ILiAD Biotechnologies

IB-201P

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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Final Statistical Analysis Plan

Version 1.0, Amendment 1.0

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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
DNT	dermonecrotic toxin
DTaP	diphtheria-tetanus-acellular pertussis
eCRF	electronic case report form
EOS	end of study
FHA	filamentous hemagglutinin
FMQ	FDA Medical Query
GCP	Good Clinical Practice
GMFR	geometric mean fold rise
GMC	geometric mean concentration
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
IRT	interactive response technology
ITT	intent-to-treat
IU	international unit(s)
MAAE	medically attended adverse event
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
PT	preferred term
RRR	reduction of relative risk
SAE	serious adverse event
SAP	statistical analysis plan
S-IgA	secretory immunoglobulin A
SMC	safety monitoring committee
SOC	system organ class

SUSAR	suspected unexpected serious adverse reaction
Tdap	tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis
WCE	whole cell extract
wP	whole cell pertussis

1. Introduction

This statistical analysis plan (SAP) describes the analyses and data presentations for ILiAD's protocol IB-201P "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™)" which was issued on 03FEB2021 (amended 02APR2021, 09JUN2021, 07JUL2021, 16AUG2021, 30MAY2022, and 30MAR2023 (still in use in countries outside Costa Rica), 10OCT2023 (applicable in Costa Rica only)). It contains definitions of analysis populations, derived variables, and statistical methods for the analysis of immunogenicity, colonization (substudy only) and safety.

These analyses include one (1) interim analysis and one final analysis. The purpose of the SAP is to ensure the credibility of the study findings by pre-specifying the statistical approaches to the analysis of study data prior to database lock and any data analysis for the interim/final analyses. This SAP is to be interpreted in conjunction with the protocol. Should the SAP and protocol be inconsistent with respect to the planned analyses, the language of the SAP is governing. This SAP will be finalized and signed prior to the clinical database lock for the interim analyses. If the final clinical study report (CSR) contains changes to any planned statistical analyses, the justification for any such differences will be fully documented in the CSR. All statistical analyses detailed in this SAP will be conducted using SAS® Version 9.4 or higher.

2. Objectives

2.1. Primary Objectives

Immunogenicity

- 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).

Safety

- To assess reactogenicity by toxicity scoring through 7 days following first study vaccination with any combination of pertussis-containing vaccines (BPZE1, BPZE1 + Boostrix, Boostrix).

2.2. Secondary Objectives

2.2.1. Key Secondary Objective

- 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).

2.2.2. Colonization (Optional Substudy Only)

- To describe colonization in each of the groups: BPZE1, BPZE1 + Boostrix, BPZE1 and BPZE1 + Boostrix, Boostrix control.

2.2.3. Mucosal Secretory Immunogenicity (S-IgA)

- 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).
- To demonstrate BPZE1 induction of mucosal S-IgA immunity is greater than baseline at Day 29 (BPZE1, BPZE1 + Boostrix).
- 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).
- 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).
- 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.
- 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).
- 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, other) may also be tested.
- To assess reverse cumulative distribution curves for S-IgA at Day 29.

2.2.4. Serum Immunogenicity (IgA and IgG)

- To demonstrate that BPZE1 (BPZE1, BPZE1 + Boostrix) induction of serum immunity is significantly greater than baseline at Day 29.
- To demonstrate that BPZE1 + Boostrix induction of serum immunity is non-inferior compared to Boostrix at Day 29 (BPZE1 + Boostrix vs Boostrix).
- 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.
- To demonstrate that BPZE1 induction of serum IgA is non-inferior to Boostrix at Day 169/EOS (BPZE1 vs Boostrix; BPZE1 + Boostrix vs Boostrix)
- 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).
- 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).
- 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).
- To assess for seroconversion at Days 29 and 85 (BPZE1, BPZE1 + Boostrix, Boostrix). Additional combinations of antibody response (WCE, PT, FHA, PRN) may also be tested.

- To assess reverse cumulative distribution curves for immune responses (IgA and IgG) at Day 29.

2.2.5. Safety

NOTE: Subjects participating in the substudy will be evaluated for safety endpoints in a separate evaluation after Day 85 re-vaccination/attenuated challenge.

