tested independently by treatment group and overall).
• To assess functional antibody response by treatment group at Day 113 compared to baseline and Day 29, as measured by an appropriate assay for bactericidal activity.	• Proportion of subjects with ≥50% killing (or equivalent) for functional antibody response at Day 113 compared to baseline and Day 29 (tested independently by treatment group and day).
Colonization (optional substudy only)	
Exploratory objective	**Exploratory endpoints**
• To demonstrate prior vaccination with BPZE1 substantially reduces colonization at Days 92 and 99.	• *B. pertussis* colony counts at each of Days 92 and 99 by treatment group (mean counts, area under the curve [AUC] or similar total bacterial load assessments will also be calculated). • Subjects with colonization at each of Days 92 and 99 by treatment group. Expressed as absolute proportion (Yes/No) and percent reduction relative to control.

Other, including cellular-mediated response	
Exploratory objectives	**Exploratory endpoints**
• To assess cellular-mediated response (by ELISpot or flow cytometry) to BPZE1 and Boostrix (Th1 and Th2) in a subset of subjects.	• Cellular-mediated response at baseline and Day 8 (safety lead-in cohort) and Days 85 and 113 (subset of substudy subjects).
• To further characterize mucosal and serum immunological responses over time, relative to baseline status and to vaccination response, with any future assays developed for BPZE1.	• Immunologic response measurement by treatment group.

Study Population:

Inclusion Criteria

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

1. The subject is male or female 6 to 17 years of age, inclusive, on Day 1 (day of vaccination).
2. The subject (and/or legal guardian) is capable of understanding the written informed consent, providing signed and witnessed written informed consent (or assent, depending on age), and understanding and complying with protocol requirements.
3. Female subjects of reproductive age must be nonpregnant and nonlactating, and if of childbearing potential (defined as any female who has experienced menarche at any time prior to or during the study), must agree to be heterosexually inactive from at least 21 days prior to enrolment and through 90 days following any study vaccination or agrees to consistently use any of the following methods of birth control from at least 21 days prior to enrolment and through 90 days following any study vaccination:
    a. Condoms (male or female) with or without spermicide*
    b. Diaphragm with or without spermicide*
    c. Cervical cap with or without spermicide*

    ***NOTE**: The highest level of protection is required, in accordance with local regulations.
    d. Intrauterine device
    e. Oral or patch contraceptives

f. Norplant®, Depo-Provera®, or other acceptable method of birth control that is designed to protect against pregnancy.

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

4. The subject has a stable health status, as established by physical examination, vital sign measurements (body temperature and heart rate), and medical history.
5. The subject (and/or legal guardian) has access to a consistent and reliable means of electronic or telephone contact, which may be in the home, workplace, school, or by personal mobile electronic device.
6. The subject is willing to refrain from routine nasal sprays (including steroid sprays) or washes for at least 7 days following any study vaccination.
7. The subject (and/or legal guardian) lives within a reasonable distance from the clinical site, is willing and able to travel to and from the clinical site for follow-up visits and agrees to medical evaluation in the event of an AE requiring acute assessment.
8. The subject (and/or legal guardian) 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 agrees to provide updated contact information, as necessary.

Exclusion Criteria

Subjects meeting any of the following criteria (by review of medical history and subject intake) will be excluded from the study:

1. The subject has a history of pertussis-containing vaccination (inclusive of school vaccination programs) or documented *B. pertussis* infection within 3 years prior to Day 1 and/or a history of Td-containing vaccination (without pertussis vaccine component) within 1 month prior to Day 1.
2. The subject has a chronic significant illness actively being treated or a history of recent intervention for worsening or fluctuating symptoms (at the discretion of the investigator).
3. The subject has a history of cancer (malignancy).

4. The subject has a congenital, hereditary, or acquired disease or disorder classified as autoimmune, immunodeficient, coagulopathy, hepatic, renal, neurologic (including a history of Bell's palsy), or moderate to severe cognitive. (Attention deficit hyperactivity disorder permitted.)
5. The subject currently uses smoking products (including vaping and e-cigarettes) and is unwilling to refrain from use from Day 1 through Day 29 following any study vaccination.
6. The subject has received immunoglobulin, blood-derived products, systemic corticosteroids (at a dose of >10 mg per day for more than 10 days in total), or other immunosuppressant drugs within 90 days prior to Day 1.
7. The subject has any chronic pulmonary disease requiring active medication or pulmonary therapies, with the following exceptions:
    a. Subjects with exercise-induced bronchospasm, if currently well controlled, who are willing to refrain from intense exercise for 7 days following study vaccination or
    b. Subjects with Step 1 (intermittent) asthma classification who have not had an exacerbation requiring oral systemic corticosteroids in the past year; have an FEV1 documented to be >80%; do not have restrictions in normal activity due to breathing issues; and have used a short-acting beta-agonist less than or equal to 2 days per week over the past 2 months.

    NOTE: Subjects in the safety lead-in cohort are not allowed to have these exceptions.
8. The subject has a history of oro/nasopharynx surgery (eg, adenoidectomy, tonsillectomy) within 60 days prior to Day 1.
9. The subject has a known hypersensitivity to latex or any component of any study vaccine. Specific to the prior use of Boostrix: the subject has known hypersensitivity to neomycin or polymyxin; hypersensitivity after previous administration of diphtheria, tetanus, or pertussis vaccines; or has experienced transient thrombocytopenia or neurological complications following an earlier immunisation against diphtheria and/or tetanus.

10. The subject has participated in any other clinical trial for the testing of an unlicensed product where such product was received within 6 months prior to Day 1 or is planning to participate in any other clinical trial during study conduct through Day 85 following last study vaccination.

    NOTE: Exceptions may be considered for an investigational COVID-19 vaccine if the product has entered into adult Phase 3 efficacy trials or is part of a mass vaccination rollout in this age group of subjects within a country. Direct guidance will be provided by the sponsor prior to study initiation.
11. The subject has routine and/or repeated contact with, or is currently living in a household with, an immunocompromised individual.
12. The subject has 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, eating disorder, major depression or anxiety disorder, history of alcohol or drug use).
13. The subject is a study team member or first-degree relative of a study team member.
14. The subject resides in a residence during the vaccination period (defined as through Day 85 following any study vaccination) where an infant less than 6 months of age resides or may reside.

Additional Exclusion Criteria for Substudy Subjects ONLY

Subjects meeting any of the following criteria will be excluded from participation in the optional substudy.

15. Has received a pertussis-containing vaccination outside of study conduct since enrolment.
16. Has received any form of immunotherapy determined to potentially have an effect on immune response (as determined by medical consultation, when necessary).

Other Considerations for Temporarily Holding Vaccination (Days 1 or Substudy Day 85)

Subjects meeting any of the following criteria may have a planned study vaccination deferred to a later date, but these criteria are not exclusionary for study enrolment/participation. For Visit 1, Day 1, subjects may be enrolled on a return visit if they remain within 45 days of consenting. Otherwise, subjects must be reconsented and reassessed per the inclusion and exclusion criteria (ie, re-enrolled).

- The subject has or reports acute respiratory tract symptoms/infection or rhinorrhoea within the past 3 days. Subject may be vaccinated once all symptoms have been resolved for at least 3 days.
- The subject has or reports a body temperature of 100.4°F (38°C) or higher within the past 3 days. The subject may be vaccinated once the fever resolves and body temperature (site-measured or subject-measured) remains below 100.4°F (38°C) for at least 3 days.
- The subject used antibiotics within the past 10 days.
- The subject received a licensed vaccine (including any vaccine under emergency use authorization) within the past 14 days.
- The subject has a positive SARS-CoV-2 test result (eg, infection is ongoing) within the 3 days preceding vaccination. For those subjects testing positive, they must have a subsequent SARS-CoV-2 test result that is negative prior to vaccination (visit window may be extended to accommodate retesting needs).

Study Design: This is a multi-centre, randomized, observer-blinded, placebo- and active comparator-controlled trial with a 6-month safety follow-up after first study vaccination. After signing the informed consent form (ICF; or providing assent when necessary) and meeting all inclusion and none of the exclusion criteria, eligible subjects will receive an intranasal vaccination with either BPZE1 vaccine or formulation buffer (placebo) via the intranasal mucosal atomization device (MAD) and an intramuscular (IM) injection of either Tdap vaccine (ie, Boostrix) or sterile normal saline (placebo) on Day 1. To maintain the blind, placebo vaccination via intranasal and IM routes, using formulation buffer and normal saline, respectively, will be included, and an unblinded team will manage vaccine logistics, preparation, and administration (when needed to maintain blinding) but will not be involved in study-related assessments or have subject contact for data collection after study vaccination.

Approximately 360 subjects will be enrolled and randomly assigned (1:1:1) to 1 of 3 treatment groups (refer to Table S1) to contribute to safety and efficacy assessments. Enrolment will be operationally managed at the study level to maintain a minimum number of subjects enrolled in 2 age groups: 6 to 10 years, inclusive; and 11 to 17 years,

inclusive. It is estimated that a 10% dropout rate will occur, allowing for approximately 108 evaluable subjects per treatment group.

Table S1 Dosing Scheme

Treatment Group	n	Intranasal Vaccination[a] (Day 1)		IM Vaccination[b] (Day 1)	
		BPZE1	Placebo	Boostrix	Placebo
A	120	X	-	-	X
B	120	X	-	X	-
C	120	-	X	X	-

[a] Intranasal application of ~0.4 mL per nostril (0.8 mL total volume delivered). Intranasal vaccination will occur prior to IM vaccination.

[b] IM injection of 0.5 mL to the deltoid region.

All subjects will be monitored for at least 30 minutes after final study vaccination. On Day 1, intranasal vaccination will occur prior to IM vaccination with a minimum of 10 minutes (minimum of 30 minutes for subjects in the safety lead-in cohort of 11 to 17 year olds) between intranasal and IM vaccinations. Immediate reactogenicity will be assessed at 30 minutes (+30 minutes) after the last study vaccination of the Day (ie, after IM vaccination on Day 1, after nasal vaccination on Day 85), prior to release from clinical observation.

All subjects will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following any study vaccination (retrospective of the highest value in the previous 24 hours). Subject diaries will be age-adjusted as appropriate. Local (arm; after IM injection only), nasal/respiratory, and general systemic reactogenicity events will be recorded, including start and stop dates and including any actual measurements (ie, body temperature, redness and/or swelling at the injection site). Trained clinical staff will review the information from the subject diary with the subjects at the following visit and apply a standard toxicity grade to any reactogenicity event. Subjects will be instructed that should they have reactogenicity with a potential toxicity grade 3 at any time, they should contact the site on the same day and be seen or referred to a qualified medical facility within 24 hours.

As this is the first study of BPZE1 in school-age children, a staggered enrolment is planned. The first 45 subjects enrolled will be in the older age group (11 to 17 years, inclusive) and designated the safety lead-in cohort. Reactogenicity will be reviewed with these subjects during a

supplemental visit 7 days following first study vaccination. After reactogenicity for the safety lead-in cohort is reviewed by the medical monitor, medical lead, and Safety Monitoring Committee (SMC) chairman (with appropriate consultation with investigators, if needed), the remainder of subjects will be enrolled.

All AEs will be monitored through 28 days after any study vaccination; all MAAEs will be monitored through 84 days following any study vaccination; and all AESIs and SAEs will be monitored through Day 169/EOS. Due to the COVID-19 pandemic, a SARS-CoV-2 infection (confirmed by testing within the public health system) and any associated symptoms will be collected on a unique COVID-19 electronic case report form through Day 169/EOS if the COVID-19 pandemic remains ongoing in the region or if there are mass vaccinations using a COVID-19 vaccine during study conduct. Vaccines received outside of the study will be specifically queried at each visit and recorded through Day 169/EOS.

An informational card will be provided to all subjects indicating their participation in this vaccination study to prevent subjects from inadvertently receiving any scheduled/planned Boostrix-containing vaccines (tetanus, pertussis, diphtheria) during study conduct. Any enrolled subject who does not receive Boostrix during the study will be offered Boostrix at the conclusion of the study (following unblinding); this Boostrix 'catch-up' will be provided outside of the study schedule, at the discretion of the investigator, with vaccine provided by the sponsor.

Nasal and blood samples for immunogenicity assessments will be collected before vaccination and at selected time points following study vaccination. Mid-turbinate/nasopharyngeal sample collections will occur at selected time points following study vaccination to assess initial colonization (safety lead-in cohort only, Day 8) and to assess for clearance (remainder of cohort, either Day 29 or Day 85). Subjects in the safety lead-in cohort and a subset of subjects in the optional substudy will also have the option to provide extra blood for cellular-mediated immunity assessments at selected time points.

Due to the uncertainty with movement and/or isolation practices associated with the COVID-19 pandemic, telemedicine and remote sampling may be initiated with proper investigator and/or medical oversight.

The primary database lock will occur after all data are clean and all planned study procedures through Day 169/EOS (6 months) are complete. An interim database lock may occur after all subjects have

completed Day 29 and sample testing for all primary and select secondary immune endpoints have been completed (mucosal and serum), but results will only be provided at treatment assignment level to maintain the blind. The site and those interacting directly with the site or subject data (eg, contract research organization [CRO], laboratories) will remain blinded to subject assignment until EOS or end of sample testing, depending on the activities of the associated entities.

Vaccination pause rules will be in place with a review by the SMC initiated if any rule is met. In addition, the medical monitor and medical lead are empowered to request a review by the SMC at any time for any safety reason.

Substudy: Attenuated Challenge With BPZE1 Following Prior Vaccination with BPZE1, BPZE1 + Boostrix, or Boostrix Alone

This is an optional open-label substudy in which enrolled subjects may elect to participate at any time from the day of enrolment through Day 85. A separate signed ICF (and separate assent when necessary) will be obtained prior to participation in the substudy. Subjects will be excluded from participating in the substudy if they meet additional exclusion criteria. Temporary vaccination holding criteria are to be followed for the Day 85 vaccination.

Up to 120 enrolled subjects may participate in the substudy. The duration of the entire study will not change; however, additional visits/procedures starting on Day 85 will include the following:

- 3 additional visits (Days 92, 99, and 113)
- 1 additional intranasal vaccination (Day 85)
- Up to 3 additional nasal samples (Days 92, 99, and 113), including 2 needed for mid-turbinate/nasopharyngeal sample collection (Days 92 and Day 99) and 1 needed for leukosorb (Day 113). Subjects will contribute to all Day 85 sampling (systemic and nasal)
- Up to 2 additional blood samples (Day 85 [if not already assigned to provide] and Day 113, including blood for cellular-mediated and systemic immunity responses)
- 1 additional subject diary (subjects to record maximum daily reactogenicity [nasal/respiratory, systemic] in a subject diary for 7 consecutive days starting the day following study vaccination)

- 1 additional test for SARS-CoV-2 within 72 hours prior to Day 85 vaccination (see exclusion criteria for a temporary hold on vaccination). All subjects participating in the open-label substudy will receive an intranasal re-vaccination/attenuated challenge with BPZE1 on Day 85. Subjects may have the planned re-vaccination/attenuated challenge deferred to a later date (up to 20 days) if pre-defined temporary exclusion criteria are met or for other personal reasons (eg, holiday, school schedule). Subjects will be monitored for at least 30 minutes after re-vaccination/attenuated challenge, with a post-vaccination evaluation for reactogenicity (nasal/respiratory, and systemic) completed prior to release from clinical observation.

Subjects will receive a subject diary on Day 85. All subjects (with assistance when needed) will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following re-vaccination/attenuated challenge (retrospective of the highest value in the previous 24 hours). Subject-reported symptom diaries will be age-adjusted as appropriate. Nasal/respiratory and general systemic reactogenicity events will be recorded, including measurements of body temperature. Trained clinical staff will review the information from the subject diary with the subjects at the following visit and apply a standard toxicity grade to any reactogenicity event. Subjects will be instructed that should they have reactogenicity with a potential toxicity grade 3 at any time, they should contact the site on the same day and be seen or referred to a qualified medical facility within 24 hours.

Nasal and blood samples for immunogenicity assessments will be collected before re-vaccination/attenuated challenge and at selected time points thereafter. Mid-turbinate/nasopharyngeal sample collections will be performed at selected time points to assess BPZE1 colonization (*B. pertussis* culture or PCR). Subjects in the substudy will also have the option to provide extra blood for cellular-mediated immunity assessments at Day 85 and Day 113 (a maximum of 45 subjects).

All AEs will be collected following re-vaccination/attenuated challenge on Day 85 through Day 113. Following Day 113, only MAAEs, AESIs, and SAEs will be collected though Day 169/EOS.

Estimated Study Duration: Study duration is approximately 9 months and duration of subject participation is approximately 6 months.