- 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.
- To describe (severity and relationship to study vaccination) all adverse events (AEs) through 28 days following any study vaccination. Primary and re-vaccination/attenuated challenge (substudy subjects) to be described separately and 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.
- To describe any AE of special interest (AESI) and SAE through Day 169/EOS including relationship to study vaccination.
- 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.
- To assess proportion of subjects (subset of subjects) cleared from colonization (present/absent) through Day 85.

2.3. Exploratory Objectives

2.3.1. Mucosal Secretory Immunogenicity (S-IgA) (Optional Substudy Only)

- To evaluate mucosal immunity of individual and combinations of pertussis antibodies (eg, WCE, 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).
- 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).
- 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).

2.3.2. Serum Immunogenicity (Optional Substudy Only)

- 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).

- 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).
- To assess if serum immunity of individual and combinations of pertussis antibodies (eg, WCE, PT, FHA, PRN, and other known anti-pertussis antibodies) at Day 29 or 85 is relational to protection against colonization by treatment group and overall (BPZE1, BPZE1 + Boostrix, Boostrix).
- 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.

2.3.3. Colonization (Optional Substudy Only)

- To demonstrate prior vaccination with BPZE1 substantially reduces colonization at Days 92 and Day 99

2.3.4. Other Exploratory Objectives

- To assess cellular-mediated response (by ELISpot or flow cytometry) to BPZE1 and Boostrix (Th1 and Th2) in a subset of 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.

3. Investigational Plan

3.1. Overall Study Design and Plan

This is a multi-center, 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 provide 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.

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).

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. Enrollment 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	-

Abbreviation: IM, intramuscular.

[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).

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.

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 will 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.

There 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 Protocol 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 midturbinate/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 in Protocol Section 4.1.2.1 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).

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 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. For the substudy, 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.

3.2. Study Endpoints

3.2.1. Primary Endpoints

Immunogenicity

- GMFR of BPZE1 induction of mucosal S-IgA against WCE at Day 29 by treatment group (BPZE1, BPZE1 + Boostrix).

Safety

- 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.

3.2.2. Secondary Endpoints

3.2.2.1. Key Secondary Endpoint

- 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).

3.2.2.2. Colonization (Optional Substudy Only)

- 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).

3.2.2.3. Mucosal Secretory Immunogenicity

WCE, FHA, PRN, and any additional anti-pertussis mucosal antibodies identified during assay development using geometric mean concentration 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.

- BPZE1 induction of mucosal S-IgA against WCE compared with Boostrix at Day 29
- BPZE1 induction of mucosal S-IgA at Day 29 compared to baseline.
- BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA compared with baseline at Days 85 and 169/EOS (tested independently).
- BPZE1 + Boostrix induction of mucosal S-IgA compared with BPZE1 at Days 29 and 169/EOS (tested independently).
- 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.

- BPZE1 and BPZE1 + Boostrix induction of mucosal S-IgA by baseline quantile at Day 29.
- 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.
- Reverse cumulative distribution curves of each anti-pertussis mucosal antibodies S-IgA GMT at Day 29 by treatment group.

3.2.2.4. Serum Immunogenicity

WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies identified during assay development using GMT and GMFR unless otherwise stated.

- BPZE1 and BPZE1 + Boostrix induction of serum immunity (IgA, IgG) at Day 29 compared with baseline (tested independently by treatment group).
- BPZE1 + Boostrix induction of serum immunity (IgA, IgG) compared with Boostrix at Day 29.
- 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).
- 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.
- 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).
- BPZE1, BPZE1 + Boostrix and Boostrix induction of serum immunity (IgA, IgG) at Day 29 by baseline quantile (tested independently by treatment group).
- BPZE1 and BPZE1 + Boostrix induction of measurable functional antibody (subset of subjects) compared with Boostrix at Day 29 (tested independently by treatment group).
- 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).
- Reverse cumulative distribution curves of GMT at Day 29 by treatment group.

3.2.2.5. Safety

Subjects participating in the substudy will be evaluated for safety endpoints in a separate evaluation after Day 85 re vaccination/attenuated challenge.

- 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).
- All AEs through 28 days following study vaccination by severity and relationship to study vaccination (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).
- All AESIs and SAEs through Day 169/EOS by severity and relationship to study vaccination, and by time since study vaccination (by treatment group).
- Incidence of SARS-CoV-2 infections or AESIs associated with COVID-19 disease by severity, and by time since study vaccination (by treatment group).
- *B. pertussis* clearance following study vaccination (subset of subjects at varying time points through Day 85).

3.2.3. Exploratory Endpoints

3.2.3.1. Mucosal Secretory Immunogenicity (Optional Substudy Only)

Note that S-IgA objectives and endpoints for the overall study (as noted above) will also be analyzed for the optional substudy and will be considered exploratory.

- 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).
- Induction of mucosal S-IgA (WCE, 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).
- 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.

3.2.3.2. Serum Immunogenicity (Optional Substudy Only)

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.

- 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).
- 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).
- BPZE1, BPZE1 + Boostrix, BPZE1 and BPZE1 + Boostrix, Boostrix induction of serum immunity (IgA, IgG) at Day 29 or Day 85 (tested independently) compared by protection against colonization (Colonizers/Non-colonizers) (tested independently by treatment group and overall).
- 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).

3.2.3.3. Colonization (Optional Substudy Only)

- Subjects with colonization at each of Days 92 and 99 by treatment group. Expressed as absolute proportion and percent reduction relative to control.

3.2.3.4. Other Exploratory Endpoints

- Cellular-mediated response at baseline and Day 8 (safety lead-in cohort) and Days 85 and 113 (subset of substudy subjects).
- Immunologic response measurement by treatment group.

3.3.Treatments

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, or similar sponsor-approved substitute) to the deltoid region 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.

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. For the safety lead-in cohort, a minimum of 30 minutes must occur between intranasal and IM vaccinations on Day 1.

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 (e.g., 30 minutes following IM vaccination).

4. General Statistical Considerations

Continuous variables will be summarized using the mean, standard deviation (SD), median, first quartile, third quartile, interquartile range (IQR), minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages, as well as a 2-sided 95% confidence interval (CI) for proportions computed using the Agresti-Coull method when appropriate.

Baseline is defined as the last non-missing value before the vaccination on Day 1.

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

For immunogenicity analysis, it is assumed that the natural log of the data is normally distributed for the GMT/GMC/GMR, and GMFR.

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

For any antibody titer, if the result is reported as lower than the minimal limit of assay detection, a value equal to half of the minimal limit of assay detection will be imputed in the calculation. The minimal limit of assay detection for each of the antibody titers will be established following assay validation and prior to database lock.

GMFR will be calculated as anti-logarithm of ∑ [log-transformed titer ratio of (Yi/Bi)/number of subjects with titer information], where Yi is the post-dose titer and Bi is the baseline titer for subject i. The same data summaries will be provided as GMT/GMC/GMR.

The mucosal and serum immunogenicity descriptive summaries (GMT/GMC/GMR and/or GMFR) will be provided by treatment group and by timepoint and across timepoints if applicable (all subjects), including a separate analysis after Day 85 for the open-label substudy. Analyses will include the GMT/GMC/GMR with 95% CIs and GMFRs with 95% CIs.

For seroconversion of mucosal S-IgA and serum IgA and IgG against WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies identified during assay development, descriptive summaries including seroconversion rates and the 95% CIs will be provided by treatment group and by timepoint. Mucosal S-IgA will be normalized as [specific S-IgA]/[Total S-IgA (mg/mL)] and it will also be summarized using same method.

Immunogenicity endpoints (seroconversion, GMT/GMC/GMR, and GMFR) will be summarized by baseline PRN negative and PRN 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. An additional age group of 10 to 17 years will be added for some analyses to be able to support a rationale for immunobridging to adults. A forest plot will be generated for subgroups.

The substudy analysis will assess immunogenicity endpoints at baseline and Days 85 and 113 (independently for substudy subjects) and relative to colonization status (colonized/non colonized) at either or both Day 92 and/or 99 following re-vaccination/attenuated challenge at Day 85.

All statistical tests will be 2 sided at 0.05 significance level.

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.

All data collected will be listed in data listings.

4.1.Sample Size

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. Enrollment 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.

4.1.1. Sample Size for 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 (BPZE1, BPZE1+Boostrix). Factoring in an estimated 10% drop-out rate, each treatment group would include approximately 120 subjects for a total of approximately 240 subjects in the study for the primary analysis. An additional 120 subjects are randomized to the Boostrix group for the assessment of non-interference resulting in a total of approximately 360 subjects in the study.