Immunogenicity and Colonization Assessments:	Immune measurements will be conducted on serum (IgG and IgA) and nasal mucosal secretion (S-IgA) samples for anti-pertussis antibodies of WCE, PT, FHA, and PRN, as well as any additional antigens included in assay development. Serum bactericidal activity (or other functional assays identified) will be measured in a subset of subjects. Cellular-mediated responses to BPZE1 and Boostrix (Th1/Th17 vs Th2) will be assessed by ELISpot or flow cytometry in subjects at baseline and Day 8 (safety lead-in cohort) and Day 85 and Day 113 (subset of substudy subjects). Additional testing for antibodies specific to *B. pertussis* may be performed at a later date as BPZE1 induces broader immunity more similar to natural infection induced immunity and does not contain the purified antigen levels of PT, FHA, and PRN found in Boostrix. Mid-turbinate/nasopharyngeal samples will be evaluated by real-time PCR or by *B. pertussis* culture for colonization of *B. pertussis*. Subjects will consent for the use of samples for further anti-pertussis antibody testing or other assay development as part of the standard consenting process. Aliquots of collected samples from this study may be retained for additional testing of biological responses (eg, antibodies, T-cell responses, microbiome) specific to future development of BPZE1 and ILiAD Biotechnologies' *B-Tech* program for a maximum of 15 years (starting from the date at which the last subject had the last study visit), unless local rules, regulations, or guidelines require different time frames or different procedures, and in accordance with subject consent.
Safety Assessments:	Safety assessments for all subjects will include the following: vital sign measurements (including body temperature and heart rate), targeted physical examinations, reactogenicity, and AEs (including MAAEs, AESIs, and SAEs).
Investigational Products, Dosage, and Route of Administration:	• BPZE1, reconstituted with sterile water to provide 10^9 colony-forming units (CFU) per 0.8 mL; administered intranasally, with approximately 0.4 mL per nostril (0.8 mL total volume delivered), by syringe with MAD • Boostrix (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed) injectable suspension, for IM use; administered as a single 0.5 mL IM injection to the deltoid region • Intranasal placebo (lyophilized BPZE1 buffer reconstituted with sterile water); administered intranasally, with approximately 0.4 mL per nostril (0.8 mL total volume delivered), by syringe with MAD

- IM placebo (sterile normal saline for injection); administered as a single 0.5 mL IM injection to the deltoid region

Sample Size: The sample size is based on calculations to achieve adequate response on Day 29 over baseline of S-IgA for WCE assay in the BPZE1 and BPZE1 + Boostrix groups. Assuming the GMT follows a log-normal distribution, by applying a natural logarithmic transformation of the GMT, a paired t-test will be used to test the difference of mucosal S-IgA against WCE at Day 29 compared with baseline (equivalent to natural logarithmic transformation of the GMFR). With an effective sample size of N=108 subjects per group, the study provides 95% power for detecting an effect size of 0.35 for each of the two primary comparisons with a two-sided significance level of 0.05. By multiplying the power for each of the two comparisons, the study provides an overall power of 90% (=95%*95%) for testing the primary endpoint in each of the two groups. Factoring in an estimated 10% drop-out rate, each treatment group would include approximately 120 subjects for a total of approximately 360 subjects in the study.

Statistical Methods: Statistical analysis will be performed using SAS software Version 9.3 or later. Continuous variables will be summarized using the mean, standard deviation, median, first quartile, third quartile, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages, as well as a two-sided 95% confidence interval (CI) for proportions computed using the Agresti-Coull method.

<u>Primary endpoints</u>
For mucosal S-IgA against WCE at Day 29, the two-sided 95% CI for change from baseline in GMFR will be calculated. Assuming the GMT follows a log-normal distribution, by applying natural logarithmic transformation of the GMT, a paired t-test will be used to test the difference of mucosal S-IgA against WCE at Day 29 compared with baseline (equivalent to natural logarithmic transformation of the GMFR). The superiority objective will be demonstrated if the lower limit of the 95% CI for the difference of mucosal S-IgA against WCE at Day 29 compared to baseline on natural log scale is greater than 0 within the treatment group. The superiority hypothesis will be tested in treatment groups BPZE1 and BPZE1 + Boostrix separately.

<u>Key secondary endpoints</u>
For comparisons of two proportions between treatment groups, eg, comparison between BPZE1 + Boostrix and Boostrix for the

proportion with titer ≥ 0.1 IU/mL (for diphtheria, tetanus), a non-inferiority test for the difference between two proportions will be used. If the lower limit of the 95% CI for the difference of two proportions (proportion for BPZE1 + Boostrix - proportion for Boostrix) is greater than the non-inferiority margin (eg, -0.1), then BPZE1 + Boostrix is considered to be non-inferior to Boostrix. For comparisons between two groups on GMT (eg, comparison between BPZE1 + Boostrix and Boostrix) to demonstrate non-interference of IgG serum responses for the Boostrix-containing antigens (PT, FHA, PRN) at Day 29 when BPZE1 is co-administered with Boostrix, a two-sample t-test will be used to test the significance of the difference between two natural logarithmic transformed GMTs. If the lower limit of the 95% CI for the difference in the natural logs of two GMTs [ln(GMT for BPZE1 + Boostrix) - ln(GMT for Boostrix)] is greater than the non-inferiority margin (eg, -0.693), then BPZE1 + Boostrix is considered to be non-inferior to Boostrix.

For the key secondary endpoints, the Holm method will be used to control for multiplicity.

A blinded sample size recalculation may occur when Day 29 immunogenicity data for approximately 40 to 60 subjects per group are available.

Version and Date of Protocol: 30 March 2023 (Amendment 6, v7.0)

List of Abbreviations

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
ANCOVA	analysis of covariance
aP	acellular pertussis
AUC	area under the curve
BMI	body mass index
CFU	colony-forming units
CI	confidence interval
CRO	contract research organization
CSR	clinical study report
DHHS	Department of Health and Human Services
DNT	dermonecrotic toxin
DTaP	diphtheria-tetanus-acellular pertussis
eCRF	electronic case report form
EOS	end of study
FHA	filamentous hemagglutinin
GCP	Good Clinical Practice
GMFR	geometric mean fold rise
GMT	geometric mean titre
IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IgA	immunoglobulin A
IgG	immunoglobulin G
IM	intramuscular
IPV	inactivated poliovirus vaccine
IRT	interactive response technology
IU	international unit(s)
MAAE	medically attended adverse event

Abbreviation	Definition
MAD	mucosal atomization device
MedDRA	Medical Dictionary for Regulatory Activities
OTC	over-the-counter
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PRN	pertactin
PT	pertussis toxin
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
S-IgA	secretory immunoglobulin A
SMC	safety monitoring committee
SUSAR	suspected unexpected serious adverse reaction
TCT	tracheal cytotoxin
Td	tetanus and diphtheria toxoids
Tdap	tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis
US	United States
WCE	whole cell extract
WFI	Water for Injection
wP	whole cell pertussis

1 Introduction

1.1 Background Information

Currently registered acellular pertussis (aP) vaccines protect against respiratory disease but not against colonizing *Bordetella pertussis* (*B. pertussis*) infection and transmission. *Bordetella 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 (ie, a natural infection confers short-term sterilizing immunity); however, vaccination with aP vaccines did not protect against subsequent challenge or transmission in animals housed in proximity (Warfel et al 2014). In contrast, a single intranasal administration of the highly attenuated live *B. pertussis* vaccine BPZE1 has demonstrated the ability to reduce the bacterial burden of a substantial *B. pertussis* challenge by more than 99.8% when compared to prior studies in nonhuman primates immunized with 3 doses of aP vaccine (Locht et al 2017). ILiAD Biotechnologies has licensed BPZE1 from the Institut Pasteur de Lille and Inserm with worldwide rights, and the company is conducting further clinical and regulatory development with the goal of an indication for active booster immunization against *B. pertussis* in adults and school-age children (ie, those who have received their childhood aP vaccination series).

Despite the dramatic decline in pertussis cases and deaths in industrialized nations during the 20th century due to public health vaccine initiatives, the last 2 decades have witnessed an increase in epidemics, and it is generally agreed that the short durability of aP vaccines along with the lack of protection against *B. pertussis* acquisition and/or transmission has contributed significantly to this resurgence (WHO 2015). In a recent outbreak in the United States (US), 82% of children who acquired pertussis had received their full complement of diphtheria-tetanus-acellular pertussis (DTaP) vaccinations. The adjusted risk of pertussis 3 or more years after vaccination was 5 times higher than the risk less than 1 year after vaccination; and among children aged 7 to 11 years, the adjusted risk of pertussis 6 or more years after vaccination was twice as high as the risk less than 3 years after vaccination (Zerbo et al 2019). In addition, the emergence of pertactin-negative *B. pertussis* strains may contribute to the reduced-effectiveness of aP vaccines (Safarchi et al 2015; Xu et al 2019). There is now a critical need for new and more effective vaccines targeting *B. pertussis*. As a potential solution to this problem, the BPZE1 vaccine has been developed

to be given as a single intranasal administration. Unlike existing aP vaccines, BPZE1 has the potential to prevent transmission of *B. pertussis*, through its mechanism of mucosal action that mimics wild-type *B. pertussis* infection, reducing the circulating burden of *B. pertussis*, and thereby disrupting the cycle of transmission that is the basis for ongoing epidemics currently observed every 3 to 5 years.

BPZE1 was engineered by genetically altering or removing three *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 (Thalen et al 2020). The safety of liquid BPZE1 has been assessed in immunocompetent and immunosuppressed animals (Mielcarek et al 2006; Feunou et al 2008; Skerry et al 2009; Mielcarek et al 2010). These results contributed to downgrading BPZE1 from a Biosafety Level 2 to a Biosafety Level 1 organism in the US and France. Good Laboratory Practice toxicology studies have confirmed the safety of BPZE1 in 2 animal models: mouse and rabbit (data on file).

BPZE1 in a liquid formulation has been studied at various doses from 10^3 to 10^9 colony-forming units (CFU) in two Phase 1 clinical studies in Sweden with no vaccine-related serious adverse events (SAEs) and comparable general adverse events (AEs) to placebo controls. Most recently, in a Phase 2b study conducted in the US, BPZE1 (or Boostrix™) was given as a single vaccination supplied in a lyophilized vial for reconstitution prior to intranasal administration via the intranasal mucosal atomization device (MAD) and then a re-vaccination/attenuated challenge was given 3 months later with BPZE1 (or placebo) to 300 healthy adults aged 18 to 50 years. BPZE1 (but not Boostrix) was shown to induce mucosal secretory immunoglobulin A (S-IgA) as measured by whole cell extract (WCE), filamentous hemagglutinin (FHA), and pertactin (PRN) assays, and these antibodies were sustained through 9 months (end of study [EOS]). Unlike Boostrix, the BPZE1-induced mucosal immunity was associated with protection from *B. pertussis* acquisition (colonization) using an attenuated challenge with BPZE1 3 months later. In addition, BPZE1 induced systemic immunoglobulin A (IgA) and immunoglobulin G (IgG) anti-pertussis antibodies (WCE, PT, FHA and PRN), which were sustained above baseline through the 9 months of study. A single vaccination appeared sufficient to induce both mucosal and systemic responses in this population. In addition, systemic and nasal/respiratory reactogenicity was overall none to mild, similar to Boostrix/placebo in intensity, with average

duration of 3 days, and was not associated with severe events. Re-vaccination/attenuated challenge did not result in worsening reactogenicity. Overall, the AE profile was similar between treatment groups and no SAEs were attributed to vaccination. The Phase 2b study was conducted in adults, in which the majority (>90%) was estimated to have received a whole cell pertussis (wP) vaccination as their primary vaccination series during infancy. In the current study, subjects will all have received aP vaccinations containing (at a minimum) PT, FHA, and PRN as their primary series and will have never received a wP vaccination. Since wP vaccines and BPZE1 induce Th1/Th17 responses, which have been associated with polarizing Th1 long-term immunity, whereas aP vaccines preferentially stimulates a polarizing Th2 pathways, administration of BPZE1 in this school-age population may be the first stimulation of the Th1/Th17 pathway for preferential T-cell-associated immunity (Warfel et al 2014; Antunes et al 2018).

1.2 Rationale

Acellular pertussis vaccines were introduced into most developed countries in the late 1990's and early 2000's, and for 2 decades, children in these countries have received only aP vaccinations. The resurgence of *B. pertussis* in these countries, despite high vaccination rates, is hypothesized to be linked to the sole use of aP vaccines, which do not alter *B. pertussis* acquisition and have limited durability (Althouse and Scarpino 2015; WHO 2015). 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 *B. pertussis* infections. The current pertussis vaccine strategies do not provide mucosal immunity or alter *B. pertussis* acquisition and therefore have not been successful in halting human-to-human transmission. As humans are the only known reservoir for *B. pertussis*, targeting colonization would provide a novel approach through mucosal-induced immunity to reduce the *B. pertussis* reservoir in the population and thereby reduce transmission. The timing of aP vaccine introduction has resulted in a school-age population who could benefit from a broader immune response achievable with a live attenuated pertussis vaccine such as BPZE1.

The intranasally administered BPZE1 live attenuated vaccine provides an opportunity to generate a locally effective mucosal antibody response at the site of potential exposure, mimic the route of entry of the wild-type pathogen, and induce a broader immune response as measured by cellular, mucosal, and serum indices. Inducing mucosal immunity with an

attenuated live vaccine delivered through intranasal vaccination could halt progression from upper airway to lower airway disease in BPZE1-vaccinated individuals as well as strengthen herd immunity by reducing upper airway person-to-person transmission. The overall effect would be improved protection of even the most vulnerable from *B. pertussis* through a robust booster vaccination strategy with the best potential to disrupt *B. pertussis* epidemics.

Acellular pertussis vaccines are currently recommended as both primary and booster vaccinations and usually given in a combination vaccine containing diphtheria (D/d), tetanus (T), and pertussis (P/p), with the level of antigen content dictated by standard nomenclature of DTaP (higher antigen content overall) or Tdap (lower antigen content for diphtheria and pertussis). The current recommendation in the United Kingdom includes the childhood booster of Tdap at 3 years of age with no adolescent booster of aP-containing vaccine, whereas some other European/Commonwealth countries and the US have a similar early childhood DTaP vaccination schedule but have added a Tdap booster during the school-age period (up to and including adolescence). This study will investigate BPZE1-induced immune responses in a background of DTaP or Tdap, with the last pertussis-containing booster occurring more than 3 years prior. BPZE1 alone as well as coadministration with Boostrix will be investigated to test safety and whether augmenting/broadening mucosal immune responses are possible in an aP primed-only population. Furthermore, the testing of interference of BPZE1 with all components found in Boostrix will be investigated as a key secondary objective. In addition, demonstrating protection against subsequent colonization using the attenuated challenge model (re-vaccination/attenuated challenge with BPZE1) will be investigated at 3 months following initial vaccination (optional substudy) as a secondary objective. As this population has never received a wP vaccination, this will be the first time that a broader immunization against *B. pertussis* is provided; therefore, additional hypotheses include the following: a single intranasal vaccination with BPZE1 will be sufficient to induce a differential immune response compared to Boostrix (eg, mucosal immunity induction and reduction in *B. pertussis* acquisition), and immunological boosting with re-vaccination/attenuated challenge is not required to achieve mucosal immunity and protection.

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

1.3.1.1 Risks of Study Participation

The risks of study participation include exposure to study vaccine, maintenance of confidentiality, and side effects of blood and nasal sample collections. All risks will be minimized to every extent possible.

1.3.1.2 BPZE1 Risks to Study Subjects

The risks of BPZE1 administration are expected to be minimal and clinically manageable. The attenuated BPZE1 bacteria colonize the upper respiratory tract similarly to wild-type *B. pertussis* and colonization is strictly limited to respiratory epithelium without dissemination of the bacteria outside the respiratory tract, which also excludes systemic bacteraemia of the BPZE1 strain. Clearance of BPZE1 follows kinetics of wild-type clearance over the course of 28 days with the majority of subjects clearing in the first 2 weeks (data on file). In the recent Phase 2b study, systemic and nasal/respiratory reactogenicity events were mainly none to mild in severity with mean duration of 3 days and did not vary between BPZE1 and Boostrix. A second dose of BPZE1 did not induce greater reactogenicity events. No grade 3 reactogenicity events occurred without a secondary cause (unrelated to vaccination). Treatment-emergent AEs were mainly mild and of similar incidence between BPZE1 and Boostrix (first vaccination) and placebo (re-vaccination). There were no SAEs attributed to vaccination in any studies to date.

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 significant coughing (as demonstrated in the Phase 2b study); therefore, transmission of the attenuated strain is highly unlikely. Furthermore, *Bordetella* species have fastidious growth requirements and have limited survival time outside the human body.

B. pertussis has not been shown to be allergenic in any preclinical or clinical studies to date, nor have any of the excipients in the lyophilized formulation been shown to induce allergic reactions. In fact, using a classical murine asthma model, nasal inoculation of BPZE1 10 days before ovalbumin sensitization reduced peri-bronchial inflammation upon ovalbumin

challenge, as compared with the nonvaccinated control group of sensitized mice (Kavanagh et al 2010). However, there remains a theoretical risk of immediate allergic reaction, as is present with any vaccine product and therefore subjects will remain in clinic for 30 minutes of observation following vaccinations.

BPZE1 vaccine or the placebo will be administered intranasally via the MAD attached to a syringe while subjects partially recline or tilt their head with their neck in retraction. The MAD atomizes the liquid vaccine as it exits the syringe to provide a uniform spray to the mucosal surface. Atomization could induce local nasal reactogenicity such as sneezing, irritated nasal passage, cough, sore throat, or in very rare cases, epistaxis. These reactogenicity parameters will be assessed daily for 7 days following any study vaccination and will be attributed to the vaccine.

Chronic carriage of *B. pertussis* has not been reported and BPZE1 has always been shown to clear in prior studies. Long-term BPZE1 colonization is therefore not expected. No cross-contamination between the subjects was observed in the previous clinical trials of BPZE1, nor has any risk to the family members of study subjects been reported; however, as a precaution, subjects who are in close (and repeated) contact with individuals with known immunodeficiency or on immunosuppressant therapy or who reside or may reside with an infant (<6 months of age) during the vaccination period will be excluded from participation in the study. In case of transmission to other humans or accidental exposure, an efficient treatment is a commercially available macrolide (BPZE1, similar to *B. pertussis*, has been shown to be sensitive to erythromycin/azithromycin).

No reproductive toxicology studies have been performed with BPZE1 to date; however, when female mice were vaccinated with BPZE1 shortly before mating, no negative effect on either the pregnancy or their offspring was observed, and the offspring were protected against *B. pertussis* challenge (Feunou et al 2016).

1.3.1.3 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. In rare cases, significant upper extremity swelling may occur after repeated vaccinations with aP vaccines. General adverse reactions include headache, fatigue, gastrointestinal symptoms, and fever.

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.

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

Syncope can occur in association with administration of injectable vaccines, including Boostrix. Syncope can be accompanied by transient neurological signs such as visual disturbance, paraesthesia, 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. For these reasons, subjects with congenital or acquired neurologic conditions will be excluded from this study.

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.

Note that the safety of Tdap-inactivated poliovirus vaccine (IPV) was similar when Tdap-IPV vaccine was administered 1 month after either Td-IPV or placebo in 500 subjects (Beytout et al 2009). At 7 days, 85.1% versus 93.4% of subjects reported at least one reaction at the injection site, mainly pain (82.6% versus 92.1%); 40.5% versus 45.0% reported at least one systemic AE (mainly headache: 26.4% versus 26.0%); fever concerned 1.7% of both groups. No serious vaccine-related AEs were reported. The pertussis immunization lead at the UK Health Security Agency has also confirmed the acceptability of the study approach (personal communication).

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.

Investigators should refer to the product insert provided for Boostrix (BOOSTRIX 2020).

The following section on the possible undesirable effects from Boostrix is provided verbatim from the SmPC (Boostrix 2021):

"*Summary of the Safety Profile*

The safety profile presented below is based on data from clinical trials where Boostrix was administered to 839 children (from 4 to 8 years of age) and 1931 adults, adolescents, and children (from 10 to 76 years of age) (Table 1-1).

The most common events occurring after Boostrix administration in both groups were local injection site reactions (pain, redness, and swelling) reported by 23.7 – 80.6% of subjects in each trial. These usually had their onset within the first 48 hours after vaccination. All resolved without sequelae.

Tabulated List of Adverse Reactions

Adverse reactions reported are listed according to the following frequency:

- *Very common:* *(≥ 1/10)*
- *Common:* *(≥ 1/100 to < 1/10)*
- *Uncommon:* *(≥ 1/1,000 to < 1/100)*
- *Rare:* *(≥ 1/10,000 to < 1/1,000)*
- *Very rare:* *(< 1/10,000)*

Within each frequency grouping, undesirable effects are presented in order of decreasing seriousness.

Clinical Trials

Table 1-1 Adverse Reactions Reported in Clinical Trials with Boostrix

System Organ Class	***Frequency***	***Adverse Reactions***	
		Subjects Aged 4 – 8 years (N=839)	***Subjects Aged 10 – 76 years (N=1931)***
Infections and infestations	*Uncommon*	*Upper respiratory tract infection*	*Upper respiratory tract infection, pharyngitis*
Blood and lymphatic system disorders	*Uncommon*		*Lymphadenopathy*
Metabolism and nutrition disorders	*Common*	*Anorexia*	
Psychiatric disorders	*Very common*	*Irritability*	
Nervous system disorders	*Very common*	*Somnolence*	*Headache*
	Common	*Headache*	*Dizziness*
	Uncommon	*Disturbance in attention*	*Syncope*
Eye disorders	*Uncommon*	*Conjunctivitis*	
Respiratory, thoracic and mediastinal disorders	*Uncommon*		*Cough*
Gastrointestinal disorders	*Common*	*Diarrhoea, vomiting, gastrointestinal disorders*	*Nausea, gastrointestinal disorders*
	Uncommon		*Diarrhoea, vomiting*
Skin and Subcutaneous tissue disorders	*Uncommon*	*Rash*	*Hyperhidrosis, pruritis, rash*
Musculoskeletal and connective tissue disorders	*Uncommon*		*Arthralgia, myalgia, joint stiffness, musculoskeletal stiffness*
General Disorders and administration site conditions	*Very Common*	*Injection site reactions (such as redness and/or swelling), injection site pain, fatigue*	*Injection site reactions (such as redness and/or swelling), malaise, fatigue, injection site pain*
	Common	*Pyrexia (fever ≥ 37.5°C including fever > 39.0°C), extensive swelling of vaccinated limb (sometimes involving the adjacent joint)*	*Pyrexia (fever ≥ 37.5°C), injection site reactions (such as injection site mass and injection site abscess sterile)*
	Uncommon	*Other injection site reactions (such as induration), pain*	*Pyrexia (fever > 39.0°C), influenza like illness, pain*

Reactogenicity After Repeat Dose

Data on 146 subjects suggest that there might be a small increase in local reactogenicity (pain, redness, swelling) with repeated vaccination according to a 0, 1, 6 months schedule in adults (> 40 years of age).

Data suggest that in subjects primed with DTP in childhood, a second booster dose might give an increase of local reactogenicity.

Post-Marketing Surveillance

Because these events were reported spontaneously, it is not possible to reliably estimate their frequency (*Table 1-2*).

Table 1-2 Adverse Reactions Reported with Boostrix During Post-Marketing Surveillance

System Organ Class	*Frequency*	*Adverse Reactions*
Immune system disorders	*Unknown*	*Allergic reactions, including anaphylactic and anaphylactoid reactions*
Nervous system disorders	*Unknown*	*Hypotonic-hyporesponsiveness episodes, convulsions (with or without fever)*
Skin and subcutaneous tissue disorders	*Unknown*	*Urticaria, angioedema*
General disorders and administration site conditions	*Unknown*	*Asthenia*

Following administration of tetanus toxoid-containing vaccines, there have been very rare reports of adverse reactions on the central or peripheral nervous systems, including ascending paralysis or even respiratory paralysis (eg Guillain-Barre syndrome)."

1.3.1.4 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 beyond that given directly into the nasal cavity, since the bacterium is strictly a respiratory

tract organism. Clinical staff members should follow appropriate biological handling procedures.

The attenuated strain of *B. pertussis* (BPZE1) was engineered by genetically altering or removing three *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*. The genetic modifications in BPZE1 strongly increase the in vivo and in vitro safety, as follows:

- 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 highly improbable since *B. pertussis* does not harbour plasmids or conjugative transposons. In addition, *B. pertussis* Tohama I (origin of BPZE1) does not harbour intact prophage genomes and is therefore incapable of producing functional phage particles.

Reversion to wild type has not been demonstrated in vivo or in vitro, including 20 continuous in vitro passages over a period of 20 weeks, as well as 9 in vivo passages through mice over a period of 27 weeks (Feunou et al 2008; Thalen et al 2020).

Due to the robust preclinical safety data, BPZE1 has been classified as a Biosafety Level 1 organism by US and French authorities Republique Francaise Ministere de l'Enseignement Superieur et de la Recherche (French Ministry of Higher Education and Research). Germany, Belgium, Spain, the US, the Netherlands, and Sweden have accepted the Level 1 rating for the purpose of manufacturing and clinical studies.

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.

1.3.2 Known Potential Benefits

The benefits of the study lie primarily in the opportunities to science and humanity. No direct personal benefit from participation in the study can be guaranteed, as the vaccine may or may not confer protection in humans. As Boostrix is an approved vaccine for boosting the immune response to pertussis, tetanus, and diphtheria, a clinical benefit of receiving this vaccine has been established. All subjects will have the benefit of receiving Boostrix either during the study (if randomized to receive Boostrix) or at study conclusion (at the discretion of the investigator).

2 Study Objectives and Endpoints

2.1 Primary Objectives and Endpoints

Immunogenicity	
Primary objectives	**Primary endpoints**
• To demonstrate BPZE1 induction of broad pertussis mucosal secretory immunoglobulin A (S-IgA) immunity is measurable at Day 29 by geometric mean fold rise (GMFR) by antibodies to whole cell extract (WCE) (BPZE1, BPZE1 + Boostrix).	• GMFR of BPZE1 induction of mucosal S-IgA against WCE at Day 29 by treatment group (BPZE1, BPZE1 + Boostrix).
Safety	
Primary objectives	**Primary endpoints**
• To assess reactogenicity by toxicity scoring through 7 days following first study vaccination with any combination of pertussis-containing vaccines (BPZE1, BPZE1 + Boostrix, Boostrix).	• Occurrence of solicited AEs including local, nasal/respiratory, and systemic reactogenicity events through 7 days following first study vaccination with any combination of pertussis-containing vaccines.

2.2 Secondary Objectives and Endpoints

Immunogenicity	
Key secondary objectives	**Key secondary endpoints**
• To demonstrate non-interference of immunoglobulin G (IgG) serum responses for all Boostrix-containing antigens (diphtheria, tetanus, pertussis toxin [PT], filamentous hemagglutinin [FHA], pertactin [PRN]) at Day 29 when BPZE1 is co-administered with Boostrix (BPZE1 + Boostrix vs Boostrix).	• BPZE1 + Boostrix induction of serum IgG against diphtheria, tetanus, and all aP antigens (PT, FHA, PRN) compared with Boostrix at Day 29 (criteria to be assessed for diphtheria and tetanus responses per WHO guidelines).
Colonization (optional substudy only)	
Secondary objective	**Secondary endpoints**
• To describe colonization in each of the groups: BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control.	• Proportion of subjects with colonization at either Day 92 or 99 (*B. pertussis* culture or polymerase chain reaction [PCR]) with prior BPZE1 immunization following re-vaccination/attenuated challenge at Day 85 (BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control). Expressed as absolute proportion (Yes/No).
Mucosal secretory immunogenicity (S-IgA): WCE, PT, FHA, PRN, and any additional anti-pertussis mucosal antibodies identified during assay development using geometric mean titre (GMT) and geometric mean fold rise (GMFR) unless otherwise stated. **NOTE: To also be tested separately in subjects who participate in the optional substudy as exploratory endpoints, to assess response following re-vaccination/attenuated challenge.**	
Secondary objectives	**Secondary endpoints**
• To demonstrate BPZE1 induction of mucosal S-IgA immunity is **non-inferior** to Boostrix at Day 29 as measured by antibodies to WCE (BPZE1 vs Boostrix).	• BPZE1 induction of mucosal S-IgA against WCE compared with Boostrix at Day 29.
• To demonstrate BPZE1 induction of mucosal S-IgA immunity is greater than baseline at Day 29 (BPZE1, BPZE1 + Boostrix).	• BPZE1 induction of mucosal S-IgA at Day 29 compared to baseline.
• To demonstrate BPZE1 induction of mucosal S-IgA immunity remains greater than baseline over the study period and at Day 169/EOS (BPZE1, BPZE1 + Boostrix).	• BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA compared with baseline at Days 85 and 169/EOS (tested independently).
• To demonstrate BPZE1 + Boostrix induction of mucosal S-IgA immunity is non-inferior to BPZE1 at Days 29 and 169/EOS (BPZE1 + Boostrix vs BPZE1).	• BPZE1 + Boostrix induction of mucosal S-IgA compared with BPZE1 at Days 29 and 169/EOS (tested independently).

• To assess if age, time since last aP vaccination (prior to study entry), or other baseline demographics have a differential response to induction of mucosal S-IgA immunity (BPZE1, BPZE1 + Boostrix) at Day 29.	• BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA in covariate analysis by age, time since last aP vaccination (prior to study entry), or other baseline demographics at Day 29.
• To assess if baseline antibody values, by baseline quantile, have variable responses at Day 29 to induction of S-IgA immunity when BPZE1 is given alone or with Boostrix (BPZE1, BPZE1 + Boostrix).	• BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA by baseline quantile at Day 29.
• To assess seroconversion for each of the anti-pertussis S-IgA antibodies at Day 29 (BPZE1, BPZE1 + Boostrix, Boostrix). Additional combinations of antibody response (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) may also be tested.	• Seroconversion rate (2-fold increase over baseline value or a 4-fold increase over the lower limit of assay detection [whenever the baseline value falls below the lower limit of assay detection]) for mucosal S-IgA at Day 29 by treatment group.
• To assess reverse cumulative distribution curves for immune response (S-IgA) at Day 29.	• Reverse cumulative distribution curves of each anti-pertussis mucosal antibodies S-IgA GMT at Day 29 by treatment group.
Serum immunogenicity (IgA and IgG): WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies identified during assay development using GMT and GMFR unless otherwise stated. **NOTE: To also be tested separately in subjects who participate in the optional substudy as exploratory endpoints, to assess response following re-vaccination/attenuated challenge.**	
Secondary objectives	**Secondary endpoints**
• To demonstrate that BPZE1 (BPZE1, BPZE1 + Boostrix) induction of serum immunity is significantly greater than baseline at Day 29.	• BPZE1 and BPZE1 + Boostrix induction of serum immunity (IgA, IgG) at Day 29 compared with baseline (tested independently by treatment group).
• To demonstrate that BPZE1 + Boostrix induction of serum immunity is non-inferior compared to Boostrix at Day 29 (BPZE1 + Boostrix vs Boostrix).	• BPZE1 + Boostrix induction of serum immunity (IgA, IgG) compared with Boostrix at Day 29.
• To demonstrate that BPZE1 (BPZE1, BPZE1 + Boostrix) induction of serum immunity remains significantly greater than baseline over the course of the study and at Day 169/EOS and to assess antibody decay curves for BPZE1, BPZE1 + Boostrix and Boostrix.	• BPZE1 and BPZE1 + Boostrix induction of serum immunity (IgA, IgG) at Day 85 and Day 169/EOS compared with baseline (tested independently by treatment group and day). • Antibody decays (IgA, IgG) over the course of the study (tested independently by treatment group).