4.1.2. Sample Size for 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.

Table 4-1 Power for Non-Inferiority of BPZE1/Boostrix to Boostrix for the proportion of titer ≥ 0.1 IU/mL

Parameter			Power**			
p_B*	$p_{BBP,0}$+	$p_{BBP,1}$#	N=120***	N=135***	N=150***	N=165***
0.92	0.82	0.92	77.3%	81.8%	85.7%	88.7%
0.95	0.85	0.95	92.1%	94.6%	96.5%	97.7%
0.97	0.87	0.97	99.1%	99.0%	99.8%	99.9%

*: p_B is the proportion of titer ≥ 0.1 IU/mL for the Boostrix treatment
+: $p_{BBP,0}$ is the proportion of titer ≥ 0.1 IU/mL for the BPZE1/Boostrix treatment under the null hypothesis H_0
#: $p_{BBP,1}$ is the proportion of titer ≥ 0.1 IU/mL for the BPZE1/Boostrix treatment under the alternative hypothesis H_1
**: with one-sided significance levels of 0.025 and non-inferiority margin of 0.1

***: Effective sample size accounts for 10% dropout. For sample size of 120, 135, 150, 165 per group, the effective sample size is 108, 121, 135, 148, respectively, after accounting for 10% dropout.

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 on the natural log scale (or 2-fold decrease on the original scale).

Table 4-2: Power for Non-Inferiority of BPZE1/Boostrix to Boostrix for serum IgG parameters

Serum IgG Parameter	Mean Diff Used for Sample Size Calculation (based on previous adult Ph2b study) (on natural log)	StdDev+ (on natural log)	Power**			
			N =120 per group*	N =135 per group*	N =150 per group*	N =165 per group*
PT	-0.234	0.8759	97.0%	98.2%	99.0%	99.4%
FHA	-0.019	0.8158	100%	100%	100%	100%
PRN	-0.075	1.4363	88.2%	91.5%	94.1%	95.8%

*: Effective sample size accounts for 10% dropout. For sample size of 120, 135, 150, 165 per group, the effective sample size is 108, 121, 135, 148, respectively, after accounting for 10% dropout.
**: with one-sided significance levels of 0.025 and non-inferiority margin of -0.693 (=natural log(0.5))

4.2.Randomization, Stratification, and Blinding

Subjects will be randomly assigned to 1 of 3 treatment groups, in a 1:1:1 ratio, as presented in Table 3-1. 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. Subjects will be operationally managed to maintain a minimum number of subjects enrolled in 2 age groups. 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. 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.

Biostatistics will generate the randomization schedule using SAS® software Version 9.4 or later (SAS Institute Inc, Cary, North Carolina) for the interactive response technology, which will link sequential subject randomization numbers to treatment codes. The randomization schedule will

be created by the dedicated randomization team, stored in a separate project area, and will be blinded to the project team with the exception of 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.

4.3.Analysis Set

4.3.1. Safety Analysis Set

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.

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

The intent-to-treat analysis set will consist of all randomized subjects who are vaccinated. Subjects will be classified according to the randomized treatment group. Subject disposition, demographics, and medical history will be summarized on this analysis set, and subject disposition and demographics will be summarized using the Per Protocol Immunogenicity Analysis Set as well.

4.3.3. Immunogenicity Analysis Set

The immunogenicity analysis set will consist of all subjects in the intent-to-treat analysis set who received 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 analyzed, and have not received a licensed pertussis vaccine in the past 3 years or through Day 85 of the study nor received a tetanus/diphtheria vaccine through 29 days post study vaccination. Subjects will be classified according to the actual vaccine received.

4.3.4. Per-Protocol (PP) Immunogenicity Analysis Set

Immunogenicity Analyses to be performed based on PP Immunogenicity Analysis Set.

The PP immunogenicity 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 subjects who received at least one dose of whole-cell pertussis (wP) containing vaccine in their life.
- 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.
- For subjects in the colonization substudy, missing Day 92 and Day 99 cultures will be removed from the Colonization endpoint.

For analyses using the PP immunogenicity analysis set, subjects will be classified according to the randomized treatment group. Determination of exclusions from the PP Immunogenicity Analysis Set will be performed by the sponsor clinical lead in a blinded fashion prior to the database lock.

4.3.5. Safety Lead-in Analysis Set

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 analyzed 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.

4.4.Handling of Missing Data

Impute Missing Colonization Data

Colonization data will be presented in the listings as reported for subjects consented to substudy only. For summaries and analysis, the following conventions apply.