• To demonstrate that BPZE1 induction of serum IgA is non-inferior to Boostrix at Day 169/EOS (BPZE1 vs Boostrix; BPZE1 + Boostrix vs Boostrix)	• BPZE1 induction of serum IgA compared with Boostrix at Day 169/EOS. • BPZE1 + Boostrix induction of serum IgA compared with Boostrix at Day 169/EOS.
• To assess if age, time since last aP vaccination (prior to study entry), or other baseline demographics have a differential response to BPZE1 or Boostrix at Day 29 (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) by age, time since last aP vaccination (prior to study entry), or other baseline demographics at Day 29 (tested independently by treatment group).
• To assess if baseline antibody values, by baseline quantile, have variable serum immunity responses at Day 29 when BPZE1 is given alone or with Boostrix or Boostrix alone (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix and Boostrix induction of serum immunity (IgA, IgG) at Day 29 by baseline quantile (tested independently by treatment group).
• To demonstrate that BPZE1 (BPZE1, BPZE1 + Boostrix) induces a measurable functional antibody response (subset of subjects) at Day 29; and to assess whether there is similar magnitude as that seen with Boostrix (BPZE1 vs BPZE1 + Boostrix vs Boostrix).	• BPZE1 and BPZE1 + Boostrix induction of measurable functional antibody (subset of subjects) compared with Boostrix at Day 29 (tested independently by treatment group).
• To assess for seroconversion at Days 29 and 85 (BPZE1, BPZE1 + Boostrix, Boostrix). Additional combinations of antibody response (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) may also be tested.	• Seroconversion rate for serum immunity (IgA, IgG) defined as a 2-fold increase over baseline value; or a 4-fold increase over the lower limit of assay detection (whenever the baseline value falls below the lower limit of assay detection) at Days 29 and 85 (tested independently by treatment group and day).
• To assess reverse cumulative distribution curves for immune responses (IgA and IgG) at Day 29.	• Reverse cumulative distribution curves of GMT at Day 29 by treatment group.
Safety **NOTE: Subjects participating in the substudy will be evaluated for safety endpoints in a separate evaluation after Day 85 re-vaccination/attenuated challenge.**	
Secondary objectives	**Secondary endpoints**

• To describe reactogenicity events during the 7 days following any study vaccination. Primary and re-vaccination/attenuated challenge (substudy subjects) to be described separately, by treatment group. Reactogenicity will be classified by local, nasal/respiratory, and systemic.	• Maximum daily severity during the 7 days following study vaccination (by treatment group). • Incidence by toxicity grade during the 7 days following study vaccination (by treatment group). • Mean response (for any measurements) and duration during the 7 days following study vaccination (by treatment group).
• To describe (severity and relationship to study vaccination) all AEs through 28 days following any study vaccination. Primary and re-vaccination/attenuated challenge (substudy subjects) to be described separately and by treatment group.	• All AEs through 28 days following study vaccination by severity and relationship to study vaccination (by treatment group).
• To describe (severity and relationship to study vaccination) any medically attended AE (MAAE) through 84 days following any study vaccination. Primary and re-vaccination/attenuated challenge to be described separately and by treatment group.	• All MAAEs through 84 days following study vaccination by severity and relationship to study vaccination, and by time since study vaccination (by treatment group).
• To describe any AE of special interest (AESI) and SAE through Day 169/EOS including relationship to study vaccination.	• All AESIs and SAEs through Day 169/EOS by severity and relationship to study vaccination, and by time since study vaccination (by treatment group).
• To describe any severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or AESI associated with COVID-19 disease if the COVID-19 pandemic remains ongoing in the region or if there are mass vaccinations using a COVID-19 vaccine during study conduct.	• Incidence of SARS-CoV-2 infections or AESIs associated with COVID-19 disease by severity, and by time since study vaccination (by treatment group).
• To assess proportion of subjects (subset of subjects) cleared from colonization (present/absent) through Day 85.	• *B. pertussis* clearance following study vaccination (subset of subjects at varying time points through Day 85).
Mucosal secretory immunogenicity (S-IgA) (optional substudy only) **Note that S-IgA objectives and endpoints for the overall study (as noted above) will also be analysed for the optional substudy and will be considered exploratory.**	
Exploratory objectives	**Exploratory endpoints**

• To evaluate mucosal immunity of individual and combinations of pertussis antibodies (eg, WCE, PT, FHA and PRN and other known anti-pertussis mucosal antibodies) are relational to protection against colonization (BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control).	• Induction of mucosal S-IgA at Day 29 or 85 (tested independently) compared by protection against colonization (Colonizers/Non-colonizers) by treatment group (BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix control).
• To measure BPZE1 induction of mucosal immunity (S-IgA) at Day 113 by treatment group; and to assess if induction of a boost response (relative to Day 85 response) is observed for those subjects with prior BPZE1 vaccination (BPZE1, BPZE1 + Boostrix).	• Induction of mucosal S-IgA (WCE, PT, FHA, PRN and any additional anti-pertussis mucosal antibodies identified during assay development) at Day 113 compared with Day 85 following BPZE1 attenuated challenge by treatment group (BPZE1, BPZE1 + Boostrix).
• To assess if Day 113 response and longer-term durability through Day 169/EOS (absolute GMT) is altered with re-vaccination/attenuated challenge when compared to those subjects who did not participate in the substudy, relative to each randomized treatment group (BPZE1, BPZE1 + Boostrix, Boostrix).	• Induction of mucosal S-IgA at Days 113 and 169/EOS (tested independently) in subjects who participated in the substudy compared with subjects who did not participate in the substudy by treatment group.
Serum immunogenicity (IgA and IgG) (optional substudy only): WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies identified during assay development using GMT and GMFR unless otherwise stated. **Note that serum IgA and IgG objectives and endpoints for the overall study (as noted above) will also be analysed for the optional substudy and will be considered exploratory.**	
Exploratory objectives	**Exploratory endpoints**
• To measure BPZE1 induction of serum immunity at Day 113; and to assess if induction of a boost response (relative to Day 85 response) is observed for those subjects with prior BPZE1 vaccination (BPZE1, BPZE1 + Boostrix).	• BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) at Day 113 compared with baseline (by treatment group). • BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) at Day 113 compared with Day 85 (by treatment group).
• To assess if Day 113 response and longer-term immune durability through Day 169/EOS is altered with re-vaccination/attenuated challenge when compared to those subjects who did not participate in the substudy, relative to each randomized treatment group (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) at Days 113 and 169/EOS in subjects who participated in the substudy compared with subjects who did not participate in the substudy (tested independently by treatment group and day).

• To assess if serum immunity of individual and combinations of pertussis antibodies (eg, WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) at Day 29 or 85 is relational to protection against colonization by treatment group and overall (BPZE1, BPZE1 + Boostrix, Boostrix).	• BPZE1, BPZE1 + Boostrix, BPZE1 **and** BPZE1 + Boostrix, Boostrix induction of serum immunity (IgA, IgG) at Day 29 or Day 85 compared by protection against colonization (Colonized/Non-Colonized) (tested independently by treatment group and overall).
• To assess functional antibody response by treatment group at Day 113 compared to baseline and Day 29, as measured by an appropriate assay for bactericidal activity.	• Proportion of subjects with ≥50% killing (or equivalent) for functional antibody response at Day 113 compared to baseline and Day 29 (tested independently by treatment group and day).
Colonization (optional substudy only)	
Exploratory objective	**Exploratory endpoints**
• To demonstrate prior vaccination with BPZE1 substantially reduces colonization at Day 92 and Day 99.	• *B. pertussis* colony counts at each of Days 92 and Day 99 by treatment group (mean counts, area under the curve [AUC] or similar total bacterial load assessments will also be calculated). • Subjects with colonization at each of Days 92 and 99 by treatment group. Expressed as absolute proportion (Yes/No) and percent reduction relative to control.

2.3 Other Exploratory Objectives and Endpoints

Other Exploratory Objectives	**Other Exploratory Endpoint(s)**
• To assess cellular-mediated response (by ELISpot or flow cytometry) to BPZE1 and Boostrix (Th1 and Th2) in a subset of subjects.	• Cellular-mediated response at baseline and Day 8 (safety lead-in cohort) and Days 85 and 113 (subset of substudy subjects).
• To further characterize mucosal and serum immunological responses over time, relative to baseline status and to vaccination response, with any future assays developed for BPZE1.	• Immunologic response measurement by treatment group.

3 Investigational Plan

3.1 Study Design

This is a multi-centre, randomized, observer-blinded, placebo- and active comparator-controlled trial with a 6-month safety follow-up after first study vaccination. After signing the informed consent form (ICF; or providing assent when necessary) and meeting all inclusion and none of the exclusion criteria, eligible subjects will receive an intranasal vaccination with either BPZE1 vaccine or formulation buffer (placebo) via the MAD and an intramuscular (IM) injection of either Tdap vaccine (i.e., Boostrix) or sterile normal saline (placebo) on Day 1. To maintain the blind, placebo vaccination via intranasal and IM routes, using formulation buffer and normal saline, respectively, will be included, and an unblinded team will manage vaccine logistics, preparation, and administration (when needed to maintain blinding) but will not be involved in study-related assessments or have subject contact for data collection after study vaccination.

Approximately 360 subjects will be enrolled and randomly assigned (1:1:1) to 1 of 3 treatment groups (refer to Table 3-1) to contribute to safety and efficacy assessments. Enrolment will be operationally managed at the study level to maintain a minimum number of subjects enrolled in 2 age groups: 6 to 10 years, inclusive; and 11 to 17 years, inclusive. It is estimated that a 10% dropout rate will occur, allowing for approximately 108 evaluable subjects per treatment group.

Table 3-1 Dosing Scheme

Treatment Group	n	Intranasal Vaccination[a] (Day 1)		IM Vaccination[b] (Day 1)	
		BPZE1	Placebo	Boostrix	Placebo
A	120	X	-	-	X
B	120	X	-	X	-
C	120	-	X	X	-

[a] Intranasal application of ~0.4 mL per nostril (0.8 mL total volume delivered). Intranasal vaccination will occur prior to IM vaccination.

[b] IM injection of 0.5 mL to the deltoid region.

All subjects will be monitored for at least 30 minutes after final study vaccination. On Day 1, intranasal vaccination will occur prior to IM vaccination with a minimum of 10 minutes (minimum of 30 minutes for subjects in the safety lead-in cohort of 11 to 17 year olds)

between intranasal and IM vaccinations. Immediate reactogenicity will be assessed at 30 minutes (+30 minutes) after the last study vaccination of the Day (ie, after IM vaccination on Day 1, after nasal vaccination on Day 85), prior to release from clinical observation.

All subjects will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following any study vaccination (retrospective of the highest value in the previous 24 hours). Subject diaries will be age-adjusted as appropriate. Local (arm; after IM injection only), nasal/respiratory, and general systemic reactogenicity events will be recorded, including start and stop dates and including any actual measurements (ie, body temperature, redness and/or swelling at the injection site). Trained clinical staff will review the information from the subject diary with the subjects at the following visit and apply a standard toxicity grade to any reactogenicity event. Subjects will be instructed that should they have reactogenicity with a potential toxicity grade 3 at any time, they should contact the site on the same day and be seen or referred to a qualified medical facility within 24 hours.

As this is the first study of BPZE1 in school-age children, a staggered enrolment is planned. The first 45 subjects enrolled will be in the older age group (11 to 17 years, inclusive) and designated the safety lead-in cohort. Reactogenicity will be reviewed with these subjects during a supplemental visit 7 days following first study vaccination. After reactogenicity for the safety lead-in cohort is reviewed by the medical monitor, medical lead, and Safety Monitoring Committee (SMC) chairman (with appropriate consultation with investigators, if needed), the remainder of subjects will be enrolled.

All AEs will be monitored through 28 days after any study vaccination; all MAAEs will be monitored through 84 days following any study vaccination; and all AESIs and SAEs will be monitored through Day 169/EOS. Due to COVID-19 pandemic, a SARS-CoV-2 infection (confirmed by testing within the public health system) and any associated symptoms will be collected on a unique COVID-19 electronic case report form (eCRF) through Day 169/EOS if the COVID-19 pandemic remains ongoing in the region or if there are mass vaccinations using a COVID-19 vaccine during study conduct. Vaccines received outside of the study will be specifically queried at each visit and recorded through Day 169/EOS.

An informational card will be provided to all subjects indicating their participation in this vaccination study to prevent subjects from inadvertently receiving any scheduled/planned Boostrix-containing vaccines (tetanus, pertussis, diphtheria) during study conduct. Any

enrolled subject who does not receive Boostrix during the study will be offered Boostrix at the conclusion of the study (following unblinding); this Boostrix 'catch-up' will be provided outside of the study schedule, at the discretion of the investigator, with vaccine provided by the sponsor.

Nasal and blood samples for immunogenicity assessments will be collected before vaccination and at selected time points following study vaccination. Mid-turbinate/nasopharyngeal sample collections will occur at selected time points following study vaccination to assess initial colonization (safety lead-in cohort only, Day 8) and to assess for clearance (remainder of cohort, either Day 29 or Day 85). A subset of subjects will also have the option to provide extra blood for cellular-mediated immunity assessments at baseline and Day 8 (safety lead-in cohort) and Days 85 and 113 (subgroup optional in substudy).

Due to the uncertainty with movement and/or isolation practices associated with the COVID-19 pandemic, telemedicine and remote sampling may be initiated with proper investigator and/or medical oversight.

The primary database lock will occur after all data are clean and all planned study procedures through Day 169/EOS (6 months) are complete. An interim database lock may occur after all subjects have completed Day 29 and sample testing for all primary and select secondary immune endpoints have been completed (mucosal and serum), but results will only be provided at treatment assignment level to maintain the blind. The site and those interacting directly with the site or subject data (eg, contract research organization [CRO], laboratories) will remain blinded to subject assignment until EOS or end of sample testing, depending on the activities of the associated entities.

Vaccination pause rules (refer to Section 6.5) will be in place with a review by the SMC initiated if any rule is met. In addition, the medical monitor and medical lead are empowered to request a review by the SMC (refer to Section 11.1.1.1) at any time for any safety reason.

3.1.1 Substudy: Attenuated Challenge With BPZE1 Following Prior Vaccination with BPZE1, BPZE1 + Boostrix, or Boostrix Alone

This is an optional open-label substudy in which enrolled subjects may elect to participate at any time from the day of enrolment through Day 85. A separate signed ICF (and separate

assent when necessary) will be obtained prior to participation in the substudy. Subjects will be excluded from participating in the substudy if they meet additional exclusion criteria listed in Section 4.1.2.1. Temporary vaccination holding criteria are to be followed for the Day 85 vaccination.

Up to 120 enrolled subjects may participate in the substudy. The duration of the entire study will not change; however, additional visits/procedures starting on Day 85 will include the following:

- 3 additional visits (Days 92, 99, and 113)
- 1 additional intranasal vaccination (Day 85)
- Up to 3 additional nasal samples, including 2 of those needed for mid-turbinate/nasopharyngeal sample collection (Days 92 and 99) and 1 needed for leukosorb (Day 113). Subjects will contribute to all Day 85 sampling (systemic and nasal)
- Up to 2 additional blood samples (Days 85 [if not already assigned to provide] and 113, including blood for cellular-mediated and systemic immunity responses)
- 1 additional subject diary (subjects to record maximum daily reactogenicity [nasal/respiratory, systemic] in a subject diary for 7 consecutive days starting the day following study vaccination)
- 1 additional test for SARS-CoV-2 within 72 hours prior to Day 85 vaccination

All subjects participating in the open-label substudy will receive an intranasal re-vaccination/attenuated challenge with BPZE1 on Day 85. Subjects may have the planned re-vaccination/attenuated challenge deferred to a later date (up to 20 days) if pre-defined temporary exclusion criteria are met (Section 4.1.2.1) or for other personal reasons (eg, holiday, school schedule). Subjects will be monitored for at least 30 minutes after re-vaccination/attenuated challenge, with a post-vaccination evaluation for reactogenicity (nasal/respiratory, and systemic) completed prior to release from clinical observation.

Subjects will receive a subject diary on Day 85. All subjects (with assistance when needed) will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following re-vaccination/attenuated challenge (retrospective of the highest value in the previous 24 hours). Subject-reported symptom diaries will be age-adjusted as appropriate.

Nasal/respiratory and general systemic reactogenicity events will be recorded, including measurements of body temperature. Trained clinical staff will review the information from the subject diary with the subjects at the following visit and apply a standard toxicity grade to any reactogenicity event. Subjects will be instructed that should they have reactogenicity with a potential toxicity grade 3 at any time, they should contact the site on the same day and be seen or referred to a qualified medical facility within 24 hours.

Nasal and blood samples for immunogenicity assessments will be collected before re-vaccination/attenuated challenge and at selected time points thereafter. Mid-turbinate/nasopharyngeal sample collections will be performed at selected time points to assess BPZE1 colonization (*B. pertussis* culture or PCR). Some subjects participating in the substudy may also provide extra blood (optional) for cellular-mediated immunity assessments on Day 85 and Day 113 (optional extra blood collection for up to 45 substudy participants).

All AEs will be collected following re-vaccination/attenuated challenge on Day 85 through Day 113. Following Day 113, only MAAEs, AESIs, and SAEs will be collected though Day 169/EOS.

4 Subject Selection and Withdrawal Criteria

4.1 Selection of Study Population

Approximately 360 subjects will be enrolled at approximately 30 centres in the United Kingdom, European/Commonwealth countries and/or Costa Rica. Subjects will be enrolled 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. The subject is male or female 6 to 17 years of age, inclusive, on Day 1 (day of study vaccination).
2. The subject (and/or legal guardian) is capable of understanding the written informed consent, providing signed and witnessed written informed consent (or assent, depending on age), and understanding and complying with protocol requirements.
3. Female subjects of reproductive age must be nonpregnant and nonlactating, and if of childbearing potential (defined as any female who has experienced menarche at any time prior to or during the study), must agree to be heterosexually inactive from at least 21 days prior to enrolment and through 90 days following any study vaccination or agrees to consistently use any of the following methods of birth control from at least 21 days prior to enrolment and through 90 days following any study vaccination:
    a) Condoms (male or female) with or without spermicide*
    b) Diaphragm with or without spermicide*
    c) Cervical cap with or without spermicide*

    * **NOTE**: The highest level of protection is required, in accordance with local regulations.

    d) Intrauterine device

e) Oral or patch contraceptives

f) Norplant®, Depo-Provera®, or other acceptable method of birth control that is designed to protect against pregnancy

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

4. The subject has a stable health status, as established by physical examination, vital sign measurements (body temperature and heart rate), and medical history.
5. The subject (and/or legal guardian) has access to a consistent and reliable means of electronic or telephone contact, which may be in the home, workplace, school, or by personal mobile electronic device.
6. The subject is willing to refrain from routine nasal sprays (including steroid sprays) or washes for at least 7 days following any study vaccination.
7. The subject (and/or legal guardian) lives within a reasonable distance from the clinical site, is willing and able to travel to and from the clinical site for follow-up visits, and agrees to seek medical evaluation in the event of an AE requiring acute assessment.
8. The subject (and/or legal guardian) 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 agrees to provide updated contact information, as necessary.

4.1.2 Exclusion Criteria

Subjects meeting any of the following criteria (by review of medical history and subject intake) will be excluded from the study:`

1. The subject has a history of pertussis-containing vaccination (inclusive of school vaccination programs) or documented *B. pertussis* infection within 3 years prior to Day 1 and/or a history of Td-containing vaccination (without pertussis vaccine component) within 1 month prior to Day 1.

2. The subject has a chronic significant illness actively being treated or a history of recent intervention for worsening or fluctuating symptoms (at the discretion of the investigator).
3. The subject has a history of cancer (malignancy).
4. The subject has a congenital, hereditary, or acquired disease or disorder classified as autoimmune, immunodeficient, coagulopathy, hepatic, renal, neurologic (including a history of Bell's palsy), or moderate to severe cognitive. (Attention deficit hyperactivity disorder permitted.)
5. The subject currently uses smoking products (including vaping and e-cigarettes) and is unwilling to refrain from use from Day 1 through Day 29 following any study vaccination.
6. The subject has received immunoglobulin, blood-derived products, systemic corticosteroids (at a dose of >10 mg per day for more than 10 days), or other immunosuppressant drugs within 90 days prior to Day 1.
7. The subject has any chronic pulmonary disease requiring active medication or pulmonary therapies, with the following exceptions:
    a) Subjects with exercise-induced bronchospasm, if currently well controlled, who are willing to refrain from intense exercise for 7 days following study vaccination or
    b) Subjects with Step 1 (intermittent) asthma classification who have not had an exacerbation requiring oral systemic corticosteroids in the past year; have an FEV1 documented to be >80%; do not have restrictions in normal activity due to breathing issues; and have used a short-acting beta-agonist less than or equal to 2 days per week over the past 2 months.

    NOTE: Subjects in the safety lead-in cohort are not allowed to have these exceptions.
8. The subject has a history of oro/nasopharynx surgery (eg, adenoidectomy, tonsillectomy) within 60 days prior to Day 1.
9. The subject has a known hypersensitivity to latex or any component of any study vaccine. Specific to Boostrix: the subject has known hypersensitivity to neomycin or

polymyxin; hypersensitivity after previous administration of diphtheria, tetanus, or pertussis vaccines; or has experienced transient thrombocytopenia or neurological complications following an earlier immunisation against diphtheria and/or tetanus.

10. The subject has participated in any other clinical trial for the testing of an unlicensed product where such product was received within 6 months prior to Day 1 or is planning to participate in any other clinical trial during study conduct through Day 85 following last study vaccination.

    NOTE: Exceptions may be considered for an investigational COVID-19 vaccine if the product has entered into adult Phase 3 efficacy trials or is part of a mass vaccination rollout in this age group of subjects within a country. Direct guidance will be provided by the sponsor prior to study initiation.

11. The subject has routine and/or repeated contact with, or is currently living in a household with, an immunocompromised individual.
12. The subject has 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, eating disorder, major depression or anxiety disorder, history of alcohol or drug use).
13. The subject is a study team member or first-degree relative of a study team member.
14. The subject resides in a residence during the vaccination period (defined as through Day 85 following any study vaccination) where an infant less than 6 months of age resides or may reside.

4.1.2.1 Additional Exclusion Criteria (Substudy Subjects ONLY)

Subjects meeting any of the following criteria will be excluded from participation in the optional substudy (Section 3.1.1):

15. Has received a pertussis-containing vaccination outside of study conduct since enrollment.
16. Has received any form of immunotherapy determined to potentially have an effect on immune response (as determined by medical consultation, when necessary).

4.1.3 Other Considerations for Temporarily Holding Vaccination (Days 1 or Substudy Day 85)

Subjects meeting any of the following criteria may have a planned study vaccination deferred to a later date, but these criteria are not exclusionary for study enrolment/participation. For Visit 1, Day 1, subjects may be enrolled on a return visit if they remain within 45 days of consenting. Otherwise, subjects must be reconsented and reassessed per the inclusion and exclusion criteria (ie, re-enrolled).

- The subject has or reports acute respiratory tract symptoms/infection or rhinorrhoea within the past 3 days. Subject may be vaccinated once all symptoms have been resolved for at least 3 days.
- The subject has or reports a body temperature of 100.4°F (38°C) or higher within the past 3 days. The subject may be vaccinated once the fever resolves and body temperature (site-measured or subject-measured) remains below 100.4°F (38°C) for at least 3 days.
- The subject used antibiotics within the past 10 days.
- The subject received a licensed vaccine (including any vaccine under emergency use authorization) within the past 14 days.
- The subject has a positive SARS-CoV-2 test result (eg, infection is ongoing) within the 3 days preceding vaccination. For those subjects testing positive, they must have a subsequent negative SARS-CoV-2 test result prior to vaccination (visit window may be extended to accommodate retesting needs).

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 (whether in person or via telemedicine).

4.2.1 Reasons for Withdrawal/Discontinuation

Subjects may withdraw/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 centre. Every effort

should be made to keep subjects in the study for safety monitoring (at a minimum), which includes telemedicine/phone call for long-term safety follow-up (in such a case subjects will not be considered 'discontinued'). The reasons for subjects not completing the study will be recorded. A subject may be withdrawn from the study for any of the following reasons:

1. Significant noncompliance with the protocol, as determined by the medical monitor(s).
2. Serious or intolerable AE(s) that in the investigator's opinion requires withdrawal or discontinuation from the study; however, every effort should be made to keep the subject in the study for safety follow-up via telemedicine (eg, telephone, internet).
3. The subject is lost to follow-up.
4. The subject withdraws consent.
5. The investigator decides it is in the subject's best interest to discontinue the study; however, every effort should be made to keep the subject in the study for safety follow-up via telemedicine (eg, telephone, internet).
6. The sponsor decides to discontinue the subject's participation in the study or to terminate the study.

Upon occurrence of a serious or intolerable AE, the investigator will confer with the sponsor prior to withdrawal of a subject. Any subject may withdraw his or her consent at any time.

4.2.2 Handling of Withdrawals

When a subject withdraws from the study, the reason(s) for withdrawal shall be recorded by the investigator on the relevant page in the eCRF.

Whenever possible, rather than lose subjects to withdrawal, any subject enrolled and vaccinated prior to withdrawal will be given the option to be followed for safety follow-up via telemedicine (eg, phone, internet) if they refuse further protocol-specified procedures. In addition, subjects who fail to return for final assessments will be contacted by the clinical site in an attempt to have them followed for safety follow-up via telemedicine. In all such cases if a phone call/telemedicine contact is made for safety follow-up then the subject is still

considered as enrolled and not discontinued. A subject will not be considered lost to follow-up until every attempt to contact the subject has been made, at minimum 2 (documented) telephone calls, followed by a registered letter. Every effort should be made to collect safety data on subjects through the Day 169/EOS visit.

4.2.3 Replacements

Subjects who subsequently withdraw, are terminated from the study, or are lost to follow-up prior to completing Day 29 assessments may be replaced during the enrolment period at the discretion of the sponsor.

5 Study Treatments

5.1 Method of Assigning Subjects to Treatment Groups

Subjects will be randomly assigned to 1 of 3 treatment groups, in a 1:1:1 ratio, as presented in Table 3-1, and operationally managed to maintain a minimum number of subjects enrolled in 2 age groups: 6 to 10 years, inclusive; and 11 to 17 years, inclusive. A staggered enrolment is planned: the first 45 subjects enrolled will be in the older age group of 11 to 17 years, inclusive, and designated the safety lead-in cohort. After reactogenicity for the safety lead-in cohort is reviewed by the medical monitor, medical lead, and SMC chairman (with appropriate consultation with investigators, if needed), the remainder of subjects will be enrolled. An interactive response technology (IRT) system will be used to administer the randomization schedule centrally. The IRT system will also be designed to distribute the full cohort into groups to provide mid-turbinate/nasopharyngeal samples for *B. pertussis* culture or PCR on Day 29 or 85; however, if a subject cannot provide a sample on Day 29 (for any reason), the subject should provide a sample on Day 85 (this will not be considered a protocol deviation). Biostatistics will generate the randomization schedule using SAS® software Version 9.3 or later (SAS Institute Inc, Cary, North Carolina) for the IRT, 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 an unblinded team 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 vaccination.

Enrolled subjects may participate in an optional substudy in which subjects may elect to participate at any time from the day of enrolment through Day 85. No randomization is planned for the substudy as all subjects participating in the open-label substudy will receive an intranasal re-vaccination/attenuated challenge with BPZE1 on Day 85. It is recognized this may introduce some subject bias due to the opt-in nature, but this has been deemed acceptable by the sponsor.

5.2 Treatments Administered

Subjects in Treatment Group A (N ~120) will receive an intranasal application of 10^9 CFU BPZE1, with approximately 0.4 mL per nostril (0.8 mL total volume delivered); and an IM injection of 0.5 mL placebo (sterile normal saline for injection) to the deltoid region on Day 1. Intranasal application of BPZE1 or placebo should precede IM injection of Boostrix or placebo by at least 10 minutes (minimum of 30 minutes for subjects in the safety lead-in cohort of 11 to 17 year olds). For the safety lead-in cohort, a minimum of 30 minutes must occur between intranasal and IM vaccinations on Day 1.

Subjects in Treatment Group B (N ~120) will receive an intranasal application of 10^9 CFU BPZE1, with approximately 0.4 mL per nostril (0.8 mL total volume delivered); and an IM injection of 0.5 mL Boostrix (aP vaccine, manufactured by GlaxoSmithKline) to the deltoid region on Day 1. Intranasal application of BPZE1 or placebo should precede IM injection of Boostrix or placebo by at least 10 minutes (minimum of 30 minutes for subjects in the safety lead-in cohort of 11 to 17 year olds).

Subjects in Treatment Group C (N ~120) will receive an intranasal application of lyophilized buffer reconstituted in sterile water, with approximately 0.4 mL per nostril (0.8 mL total volume delivered); and an IM injection of 0.5 mL Boostrix (aP vaccine, manufactured by GlaxoSmithKline) to the deltoid region on Day 1. Intranasal application of BPZE1 or placebo should precede IM injection of Boostrix or placebo by at least 10 minutes (minimum of 30 minutes for subjects in the safety lead-in cohort of 11 to 17 year olds).

All subjects participating in the optional substudy will receive a second intranasal application of 10^9 CFU BPZE1, with approximately 0.4 mL per nostril (0.8 mL total volume delivered) on Day 85.

All subjects will be monitored for study vaccination-related events for at least 30 minutes following the last vaccination (eg, 30 minutes following IM vaccination).

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. Refer to the most recent version of the investigator's brochure (IB) for additional details.

The sponsor will provide the CRO with the investigational vaccine and lyophilized buffer, properly labelled, and the CRO will ensure labelling complies with local regulations and is distributed to the clinical sites.

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

Product	**Dosage and Route of Administration:**
BPZE1	Reconstituted with sterile water to provide 10^9 CFU per 0.8 mL; administered intranasally, with approximately 0.4 mL per nostril (0.8 mL total volume delivered), by syringe with MAD
Boostrix (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed) injectable suspension, for IM use	Administered as a single 0.5 mL IM injection to the deltoid region
Intranasal placebo (lyophilized BPZE1 buffer reconstituted with sterile water)	Administered intranasally, with approximately 0.4 mL per nostril (0.8 mL total volume delivered), by syringe with MAD
IM placebo (sterile normal saline for injection)	Administered as a single 0.5 mL IM injection to the deltoid region

The intranasal placebo consists of lyophilized buffer reconstituted with sterile Water for Injection (WFI), which has the same constituents in approximately the same quantities as the BPZE1 investigational vaccine excluding the attenuated *B. pertussis* cells, Tris buffer, and sodium chloride. The IM placebo is sterile normal saline for injection.

A detailed description regarding the storage, reconstitution, and handling of investigational vaccines will be in the pharmacy manual.

5.3.1 Investigational Product Packaging and Storage

BPZE1 and lyophilized placebo will be shipped from the sponsor's European contract manufacturing organization to the CRO's repository and distribution centre. 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 aluminium 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 will be packaged in a similar fashion. A 1:1 subject to vial randomization is planned.

Boostrix will be procured from commercial stock and supplied as either single dose vials (ideally) or prefilled syringes containing a 0.5 mL suspension for injection. The IM placebo will be sterile normal saline for injection from a commercial supplier, supplied in single- or multi-dose vials, and must be appropriately blinded at the syringe level to coincide with Boostrix supply.

Study vaccines must be stored in a secure area (eg, locked refrigerator/freezer), protected from light and moisture, and kept at a controlled temperature. Refer to the IB and pharmacy manual for specific storage conditions as appropriate for BPZE1 and comparators. BPZE1 and formulation buffer control is to be stored at 8°C or lower at all times.

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.

Supplies for sample collection will be provided in sufficient quantity. 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 reconstitution, 25G or smaller needles for vaccination (age-appropriate), syringes (1-mL tuberculin) for IM and intranasal vaccinations and for reconstitution purposes, sterile WFI, normal saline, the MAD, and blinding tape, will be supplied. All supplies can be stored at ambient temperature in a secure area.

Mucosal atomization devices (MADs) with CE marking will be provided to be used with a 1-mL tuberculin syringe for each intranasal vaccination. The MAD is a conical shape plastic component that Luer-locks onto a 1-mL syringe. The MAD 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 to provide broad mucosal surface delivery.

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 centre. 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 deviation 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. BPZE1 is

a live attenuated *B. pertussis* bacterium and is susceptible to an erythromycin/azithromycin antibiotic treatment.

5.5 Blinding

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

The optional substudy includes an open-label re-vaccination/attenuated challenge with BPZE1.

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 will have direct access to unblinding and may unblind an individual subject's treatment allocation.

The treatment assignment for a subject can be unblinded by request of the investigator using the IRT system at the site. 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. An IRT-triggered notification indicating that a code break has occurred will be sent to the designated team following unblinding, which will include the role performing the code break.

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 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 (refer to Table 13-1)

will be flagged in the clinical database unless noted as an allowance in the protocol (eg, acute illness, vaccination pause).

5.7 Prior and Concomitant Medications, Therapies, or Vaccines

Restrictions for prior medications, therapies, or vaccines are provided in Section 4.1.

Administration of concomitant medications, therapies, or vaccines will be recorded in the eCRF. Concomitant medications will include all medications and over-the-counter (OTC) products (including herbal supplements and multivitamins) taken by the subject from the time of signing the ICF through Day 29 (and from Day 85 [following study vaccination] through Day 113 for subjects participating in the optional substudy), or through an early termination visit, if prior to these time points. The use of anti-pyrogenic medication will be specifically queried by diary during the 7 days following any study vaccination. Concomitant medications received associated with any MAAE will be recorded through 84 days following any study vaccination; and concomitant medications received associated with any AESI or SAE will be recorded through Day 169/EOS.

Vaccines received outside of the study will be specifically queried during each visit and recorded through Day 169/EOS. Necessary vaccinations (eg, seasonal influenza, COVID-19, standard pediatric vaccines such as HPV, IPV, MenACWY) are allowed during the study, but should ideally be delayed until at least 28 days after any study vaccination whenever possible. Administration of tetanus and diphtheria toxoids (Td) vaccine during the first 90 days of the study is allowed only for emergency needs, but not as a standard schedule.