When only one day out of two days (Day 92 or Day 99) is missing:

- If Day 92 is negative but Day 99 is missing, Day 99 will be imputed as negative;
- If Day 92 is missing but Day 99 is negative, Day 92 will be imputed using fully conditional specification multiple imputation.
- If any day is positive, the remaining missing data will not be imputed.

When subjects drop out before they visit on Day 92 but consent to the substudy, i.e., all two days (Day 92 and Day 99) are missing:

- Status of being positive or negative for a given day will be imputed using fully conditional specification multiple imputation.

Multiple imputation method for missing binary outcome of colonization

Imputation of the missing binary variable of being a colonizer or non-colonizer will be done using fully conditional specification with a logistic model. The multiple imputation procedure and the analysis will be conducted in three separate steps: 1) Imputation 2) Analysis of imputed complete data sets 3) combination of results using Rubin’s rule.

1. Imputation (using SAS PROC MI): Use the logistic method with age group as covariate to impute the missing colonization status of being positive or negative by treatment group. The FCS LOGISTIC statement will be used with a seed of 57808563.
   In this MI approach, SAS® procedure PROC MI performs MI by replacing each missing value with a set of plausible values by treatment group. The missing data will be filled in multiple times to generate multiple complete data sets. The number of imputed complete data sets will be 50.
2. Analysis of imputed complete data sets: imputed data sets will then be analyzed separately using the same method for the colonization endpoint as described in Section 8.4.1.
3. Combination of estimates across imputed datasets (can be obtained using SAS PROC MIANALYZE): the results obtained from Step 2 (one for each imputation), which are difference in response proportion and its standard errors, will then be combined per Rubin's rule (t statistics and standard errors), p-values for combined t-statistics will be derived using Student's t distribution with error degrees of freedom.

Impute Missing Adverse Events/ Prior or Concomitant Medications

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

A. Incomplete Start Date:

Missing day and month

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

Missing day only

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

Missing day, month, and year

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

B. <u>Incomplete End Date:</u>

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

Missing day and month

- If the year of the incomplete stop date is the **same** as the year of the last dosing date, then the day and month of the last dosing date will be assigned to the missing fields.
- If the year of the incomplete stop date is **prior to** the year of the last dosing date, then December 31 will be assigned to the missing fields.
- If the year of the incomplete stop date is **prior to** the year of the last dosing date but is the same as the year of the first dosing date, then the first dosing date will be assigned to the missing date.
- If the year of the incomplete stop date is **after** the year of the last dosing date, then January 1 or EOS date which ever occurred later will be assigned to the missing fields.

Missing day only

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

4.5.Analysis Window/Visit

Visit windows for each visit are defined in Section 13.1. Additionally, analysis windows will be derived using below rules:

Study Visit/Study Day	Main Study	Substudy
Visit 1/Day 1	Day 1	
Visit S1/Day 8[a]	Day 6 to Day 18	
Visit 2/Day 29	Day 19 to Day 57	
Visit 3/Day 85	Day 58 to Day 127	Day 58 to Day 115
SS Visit 4[b]/Day 92	N/A	3 days before SS Visit 4 to 4 days after SS Visit 4

SS Visit 5[b]/Day 99	N/A	3 days before SS Visit 5 to 7 days after SS Visit 5
SS Visit 6[b]/Day 113	N/A	7 days before SS Visit 6 to 28 days after SS Visit 6
EOS/Day 169	Day 128 to Day 199	29 days after SS Visit 6 to Day 199

[a] Safety Lead-in subject only

[b] Subjects in substudy only

5. Subject Disposition

The number of subjects who were randomized and eligibility criteria met/failed will be summarized based on all enrolled subjects. Eligibility criteria met/failed will be listed based on all screened subjects.

Disposition of all enrolled subjects will be summarized by treatment groups, age groups (6 - 10 years, and 11 – 17 years, and 10 – 17 years) and overall including:

- Number screened
- Number screen failures
- Number of subjects randomized
- Number of subjects randomized, not vaccinated
- Number of subjects vaccinated
- Safety Analysis Set
- ITT Analysis Set
- Immunogenicity Analysis Set
- Per Protocol Immunogenicity Analysis Set
- Safety Lead-in Analysis Set
- Number of subjects who participated in substudy
- Number of subjects who completed substudy
- Number of subjects who completed study (EOS Visit)
- Number of subjects who prematurely discontinued study
- Primary reason for premature discontinuation of study

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

- Adverse Event

- Lost to Follow-Up
- Withdrawal of Consent
- Investigator Decision
- Sponsor Decision
- Death
- Other

A by subject listing will be provided based on the ITT Analysis set, subjects who are willing to participate in the substudy will also be included.