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

6 Study Assessments and Procedures

6.1 Demographic and Baseline Data

Demographic and baseline data, such as date of birth (where allowed by local regulations), sex, race, ethnicity, weight, and height (with derived body mass index [BMI] and Z-score when appropriate for age), should be collected at screening and recorded in the eCRF.

6.2 Medical History

A medical history (including vaccination history, prior and concomitant medical conditions, and surgeries/significant procedures for medical conditions [eg, endoscopy, tonsillectomy]) will be collected at screening and reviewed and/or updated on Day 1 if screening occurs prior to Day 1.

Ideally, the subject will provide a vaccination card (or equivalent) or the subject's vaccination history can be accessed via a centralized vaccination register to record last known pertussis vaccination. If the subject's vaccination history is not available, the timing of prior pertussis vaccine may be estimated based on the national vaccination schedule appropriate for the age of subject.

6.3 Safety Assessments

Safety assessments for all subjects will include the following: vital sign measurements (including body temperature and heart rate), targeted physical examinations, reactogenicity, and AEs (including MAAEs, AESIs, and SAEs). The timing and frequency of all safety assessments are listed in the schedules of events (refer to Table 13-1).

6.3.1 Vital Sign Measurements

Vital sign measurements will include body temperature (oral, tympanic, or noncontact) and heart rate. If temperature is to be taken orally, the subject must not eat or drink anything hot or cold within 10 minutes prior to measurement.

6.3.2 Post-Vaccination Evaluation

All subjects will be monitored for at least 30 minutes after final study vaccination. The following evaluations will be performed:

1. Obtain heart rate (refer to Table 13-1).
2. Assess for reactogenicity based on injection site examination with FDA toxicity grade (refer to Section 6.3.5.1.2)
3. Assess for reactogenicity based on nasal/respiratory and systemic (including oral temperature) FDA toxicity grading scales (refer to Section 6.3.5.1.2)

Immediate reactogenicity (including grade any actual measurements) will be recorded at 30 (+30) minutes following study vaccination. On Day 1, intranasal vaccination will occur prior to IM vaccination with a minimum of 10 minutes (minimum of 30 minutes for subjects in the safety lead-in cohort of 11 to 17 year olds) between intranasal and IM vaccinations. Immediate reactogenicity will be assessed at 30 minutes (+30 minutes) after the last study vaccination of the Day (ie, after IM vaccination on Day 1, after nasal vaccination on Day 85), prior to release from clinical observation.

All subjects will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following any study vaccination (retrospective of the highest value in the previous 24 hours). Subject diaries will be age-adjusted as appropriate. In addition, a measuring tool for measuring injection site erythema and swelling (after IM injection only) and a thermometer for measuring body temperature (oral or tympanic) will also be distributed to subjects. The use of anti-pyrogenic medication will also be specifically queried by subject diary during the 7 days following study vaccination.

Local (arm; after IM injection only), nasal/respiratory, and general systemic reactogenicity events will be recorded, including start and stop dates, and including actual measurements of body temperature, and if applicable, swelling and redness at the injection site (after IM injection only). Trained clinical staff will review the information from the subject diary with the subjects at the following visit and apply a standard toxicity grade (refer to Section 13.2) to any reactogenicity event. Subjects will be instructed that should they have reactogenicity with a potential toxicity grade 3 at any time, they should contact the site on the same day and be seen or referred to a qualified medical facility within 24 hours. Any discrepancies between

investigator- and subject-reported outcomes of reactogenicity will be noted in the source documents prior to entry into the clinical database.

6.3.3 Physical Examination

A targeted physical examination will include the following: head and neck (inclusive of nasal cavity and oropharynx), respiratory, cardiac, abdomen, and lymph nodes associated with the upper extremity, head, and neck. Height and weight will be measured at the screening visit only.

Additional targeted physical examinations will be symptom-directed and performed at the discretion of the investigator, including unscheduled visits, if necessary, to evaluate AEs.

6.3.4 Clinical Safety Laboratory Assessments

6.3.4.1 Urine Pregnancy Test

For female subjects of childbearing potential who have been heterosexually active in their lifetime, a urine pregnancy test will be performed at screening and prior to any study vaccination (at the discretion of the investigator based on history taking).

6.3.4.2 SARS-CoV-2 Test

Given the ongoing pandemic, a SARS-CoV-2 test for active infection (an approved local test accepted by public health authorities and readily available) will be administered to subjects within 72 hours prior to vaccination (Day 1 for all subjects or Day 85 if in substudy). Should the subject have a positive SARS-CoV-2 test result, regardless of symptomatology, then vaccination will be temporarily held until the subject has a negative test result.

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 study vaccination if any symptoms develop.

6.3.5.1.1 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, additional local laboratory or diagnostic assessments (including additional blood sampling) may be performed on any subject who experiences an SAE.

6.3.5.1.2 Local (Arm), Nasal/Respiratory, and General Systemic Reactogenicity

Reactogenicity events are common and known to occur following administration of vaccines, indicating a positive immune response is being mounted. Site-specific local (arm), nasal/respiratory, and general systemic reactogenicity events including start and stop dates/times will be recorded and a standard toxicity grade (refer to Section 13.2) will be applied to any reactogenicity event.

6.3.5.1.3 Medically Attended Adverse Events

A medically attended AE is defined as any unsolicited AE that leads to an unscheduled visit to a healthcare provider, or any grade 3 solicited reactogenicity event that leads to an unscheduled visit to a healthcare provider.

6.3.5.1.4 Adverse Events of Special Interest

If the COVID-19 pandemic remains ongoing in the region or if there are mass vaccinations using a COVID-19 vaccine during study conduct, any symptoms associated with COVID-19 infection or vaccination will be collected as an AESI on a unique COVID-19 eCRF through Day 169/EOS.

6.3.5.2 Assessing and Documenting Adverse Events

Safety will be assessed by the frequency and severity of:

1. All AESIs and SAEs occurring from the time of vaccination through Day 169/EOS.
2. All MAAEs occurring through 84 days following any vaccination.
3. Solicited reactogenicity events occurring from the time of any study vaccination for 7 consecutive days starting the day following study vaccination, including:
    a. Nasal/respiratory reactions (runny/congested nose, sore/irritated throat, cough, sneeze, nasal or sinus pain/irritation, difficulty breathing/wheezing; if epistaxis occurs it should be listed as an AE);
    b. Systemic reactions (fever, headache, fatigue/malaise, loss of appetite/anorexia, vomiting, diarrhoea, nausea, myalgia, acute allergic reaction/urticaria); and
    c. Local reactions (pain/tenderness, redness, swelling [after IM injection only]).
4. Unsolicited AEs – all AEs regardless of causality, will be collected for 28 days after any vaccination. Between screening (signing of ICF) and Day 1, AEs will be collected if classified as serious or if considered related to study procedure or study involvement. Any event that results in vaccination delay (eg, temporary exclusions due to acute upper respiratory symptoms/illness or fever, or investigator determined medical condition) during screening or during study conduct will also be recorded as an AE. Should any reactogenicity event extend beyond 7 days following vaccination, it will be recorded as an AE with the same start date as the reactogenicity event and followed until considered resolved.

At every study visit, subjects will be asked a standard non-leading question to elicit any medically related changes in their well-being that would pertain to the AEs being collected at the time. They will also be asked if they have been hospitalized, had any accidents, used any new medications, received a vaccination or changed concomitant medication regimens (both prescription and OTC medications).

6.3.5.3 Reporting Adverse Events

Adverse events reported or observed during the study will be recorded on the AE page in the eCRF.

An AE is any unfavourable and unintended sign, symptom, or disease. 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. A single AE may have a constellation of signs/symptoms and the single most precise Medical Dictionary for Regulatory Activities (MedDRA) term should be utilized as the AE classification to avoid duplication of AEs.

Adverse events 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 vaccination and alternate aetiology (if not related to study vaccination), 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 6.3.5.7).

Any medical condition that is present at the time that the subject is screened will be considered as baseline (recorded as medical history) 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 vaccination. 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.

6.3.5.4 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.

6.3.5.5 Suspected Unexpected Serious Adverse Reactions

The sponsor will promptly evaluate any new suspected unexpected serious adverse reactions (SUSARs) and expeditiously communicate possible new safety findings to investigators, institutional review boards or independent ethics committees, and applicable health authorities based on applicable legislation. Any SAE considered to be related to BPZE1 will be classified as a SUSAR.

To determine reporting requirements for single AE cases, the sponsor will assess the expectedness of these events using the most recent version of the IB or the package insert for Boostrix (BOOSTRIX 2020). A summary of the possible undesirable effects from Boostrix (Boostrix 2021) is provided in Section 1.3.1.3.

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

6.3.5.6 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: These events require minimal or no treatment and do not interfere with the subject’s normal daily activity.

Moderate:	These events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with normal functioning.
Severe:	These events interrupt a subject's normal 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.

6.3.5.7 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 study vaccination caused the event.

6.3.5.8 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, considered stable or when subject completes EOS and based on Section 6.3.5.4. Serious AEs will be collected, assessed, and followed through EOS or until satisfactory resolution or clinically stable.

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

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

6.4 Immunogenicity and Colonization Assessments

Immune measurements will be conducted on serum (IgG and IgA) and nasal mucosal secretion (S-IgA) samples for anti-pertussis antibodies of WCE, PT, FHA, and PRN as well as any additional anti-pertussis antibodies included in assay development. Serum bactericidal activity (or other functional assays identified) will be measured in a subset of subjects. Cellular-mediated response to BPZE1 and Boostrix (Th1/Th17 vs Th2) will be assessed by ELISpot or flow cytometry at baseline and Day 8 (safety lead-in cohort) and Day 85 and Day 113 (subset of substudy subjects). Additional testing for antigens specific to *B. pertussis* may be performed at a later date as BPZE1 induces broader immunity more similar to natural infection induced immunity and does not contain the purified antigen levels of PT, FHA, and PRN found in Boostrix. Mid-turbinate/nasopharyngeal samples will be evaluated by real-time PCR or by *B. pertussis* culture for colonization of BPZE1.

Subjects will consent for the use of samples for further anti-pertussis antibody testing or other assay development as part of the standard consenting process. Aliquots of collected samples from this study may be retained for additional testing of biological responses (eg, antibodies, T-cell responses, microbiome) specific to future development of BPZE1 and ILiAD Biotechnologies' *B-Tech* program for a maximum of 15 years (starting from the date at which the last subject had the last study visit), unless local rules, regulations, or guidelines require different time frames or different procedures, and in accord with subject consent.

Procedures for the handling and processing of immunogenicity samples are provided in the laboratory manual or the site manual.

6.5 Vaccination Pause Rules

Further enrolment 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 related to study vaccination, through 28 days following study vaccination.
- Any subject experiences severe respiratory symptoms of laryngospasm, shortness of breath or wheezing (grade 3 or greater) within 3 days of study vaccination.
- Any subject experiences an SAE considered related to study vaccination from the time of the vaccination through the subject's last study visit.
- More than 12 subjects report a grade 3 fever within 7 days of study vaccination that is attributed to study vaccination when no other cause can be found (corresponding to an incidence rate of approximately 3% for Boostrix).

Grading scales for solicited reactogenicity events are included in Section 13.2.

If any of the vaccination pause rules are met following any subject receipt of study vaccination, then this study will not continue with the remaining enrolments or future study vaccinations without a review by the SMC and a recommendation from the SMC to proceed (with or without alteration of protocol). 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 vaccination pause rules will be entered as AEs.

The sponsor retains the authority to suspend additional enrolment and study interventions or administration of study vaccine during the entire study, as applicable.

6.6 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 in a study participant and during study participation must be reported using a clinical study pregnancy form. To ensure subject safety, each pregnancy must be reported to the sponsor within 2 weeks of learning of its occurrence. 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 the sponsor.

Pregnancy is an exclusion criterion, and a urine pregnancy test is required in sexually active heterosexual females a) at the time of enrolment; and b) on the day of and preceding any study vaccination.

7 Statistical and Analytical Plan

7.1 Sample Size Calculations

Approximately 360 subjects will be enrolled and randomly assigned (1:1:1) to 1 of 3 treatment groups (refer to Table 3-1) to contribute to safety and efficacy assessments. Enrolment will be operationally managed at the study level to maintain a minimum number of subjects enrolled in 2 age groups: 6 to 10 years, inclusive; and 11 to 17 years, inclusive. It is estimated that a 10% drop-out rate will occur, allowing for approximately 108 evaluable subjects per treatment group.

Primary immunogenicity endpoints: The sample size is based on calculations to achieve adequate response on Day 29 over baseline of mucosal S-IgA for WCE assay in the BPZE1 and BPZE1 + Boostrix groups. Assuming the GMT follows a log-normal distribution, by applying a natural logarithmic transformation of the GMT, a paired t-test will be used to test the difference of mucosal S-IgA against WCE at Day 29 compared with baseline (equivalent to natural logarithmic transformation of the GMFR). With an effective sample size of N=108 subjects per group, the study provides 95% power for detecting an effect size of 0.35 for each of the two primary comparisons with a two-sided significance level of 0.05. By multiplying the power for each of the two comparisons, the study provides an overall power of 90% (=95%*95%) for testing both primary comparisons. Factoring in an estimated 10% drop-out rate, each treatment group would include approximately 120 subjects for a total of approximately 360 subjects in the study.

Key secondary endpoints: To demonstrate non-interference of IgG serum responses for the Boostrix-containing antigens (diphtheria, tetanus) at Day 29 when BPZE1 is co-administered with Boostrix (BPZE1 + Boostrix vs Boostrix), the test for non-inferiority of BPZE1 + Boostrix to Boostrix for the proportion with titer ≥ 0.1 IU/mL (for diphtheria, tetanus) has power exceeding 92% with approximately N=120 subjects per treatment group, assuming a noninferiority margin of -0.1, one-sided significance level of 0.025, 10% drop-out rate and the proportion with titer ≥ 0.1 IU/mL for the Boostrix treatment being at least 0.95.

To demonstrate non-interference of IgG serum responses for the Boostrix-containing antigens (PT, FHA, PRN) at Day 29 when BPZE1 is co-administered with Boostrix (BPZE1 + Boostrix vs Boostrix), the tests for non-inferiority of BPZE1 + Boostrix to Boostrix for each of the three IgG parameters (PT/FHA/PRN) have power of 97%, 100%, and 88%,

respectively, for detecting between group differences as observed in study IB-200P, with approximately 120 subjects per treatment group, one-sided significance levels of 0.025, 10% drop-out rate and non-inferiority margin of -0.693.

7.2 Blinded Sample Size Recalculation

A blinded sample size recalculation may occur when immunogenicity data for approximately 40 to 60 subjects per group are available. The standard deviation of the Day 29 change from baseline of S-IgA for WCE of natural logarithmic transformed GMFR will be computed. The standard deviation of natural logarithmic transformed GMTs of the IgG serum responses for the Boostrix-containing antigens (PT, FHA, PRN) at Day 29 will also be computed. Proportions with titer ≥ 0.1 IU/mL for the IgG serum responses for the Boostrix-containing antigens (diphtheria, tetanus) at Day 29 will be estimated.

The standard deviations and proportions with titer ≥ 0.1 IU/mL will be computed in the full dataset and not by treatment group to ensure that the blinding is maintained. The sample size will then be recalculated with the potential to adjust the sample size accordingly.

A small committee consisting of select ILiAD leadership and an independent blinded statistician will evaluate the need to change sample size. The independent blinded statistician will use the standard deviation values estimated from the full dataset to recalculate the sample size. In addition to these pooled standard deviation values, the committee will also assess data completeness for the primary endpoint and drop-out rate.

The committee will meet and document its decision in a memorandum. This memorandum will include the pooled standard deviations, the updated power/sample size computations, and the final decision regarding any change in sample size.

7.3 Analysis Sets

The safety analysis set will consist of all randomized subjects who receive study vaccine and have any safety data available. Subjects will be classified according to the actual vaccine received. The safety analysis will be done on this analysis set.

The intent-to-treat analysis set will consist of all randomized subjects. Subjects will be classified according to the randomized treatment group. Subject disposition, demographics, and medical history will be summarized on this analysis set.

The immunogenicity analysis set will consist of all subjects in the intent-to-treat analysis set who receive study vaccine, have a baseline and at least 1 post-vaccination sample (mucosal or serum immunogenicity testing, respectively) for which valid results were reported for the test being analysed, and have not received any component of a licensed pertussis vaccine in the past 3 years or through Day 85 of the study. Subjects will be classified according to the actual vaccine received. All immunogenicity analyses will be done on this analysis set. The substudy analysis set will also be based on this analysis set but including only subjects who are enrolled in the optional open-label substudy.

The per-protocol analysis set will consist of all subjects in the immunogenicity analysis set, with the following exclusions:

- Data from all available visits for subjects following the receipt of unsuitable investigational product (either via dispensed and/or assigned or delivered).
- Data from all available visits for subjects found to be ineligible at baseline per-protocol.
- Data from all available visits for subjects following the use of major immune modulators or immunosuppressants or following the receipt of blood products.
- Data from all available visits for subjects following clinically significant protocol deviations that can affect the efficacy (immunogenicity) results as determined by clinical review.
- 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-up visit should have occurred, whichever is most restrictive. Standard visit windows are described in schedule of events (Table 13-1).

For analyses using the per-protocol analysis set, subjects will be classified according to the randomized treatment group.

The safety lead-in analysis set will consist of the first 45 subjects who are randomized to the study. Subjects will be classified according to the actual vaccine received. The first

45 subjects enrolled will be in the older age group of 11 to 17 years, inclusive. Cellular-mediated responses in this group following study vaccination will be analysed if subjects contribute a baseline sample and the required post-vaccination sample (ie, Day 8) that has at least 85% recovery following peripheral blood mononuclear cell (PBMC) procedures. Similarly, cellular-mediated immunity of the optional substudy will occur in a subset of subjects who contribute to Day 85 and Day 113 samples and have a similar recovery of PBMCs.

7.4 Statistical Analysis Methodology

Statistical analysis will be performed using SAS software Version 9.3 or later. Continuous variables will be summarized using the mean, standard deviation, median, first quartile, third quartile, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages, as well as a two-sided 95% confidence interval (CI) for proportions computed using the Agresti-Coull method when appropriate. Data will be listed in data listings.

Immunogenicity endpoints (seroconversion, GMT, and GMFR) will be summarized by baseline negative and positive status (by quantiles) of each anti-pertussis antibody (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) and by antibody type (eg, S-IgA, IgA, and IgG separately), by the 2 age groups (6 to 10 years and 11 to 17 years, inclusive) and across age groups. Substudy analyses will assess immunity at baseline and Days 85 and 113 (independently for substudy subjects) and relative to colonization status (colonized/non-colonized) at either Day 92 or Day 99 following re-vaccination/attenuated challenge at Day 85. The mucosal and serum immunogenicity endpoints will be summarized by treatment group and by day (all subjects), including a separate analysis after Day 85 for the open-label substudy. Analyses will include the GMTs with 95% CIs and GMFRs with 95% CIs. The 95% CIs will also be provided for mucosal and serum seroconversion. For immunogenicity analysis, it is assumed that the natural log of the data is normally distributed. All statistical tests will be two-sided at 0.05 significance level.

There are 2 primary comparisons that will be tested at a two-sided significance level of 0.05. The study is considered a success if both primary comparisons meet statistical significance. The Holm procedure (also known as a stepdown Bonferroni procedure) will be used to control for multiplicity when evaluating the key secondary endpoints.

Further details of the planned statistical analyses, methods, and data conventions will be described in the SAP.

7.4.1 Analyses of Primary Endpoints

7.4.1.1 Analyses of Primary Immunogenicity Endpoints

For mucosal S-IgA against WCE at Day 29, the two-sided 95% CI for change from baseline in GMFR will be calculated. A superiority hypothesis will be tested to demonstrate BPZE1 can adequately induce mucosal S-IgA against WCE at Day 29 by GMFR.

The formal superiority hypothesis is:

$$H_0: GMFR \leq 1 \; vs. H_A : GMFR > 1$$

The GMFR is defined as the ratio of GMT at Day 29 and GMT at baseline, i.e., $GMFR = \left(\frac{\mu_{D29}}{\mu_{BL}}\right)$, with μ_{D29} and μ_{BL} being the GMTs at Day 29 and baseline, respectively.

After natural logarithmic transformation, $ln(GMFR) = ln(\mu_{D29}) - ln(\mu_{BL})$. Therefore, the hypothesis can be expressed in the natural log scale as:

$$H_0: ln(\mu_{D29}) - ln(\mu_{BL}) \leq 0 \text{ vs. } H_A: ln(\mu_{D29}) - ln(\mu_{BL}) > 0$$

After natural logarithmic transformation, a paired t-test will be used to test the difference of mucosal S-IgA against WCE at Day 29 compared with baseline. The superiority objective will be demonstrated if the lower limit of the 95% CI for the difference of mucosal S-IgA against WCE at Day 29 compared to baseline on the natural log scale is greater than 0 within the treatment group. The superiority hypothesis will be tested in treatment groups BPZE1 and BPZE1 + Boostrix separately.

7.4.1.2 Analyses of Primary Safety Endpoints

The number and percentage of subjects with solicited AEs (local, nasal/respiratory, and systemic reactogenicity events) through 7 days following first study vaccination with any combination of pertussis-containing vaccines (BPZE1, BPZE1 + Boostrix, Boostrix) will be calculated by treatment group.

7.4.2 Analyses of Key Secondary Serum Immunogenicity (IgG) Endpoints

Descriptive statistics (GMT, GMFR, seroconversion) for serum IgG against PT, FHA, and PRN will be presented at baseline and Day 29 and by treatment group (BPZE1 + Boostrix, Boostrix). For serum IgG against PT, FHA, and PRN at Day 29, the two-sided 95% CI for group difference in GMT in natural log will be calculated. A two-sample t-test will be used to compare the natural log-transformed GMTs between groups for serum IgG against each of PT, FHA, and PRN at Day 29. The non-inferiority objective will be demonstrated if the lower limit of the 95% CI of group difference in natural log is greater than -0.693.

For serum IgG against diphtheria and tetanus, the two-sided 95% CI for group difference in the proportion of subjects with antibody concentration ≥0.1 international units (IU)/mL will be calculated using the Agresti-Caffo method. The non-inferiority objective will be demonstrated if the lower limit of 95% CI of group difference is greater than -0.1.

The Holm procedure (also known as a stepdown Bonferroni procedure) will be used to control for multiplicity when evaluating the key secondary endpoints.

7.4.3 Analyses of Secondary Endpoints

7.4.3.1 Analyses of Secondary Mucosal Secretory Immunogenicity (S-IgA) Endpoints

Descriptive statistics (GMT, GMFR, seroconversion) for mucosal S-IgA against WCE, PT, FHA, PRN, and any other anti-pertussis mucosal antibodies identified during assay development will be presented at each time point and by treatment group.

Comparisons between two time points (eg, Day 29 and baseline) will be conducted using a paired t-test as outlined in Section 7.4.1.1. Non-interference or non-inferiority comparisons between two treatment groups (eg, BPZE1 + Boostrix being non-inferior to Boostrix by GMT at Day 29) will be conducted using a two-sample t-test as outlined in Section 7.4.2. Non-inferiority comparisons between two proportions will be conducted using the Agresti-Caffo method as outlined in Section 7.4.2.

Analysis of covariance (ANCOVA) model will be used to examine the effects of age or other baseline demographics on differential responses to induction of mucosal S-IgA immunity.

Subgroup analyses by age (6 to 10 years and 11 to 17 years, inclusive), time since last aP vaccination (prior to study entry), or other baseline demographics and by baseline antibody level (by quantile) will be conducted for BPZE1, BPZE1 + Boostrix, and Boostrix treatment groups. In addition to descriptive summaries, a forest plot of the corresponding 95% CIs will be generated for each subgroup.

Mucosal S-IgA seroconversion rate (2-fold increase over baseline value or a 4-fold increase over the lower limit of assay detection [whenever the baseline value falls below the lower limit of assay detection]) with 95% CI will be calculated by treatment group at Day 29. A chi-square test will be used to compare seroconversion rates between treatment groups. Additional combinations of antibody response (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) may also be tested.

Plots of the reverse cumulative distribution curves of each anti-pertussis mucosal antibody titre for mucosal S-IgA GMT will be generated for Day 29 by treatment group.

Further details will be provided in the SAP.

7.4.3.2 Analyses of Secondary Serum Immunogenicity (IgA and IgG) Endpoints

All analyses will use the approaches outlined in Section 7.4.1.1 for the primary analyses, in Section 7.4.2 for the key secondary analyses and those outlined in Section 7.4.3.1 for secondary analyses.

7.4.3.3 Analyses of Secondary Safety Endpoints

The number and percentage of subjects will be summarized for each solicited AE by timepoint, severity grade, and treatment group. Additionally, solicited AEs will be analysed by taking the maximum severity through 7 days following any study vaccination to summarize the frequency and percentage of subjects reporting each local [if applicable; after IM injection only], nasal/respiratory, or systemic event. Mean response (for any measurement) and duration through 7 days following any study vaccination will be summarized daily using descriptive statistics. Solicited AEs will be reported separately following study vaccination on Day 85 in the substudy.

Adverse events will be coded by preferred term and system organ class using the latest version of MedDRA.

The following unsolicited AEs will be summarized as number and percentage of subjects reporting at least 1 event in each preferred term and system organ class and cross-tabulated by severity and relationship to study vaccination.

- All AEs through 28 days following first study vaccination and following re-vaccination/attenuated challenge (substudy) by severity and relationship to study vaccination, and by treatment group.
- All MAAEs through 84 days following first study vaccination and re-vaccination/attenuated challenge (substudy) by treatment group by severity and relationship to study vaccination, and by treatment group and time since study vaccination.
- All AESIs and SAEs through Day 169/EOS by treatment group by severity and relationship to study vaccination, and by treatment group and time since study vaccination.

Serious AEs will be summarized by detailed listing showing the event description, preferred term and system organ class, relevant dates (study vaccinations and AEs), severity, relatedness, and outcome for each event. Any SAE considered related to BPZE1 will be classified as a SUSAR.

The number and percentage of subjects with SARS-CoV-2 infections or AESIs associated with COVID-19 infection or SARs-CoV-2 vaccine-related events will be summarized, if the COVID-19 pandemic remains ongoing in the region or if there are mass vaccinations using a COVID-19 vaccine during study conduct.

The number and percentage of subjects with colonization or clearance by real-time PCR or *B. pertussis* culture following study vaccination (subsets of subjects) will be summarized over time using the methods outlined in Section 7.4.2 for proportions.

Secondary safety endpoints following re-vaccination/attenuated challenge for the optional substudy will be analysed in a similar fashion as the secondary safety endpoints for the main study.

7.4.4 Analyses of Endpoints for the Optional Substudy

All analyses will use the approaches outlined in Section 7.4.1.1 for the primary analyses, in Section 7.4.2 for the key secondary analyses and those outlined in Section 7.4.3.1 for secondary analyses.

The number and percentage of subjects without colonization at either Day 92 or Day 99 will be calculated by treatment group (BPZE1, BPZE + Boostrix, BPZE1 and BPZE1 + Boostrix, Boostrix control). The number and percentage of subjects without colonization at Day 92 and Day 99 will also be calculated by treatment group (see Section 7.4.2 for a description of the approach).

BPZE1, BPZE1 + Boostrix, BPZE1 and BPZE1 + Boostrix, and Boostrix control of induction of mucosal S-IgA at Day 29 and Day 85 will be summarized and compared by protection against colonization (Colonizers/Non-colonizers) using a two-sample t-test.

BPZE1 induction of mucosal S-IgA immunity at Day 113 will be compared with that at Day 29 and Day 85 (by treatment group) using a paired t-test to assess if a re-vaccination/attenuated challenge with BPZE1 provides significantly more mucosal S-IgA induction by treatment group (BPZE1, BPZE1 + Boostrix). To demonstrate that BPZE1 following Boostrix provides a similar level of induction as BPZE1 alone, a two-sample t-test will be used to test for the difference of mucosal S-IgA immunity at Day 113 (Boostrix versus BPZE1).

BPZE1, BPZE1 + Boostrix, or control (Boostrix) induction of mucosal S-IgA at Day 113 and Day 169/EOS will be summarized and compared using a two-sample t-test between subjects who participated in the substudy and subjects who did not participate in the substudy by treatment group.

Bordetella pertussis colony counts at each of Day 92 and Day 99 (individual and total) will be summarized descriptively and the Wilcoxon Rank-Sum test will be used to compare colony counts. Mean counts and AUC will be calculated across Day 92 and Day 99. Colonization versus non-colonization status for Day 92, Day 99, and for the combined result over Day 92 and Day 99 will be tabulated.

To assess if prior BPZE1 induced mucosal immunity substantially reduces colonization at Day 92 and Day 99, the number and percentage of subjects with colonization at each of Day 92 and Day 99 will be analysed based on mucosal S-IgA at Day 29 and Day 85 (see Section 7.4.2 for analysis approach). The percent reduction (with 95% CI) relative to the Boostrix control group at each of Day 92 and Day 99 will be summarized.

7.4.5 Other Analyses

Summary statistics will be provided for exploratory endpoints, demographic and baseline data, medical history, vital sign measurements, physical examination, study vaccination compliance, and prior and concomitant medications.

Cellular immune response (Th1 and Th2) following study vaccination will be evaluated in the safety lead-in analysis set using baseline and Day 8 and in the substudy using Day 85 and Day 113 (subset). Parameters will be summarized using descriptive statistics and a formal test of differences in Th1 vs Th2 pathway induction between treatment groups. The 95% CI of median will be provided. Median of fold rise over baseline will also be provided.

If a substantial number of subjects receive a COVID-19 vaccine that is under an emergency use authorization only, then safety analysis may also assess any AEs that may be confounded by such concomitant vaccine administration during the study.

7.4.6 Interim Database Lock

An interim database lock may occur after all subjects have completed Day 29 and sample testing for all primary and select secondary immune endpoints has been completed (mucosal and serum), but results will only be provided at treatment assignment level to maintain the blind. The site and those interacting directly with the site or subject data (eg, CRO, laboratories) will remain blinded to subject assignment until EOS or end of sample testing, depending on the activities of the associated entities.

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 subject diaries for reactogenicity reporting.

Investigative site staff will enter subject data into an approved and auditable data management system. The analysis data sets 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 CRO and/or sponsor 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 MedDRA, an internal validated medical dictionary, and concomitant medications will be coded using the WHO Drug Dictionary.

9 Ethics

9.1 Independent Ethics Committee

Federal regulations and the International Council for Harmonisation (ICH) guidelines require that approval be obtained from an independent ethics committee (IEC) 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 IEC. Documentation of all IEC approvals and of the IEC compliance with ICH harmonised tripartite guideline E6 (current version): Good Clinical Practice (GCP) will be maintained by the site and will be available for review by the sponsor or its designee.

All IEC approvals should be signed by the IEC chairman or designee and must identify the IEC name and address, the clinical protocol by title or protocol number or both, and the date approval or a favourable 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 IEC. The investigator must promptly supply the sponsor or its designee, the IEC, 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 (and assent, if applicable) in compliance with regulatory authority regulations shall be obtained from each subject and/or his or her legal guardian before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An ICF and subject information sheet/authorization form 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 ICF and subject information

sheet/authorization form should be reviewed by the sponsor or its designee or both before IEC submission. Once reviewed, the ICF and subject information sheet/authorization form will be submitted by the investigator to his or her IEC for review and approval before the start of the study. If the ICF or subject information sheet/authorization form is revised during the course of the study, all active participating subjects and or legal guardians must sign the revised form(s). A separate ICF will be utilized for subjects participating in the substudy.

Before recruitment and enrolment, each prospective subject and/or his or her legal guardian will be given a full explanation of the study, be allowed to read the approved ICF (or subject information sheet/authorization form, if applicable), and have any questions answered. 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 (and assent, if applicable) to participate in the study by signing the ICF (and subject authorization form, if applicable). The authorized person obtaining the informed consent also signs the ICF (and subject authorization form, if applicable).

The ICF and subject information sheet/authorization form will contain separate sections that address the use of remaining mandatory samples for optional exploratory research (if applicable) and explain/address the exploratory research portion of the study. Subject medical records need to state that written informed consent was obtained.

The investigator shall retain the signed original ICF(s) (and subject authorization form[s], if applicable) and give copies of the signed original form(s) to the subject and/or legal guardian.

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 IEC 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, or the IEC.

The investigator and all employees and co-workers 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

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 (current version) by providing all essential documents.

10.4 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH E6 (current version). 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 enrolment of participants begins.

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

10.5 Adverse Events and Study Report Requirements

The investigator agrees to submit reports of SAEs to the sponsor and/or IEC according to the timeline and method outlined in the protocol. In addition, the investigator agrees to submit annual reports to the study site IEC as appropriate.

10.6 Investigator's Final Report

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

10.7 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. 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. No records may be transferred to another location or party without written notification to the sponsor.

10.8 Publications

After completion of the study, the data may be considered for reporting at scientific meetings or for publication in scientific journals. 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) and an external SMC.