Disposition of all subjects who participate in substudy will be summarized by treatment groups, age groups (6 - 10 years, and 11 – 17 years) and overall including:

- Number of subjects who participated in substudy
- Number of subjects vaccinated on Day 1
- Number of subjects vaccinated on Day 85
- Number of subjects who are also in the following analysis sets:
  - Safety Analysis Set
  - Immunogenicity Analysis Set
  - Per Protocol Immunogenicity Analysis Set
- Number of subjects who prematurely discontinued study
- Primary reasons for premature discontinuation of study

Primary reasons for study discontinuation for subjects who are willing to participate in substudy include:

- Adverse Event
- Lost to Follow-Up
- Withdrawal of Consent
- Investigator Decision
- Sponsor Decision
- Death
- Other

5.1.Protocol Deviations

A deviation from the protocol is an unintended or unanticipated departure from the agreed procedures or processes. 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 or non-significant prior to database lock.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Any overdose should be recorded as a protocol deviation. For subjects who are designated to provide mid-turbinate/nasopharyngeal samples on Day 29, it will not be considered a protocol deviation if subjects provide samples on Day 85 instead. Significant protocol deviations will be summarized using the ITT Analysis Set.

A by-subject listing will be provided based on the ITT Analysis Set including both the significant and non-significant protocol deviations. A separate listing will be provided to list the reasons subjects were excluded for the Per-Protocol Immunogenicity Analysis Set based on Immunogenicity Analysis Set.

6. Demographics and Baseline Characteristics

6.1.Demographics

Demographic and baseline characteristics data to be analyzed will include age, sex, race, ethnicity, weight, height, and body mass index (BMI) on the ITT Analysis set and PP Immunogenicity Analysis set by treatment group and overall, and by age group (6 - 10 years, and 11 – 17 years, and 10 – 17 years). Z-scores of height, weight, and BMI will be calculated using a SAS macro and the reference data file from the CDC website (https://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm)

The following characteristics will be summarized as continuous variables:

- Age (years)
- Weight (kg)
- Weight Z-score
- Height (cm)
- Height Z-score
- BMI (kg/m^2)
- BMI Z-score
- Time since last aP vaccination

Time since last aP vaccination prior to study entry will be derived based on the country`s immunization schedule. The schedule is as below:

1) UK – 3 years old
2) Australia – 4 years old and 12-13 years old
3) Costa Rica – 4 years old

The estimated time since last aP vaccination derive method is:

1) UK site: estimated time since last aPV = Age -3
2) Costa Rica site: estimated time since last aPV = Age -4

3) Australia if age is <= 12: estimated time since last aPV = Age -4

4) Australia if age is > 12: estimated time since last aPV = Age -12

The following characteristics will be summarized as categorical variables:

- Sex (Male, Female)
- Age group (6 - 10 years, and 11 – 17 years, and 10 – 17 years)
- Race (White, Black, Asian, Pacific Islander, Aboriginal or Torres Strait Islander, Mixed or Multiple ethnic groups, Other)
- Ethnicity (Hispanic, non-Hispanic, Not reported, Unknown)
- County

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

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.

Medical history will be summarized based on the ITT Analysis Set and will be coded according to the latest version of Medical Drug Regulatory Activities (MedDRA). Medical history will be summarized by system organ class (SOC) and preferred term (PT), with SOCs sorted alphabetically and PTs within each SOC in descending order of frequency.

A by-subject listing will be provided for medical history based on the ITT Analysis set.

7. Treatments and Medications

7.1.Prior and Concomitant Medications

The Anatomical Therapeutic Chemical (ATC) coding scheme of the latest World Health Organization Drug Dictionary (WHODrug) will be used to group medications into relevant categories.

7.1.1. Prior Medications

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

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

7.1.2. Concomitant Medications

Concomitant medications are defined as 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 associated with any MAAE will be recorded through 84 days following any study vaccination. Concomitant medications associated with any AESI or SAE will be recorded through Day 169/EOS.

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

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

7.1.3. Concomitant Vaccination

Vaccines received outside of the study will be specifically queried during each visit and recorded through Day 169/EOS. Concomitant vaccinations will be summarized based on the Safety Analysis Set.