11.1 Monitoring

11.1.1 External Data Monitoring Committee

11.1.1.1 Safety Monitoring Committee

Safety oversight will be conducted by an SMC that is an independent group of experts that monitors subject safety and advises the sponsor. 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 convene prior to study initiation, if a pause is triggered, or upon the request of the medical monitor and/or sponsor. The medical monitor and sponsor are empowered to request a review by the SMC for any safety reason.

The SMC will operate under the rules of a sponsor-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 subject's clinical course or safety results (eg AEs, reactogenicity). Reports may include enrolment 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 the sponsor. The SMC may receive data in aggregate and presented by treatment group.

The SMC will review grouped and unblinded data in the closed session only. 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.

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 clinical monitor will visit the investigator and study site at periodic intervals (including remote monitoring activities), in addition to maintaining necessary telephone and letter contact. The clinical 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, IEC 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 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 the required procedures defined in this protocol, 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 IEC before subjects can be enrolled into an amended protocol. Letters of amendment or clarification do not need to be approved by the IEC.

11.2.2 Protocol Deviations

A deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the sponsor and the IEC and agreed to by the

investigator. A significant deviation occurs when there is nonadherence to the protocol or to local regulations or ICH GCP guidelines that may or may not result in a significant, additional risk to the participant or impacts the integrity of study data. Deviations will be assessed with Sponsor involvement and deemed significant (major) or of minor significance prior to database lock.

The investigator or designee must document and explain in the participant'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/safety risk to study participants without prior IEC 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 IEC for review and approval, to the sponsor for agreement, and to the regulatory authorities, where required.

In order to keep deviations from the protocol to a minimum, the investigator and relevant site personnel will be trained in all aspects of study conduct by the sponsor/sponsor representative. This training will occur either as part of the investigator meeting or site initiation. Ongoing training may also be performed throughout the study during routine site monitoring activities.

11.3 Study Termination

Although the sponsor has every intention of completing the study, the sponsor 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 the last visit (includes follow-up telephone call for long-term safety).

11.4 Final Report

Whether the study is completed or prematurely terminated, the sponsor will ensure that the clinical study report (CSR) is 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.

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 a summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate. The study summary results will be posted on publicly available clinical trial registers.

12 Reference List

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13 Appendices

13.1 Appendix: Schedule of Events

Table 13-1 Schedule of Events

	Screening	Treatment Period									EOS
Subject Population	**All Subjects**	**All Subjects**	**Safety Lead-In Subjects ONLY**	**All Subjects**	**Subjects NOT in Substudy**	**Subjects Participating in Substudy ONLY[d]**				**All Subjects**	**All Subjects**
Study Visit	**Screening**	**V1**	**S1[b]**	**V2**	**V3[c]**	**SSV3**	**SSV4**	**SSV5**	**SSV6**	**Unscheduled[e]**	**EOS/Early Termination**
Study Day	**–30 to 1[a]**	**1**	**8**	**29**	**85**	**85**	**92**	**99**	**113**	-	**169**
Window allowance	**30**	**0**	**+3**	**+7**	**+14**	**+20**	**+3**	**±2**	**+7**	-	**+30**
Informed consent	X					X[f,g]					
Inclusion/exclusion criteria	X	X[f,g]				X[f,g]					
Demographic and baseline data[h]	X										
Medical history[i]	X	X[f,g]									
Concomitant medication[j]	X	X[g]	X	X	X[k]	X[g]	X	X	X	X[k]	X[k]
Concomitant vaccines[l]	X	X[g]	X	X	X	X[g]	X	X	X	X	X
Targeted physical examination[m]	X	X[g]	X	X	X	X[g]	X	X	X	X	X
Vital sign measurements[n]	X	X[g]				X[g]				X	
Urine pregnancy test[o]	X	X[g]				X[g]				X	
SARS-CoV-2 test for active infection (within 72 hrs prior to vaccination)[p]		X				X					
Study vaccination[q]		X				X					
Immediate reactogenicity		X[r]				X[r]					
Subject diary dispensed[s]		X				X					

	Screening	Treatment Period									EOS
Subject Population	**All Subjects**	**All Subjects**	**Safety Lead-In Subjects ONLY**	**All Subjects**	**Subjects NOT in Substudy**	**Subjects Participating in Substudy ONLY[d]**				**All Subjects**	**All Subjects**
Study Visit	**Screening**	**V1**	**S1[b]**	**V2**	**V3[c]**	**SSV3**	**SSV4**	**SSV5**	**SSV6**	**Unscheduled[e]**	**EOS/Early Termination**
Study Day	**–30 to 1[a]**	**1**	**8**	**29**	**85**	**85**	**92**	**99**	**113**	-	**169**
Window allowance	**30**	**0**	**+3**	**+7**	**+14**	**+20**	**+3**	**±2**	**+7**	-	**+30**
Subject diary reviewed[t]			X	X			X			X	
Nasal sampling for immunogenicity laboratory tests		X[g]		X	X	X[g]			X		X
Mid-turbinate/nasopharyngeal sampling for *B. pertussis* culture or PCR			X	X[u]	X[u]		X	X			
Blood sampling for immunogenicity laboratory tests		X[g]		X	X	X			X		X
Blood sampling for cellular-mediated Th1 and Th2 responses		X[b]	X			X[v]			X[v]		
Any TEAE (following most recent vaccination)		X	X	X		X	X	X	X	X	
Any MAAE[w], AESI, or SAE (following first vaccination)		X	X	X	X	X	X	X	X	X	X
EOS form											X

[a] Screening visit may occur on Day 1 prior to study vaccination.

[b] Safety lead-in subjects (only) to complete procedures listed under Study Visit S1; PBMCs to be collected in a subset of subjects.

[c] Subjects not participating in the substudy are to complete procedures listed under Study Visit V3 for Day 85. Subjects in the safety lead-in cohort who are not participating in the substudy are not required to provide samples on Day 85 and may complete this visit remotely.

[d] Subjects participating in the substudy (only) to complete procedures listed under Study Visits SSV3, SSV4, SSV5, and SSV6.

[e] Procedures listed for an Unscheduled study visit are optional and dependent on the cause for the visit itself. An unscheduled study visit may occur at any time between enrolment and Day 169/EOS.

[f] Reviewed and/or updated.

[g] Performed prior to vaccination.

[h] Such as date of birth (where allowed by local regulations), sex, race, ethnicity, weight, and height (with derived BMI and Z-score).

[i] Including prior and ongoing chronic medical conditions and past surgeries.

[j] Concomitant medications will include all medications and OTC products (including herbal supplements and multivitamins) taken by the subject from the time of signing the ICF through Day 29 (and from Day 85 [following study vaccination] through Day 113 for subjects participating in the optional substudy), or through an early termination visit, if prior to these time points. The use of anti-pyrogenic medication will be specifically queried by diary during the 7 days following any study vaccination.

[k] Only concomitant medications associated with any MAAE (through 84 days following any study vaccination); or AESI or SAE (through Day 169/EOS) will be recorded.

[l] Vaccines received outside of the study will be specifically queried during each visit and recorded through Day 169/EOS.

[m] Targeted physical examination at screening and Day 1 (prior to vaccination) to include head and neck (inclusive of nasal cavity and oropharynx), respiratory, cardiac, abdomen, and lymph nodes associated with the upper extremity, head, and neck. Targeted physical examination at all other times will be symptom-directed and performed at the discretion of the investigator. Height and weight will be measured at screening only.

[n] Vital sign measurements (heart rate, body temperature) will be collected once before vaccination and then at 30 (+30) minutes after the last study vaccination (i.e., after IM vaccination on Day 1). For the safety lead-in cohort, a minimum of 30 minutes must occur between intranasal and IM vaccinations on Day 1, and medical assessment will occur prior to the second vaccination to allow for advancement. Temperature (oral or tympanic) will also be recorded daily in a subject diary for 7 consecutive days starting the day following any study vaccination (retrospective of the highest value in the previous 24 hours).

[o] Female subjects of childbearing potential only who have become heterosexually active. To be performed at the discretion of the investigator based on history taking.

[p] SARS-CoV-2 testing should be performed within approved national guidelines with tests that are approved through a (minimum) of emergency use authorization. Minimally invasive testing should be considered for the comfort of subjects. Subjects need to have accepted negative test results within 72 hours prior to vaccination. Repeat testing is allowed on multiple occasions. For the substudy, subjects are not considered to have a protocol violation if a retest for SARS-CoV-2 exceeds the study window.

[q] On Day 1, intranasal vaccination will occur prior to IM vaccination with a minimum of 10 minutes (minimum of 30 minutes for subjects in the safety lead-in cohort of 11 to 17 year olds) between intranasal and IM vaccinations.

[r] Immediate reactogenicity will be assessed at 30 minutes (+30 minutes) after the last study vaccination of the Day (i.e., after IM vaccination on Day 1, after nasal vaccination on Day 85), prior to release from clinical observation.

[s] All subjects will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following any study vaccination (retrospective of the highest value in the previous 24 hours). Subjects will be instructed that should they have reactogenicity with a potential toxicity grade 3 at any time, they should contact the site on the same day and be seen or referred to a qualified medical facility within 24 hours.

[t] Trained clinical staff will review the information from the subject diaries with the subjects and apply a standard toxicity grade (refer to Section 13.2) to any reactogenicity event. The diary will be reviewed following the first visit after vaccination for all subjects.

[u] Subjects not in the safety lead-in cohort will be randomly assigned (1:1) to provide mid-turbinate/nasopharyngeal samples on either Day 29 or Day 85; however, if a subject cannot provide a sample on Day 29 (for any reason), the subject should provide a sample on Day 85 (this will not be considered a protocol deviation).

[v] Collection of an extra 20 mL of blood for cellular-mediated immunity may be an option in a select number of individuals who are in the substudy (up to 45 subjects). This will not involve extra venipuncture, but only collection of additional volume.

[w] Medically attended AEs will be monitored through 84 days following any study vaccination.

13.2 Appendix: Toxicity Grading Scales

Table 13-2 Local Reactogenicity Grading Scale

Variable	Grade 1	Grade 2	Grade 3
Pain/tenderness[a]	Easily tolerated, causes no or minimal limitation of use of limb	Sufficiently discomforting, interferes with greater than minimal limitation of use of limb	Causing inability to perform usual social and functional activities
Redness[b,c]	Evidence of redness that is ≤2.5 cm in diameter	>2.5 cm in diameter with <50% of upper extremity surface area segment involved	≥50% upper extremity surface area segment involved OR ulceration OR infection OR phlebitis OR sterile abscess OR drainage
Swelling[b,c]	Evidence of swelling that is ≤2.5 cm in diameter	>2.5 cm in diameter with <50% of upper extremity surface area segment involved	≥50% upper extremity surface area segment involved OR ulceration OR infection OR phlebitis OR sterile abscess OR drainage

Note: Local reactogenicity to be evaluated after an IM injection only. Grade 0 will be the classification if the observation is less than a grade 1. Any grade 3 reactogenicity event that leads to an unscheduled visit to a healthcare provider will be recorded as an MAAE.

[a] To be graded by investigator based on subject diary.

[b] Grade to be applied by data management based on measurement recording unless grade 3 is noted with other characteristics by the investigator.

[c] If redness or swelling exceeds 100 mm or >50% of upper extremity surface area, or involves the shoulder, elbow, or chest, or prevents everyday activity, then subjects must be evaluated and a swelling assessment eCRF will be completed.

Source: Adapted from DAIDS Version 2.1, 2017.

Table 13-3 Nasal/Respiratory Reactogenicity Grading Scale

Variable	Grade 1	Grade 2	Grade 3
Runny nose/nasal congestion	Noticeable but does **not** interfere with normal everyday activity (eg, aware but not troubled, rarely wipes/blows nose, breathing through nose noted)	Moderate discomfort that interferes with normal everyday activity, troublesome (eg, wipe/blow nose occasionally, noisy nose breathing, occasional mouth breathing, "nasally" speech)	Significant discomfort, prevents normal everyday activity, and/or seeks medical care (eg, wipe/blow nose frequently, mouth breathing most of the time due to congestion, consistent "nasally" speech)
Sore/irritated throat ('scratchy')	Noticeable but does **not** interfere with eating or drinking (eg, aware but not troubled)	Moderate discomfort that interferes with eating or drinking, troublesome, affects oral intake	Significant discomfort, prevents eating or drinking, and/or seeks medical care; troublesome to the level of preventing oral intake
Cough	Noticeable but does **not** interfere with normal everyday activity or sleeping (eg, aware but not troubled, a few short episodes)	Frequent cough that interrupts normal everyday activity or sleeping, troublesome (eg, coughing but only rare episodes of prolonged coughing)	Significant and repetitive coughing that prevents everyday activity or sleep, or seeks medical care (eg, frequent coughing, occasional episodes of prolonged coughing)
Sneezing	Noticeable but does **not** interfere with normal everyday activity (eg, aware but not troubled, a few short episodes)	Moderate discomfort that interferes with normal everyday activity, troublesome (eg, occasional bouts of sneezing)	Significant discomfort, prevents everyday activity, and/or seeks medical care (eg, frequent/repeated sneezing episodes)
Nasal or sinus pain/irritation	Noticeable but does **not** interfere with normal everyday activity (eg, aware but not troubled, noted discomfort)	Moderate discomfort that interferes with normal everyday activity, troublesome (facial pain/pressure)	Significant discomfort, prevents everyday activity, and/or seeks medical care; or induces nosebleed[a]
Difficulty breathing/wheezing	Audible wheeze (whistling sound) occasionally noted with exhale or inhale but does **not** interfere with normal everyday activity	Audible wheeze and or shortness of breath with activity (eg, nasal flaring, chest tightness, rapid breathing at rest/minimal activity), interferes with normal everyday activity	Requires bronchodilator use or accompanied by respiratory distress or hypoxemia

Note: Grade 0 will be the classification if the observation is less than a grade 1. Any grade 3 reactogenicity event that leads to an unscheduled visit to a healthcare provider will be recorded as an MAAE.

[a] Epistaxis (nosebleed) should be entered as an AE and graded accordingly.

Source: Adapted for age and aligned with FluMist Monograph.

Table 13-4 Systemic Reactogenicity Grading Scale

Variable	Grade 1	Grade 2	Grade 3
Fever[a]	38.0 – 38.4 °C (100.4°F – 101.1°F)	38.5 – 38.9 °C (101.2°F – 102.0°F)	39.0 – 40.0 °C (102.1°F – 104.0°F)
Headache	Easily tolerated, causing no or minimal interference with daily activities	Causing greater than minimal interference with usual social and functional activities Interferes with normal everyday activity	Prevents performance of normal social and functional activities
Fatigue/malaise (decreased activity)	Easily tolerated	Interferes with normal everyday activity	Prevents normal everyday activity
Loss of appetite/anorexia	loss of appetite but no decrease in oral intake	Decreased oral intake but no weight loss	Decreased oral intake with weight loss or evidence of dehydration
Vomiting	Transient or intermittent AND no or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in need of aggressive rehydration (eg IV hydration)
Diarrhoea	Transient or intermittent episodes of unformed stools OR increase of ≤3 stools over normal in a 24-hr period	Persistent episodes of unformed or watery stools OR increase of 4-6 stools over normal in a 24-hr period	Increase of ≥7 stools a 24-hr period OR IV fluid replacement indicated
Nausea	Transient or intermittent AND no or minimal interference with oral intake	Persistent nausea with decrease in oral intake for 24 hrs	Persistent nausea with need for fluid replacement (eg, IV hydration)
Myalgia (muscle aches)	Muscle pain beyond the site of injection causing no or minimal interference with usual social and functional activities	Muscle pain beyond the site of injection causing greater than minimal interference with usual social and functional activities	Muscle pain causing inability to perform usual social and functional activities
Acute allergic reaction/urticaria	Localized without medical intervention	Localized with medical intervention OR mild angioedema without intervention	Generalized OR angioedema with intervention

Note: Grade 0 will be the classification if the observation is less than a Grade 1. Any grade 3 reactogenicity event that leads to an unscheduled visit to a healthcare provider will be recorded as an MAAE.

[a] Temperature may be oral, tympanic, or noncontact (clinic only). Temperature greater than 40.0 °C (104.0 °F) is considered an SAE and subjects should seek medical attention immediately.

Source: Adapted from DAIDS Version 2.1, 2017.

13.3 Appendix: Expected Normal Ranges in School-Age Children and Adolescents

Table 13-5 Expected Normal Ranges for Heart Rate and Respiratory Rate in School-Age Children and Adolescents

Population	Respiratory Rate (breaths per minute)	Heart Rate (beats per minute)
School-age children (6 to 12 years of age; 20 to 42 kg)	18-30	60-140
Adolescents (13 years of age or older; and/or >42 kg)	12-16	50-100

Source: Adapted from ACLS 2021.