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

7.2.Study Treatments

7.2.1. Treatment Compliance and Modifications

A by-subject listing will be provided based on the ITT Analysis Set, and subjects in the optional substudy will also be included.

8. Immunogenicity Analysis

Immunogenicity Analyses will be performed based on Per Protocol Immunogenicity Analysis Set, and sensitivity analyses will be performed where specified.

8.1.Analyses of Primary Endpoints

The estimand description for the primary endpoint analysis is provided in the following table.

Estimand attributes for the primary endpoint analysis	
Estimand Label	Estimand
Estimand Description	GMFR (from baseline) of BPZE1 induction of mucosal S-IgA against WCE at Day 29 following vaccination at Day 1 in the BPZE1 treatment group (BPZE1 is administered intranasally via a MAD) and BPZE1+Boostrix treatment group (BPZE1 is administered intranasally via a MAD and Boostrix is administrated by IM injection).
Target population	All School-Age Children subjects in the Per Protocol Immunogenicity Analysis Set
Treatment Condition	Treatment group A: BPZE1 (2 x 0.4 mL (0.4 mL per nostril containing half the dose (5 x 10^8 CFU) to give a total dose of 10^9 CFU) administered intranasally via a MAD) on Day 1; and an IM injection of 0.5 mL placebo (sterile normal saline for injection, or similar sponsor-approved substitute) to the deltoid region on Day 1. Treatment group B: BPZE1 (2 x 0.4 mL (0.4 mL per nostril containing half the dose (5 x 10^8 CFU) to give a total dose of 10^9 CFU) administered intranasally via a MAD) on Day 1; and an IM injection of 0.5 mL Boostrix (includes aP vaccine, manufactured by GlaxoSmithKline) to the deltoid region on Day 1.
Endpoint	GMFR of BPZE1 induction of mucosal S-IgA against WCE at Day 29 by treatment group [BPZE1 (Treatment group A), BPZE1 + Boostrix (Treatment group B)]. Mucosal S-IgA will be normalized as [specific S-IgA]/[Total S-IgA (mg/mL)].
Population-level summary	GMFR (from baseline) at Day 29 in each BPZE1 and BPZE1+Boostrix treatment groups.
Intercurrent Events and Strategies	None.

For mucosal S-IgA against WCE at Day 29, GMFR will be summarized for all treatment groups using descriptive statistics, and the 2-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 hypotheses can be expressed on the natural log scale as:

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

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. The superiority hypothesis will be tested in treatment groups BPZE1 and BPZE1 + Boostrix separately. These 2 primary comparisons will be tested at a one-sided significance level of 0.025. The study is considered a success if both primary comparisons meet statistical significance.

Sensitivity analysis for primary endpoints will be performed based on the Immunogenicity Analysis Set.

8.2.Analyses of Key Secondary Serum Immunogenicity (IgG) Endpoints

Descriptive statistics (GMT, GMFR) for serum IgG against PT, FHA, and PRN will be presented at baseline and Day 29 and by treatment group (BPZE1 + Boostrix, Boostrix).

The non-interference objective will be tested as non-inferiority of BPZE1+Boostrix group compared to the Boostrix group.

The formal non-inferiority hypothesis is:

$$H_0: \frac{\mu_{BBP}}{\mu_B} \leq 0.5 \; vs. \; H_A: \frac{\mu_{BBP}}{\mu_B} > 0.5$$

Taking the natural log transformation,

$$H_0: \ln(\mu_{BBP}) - \ln(\mu_B) \leq -0.693 \; vs. \; H_A: \ln(\mu_{BBP}) - \ln(\mu_B) > -0.693$$

Where μ_{BBP} is the GMT for the BPZE1+Boostrix group and μ_B is the GMT for the Boostrix group.

For serum IgG against PT, FHA, and PRN at Day 29, separate ANCOVA models that include baseline IgG levels and treatment group as covariates will be used to compare the natural log-transformed GMTs between BPZE1 + Boostrix and Boostrix groups. The 95% CI for between group difference in GMT will be calculated in two steps: 1) calculate the 95% CI on natural log scale; 2) exponentiate the lower and upper limits to get the 95% CI on the original scale.

Non-inferiority, or non-interference, will be demonstrated if the lower limit of the 95% CI of between group difference in natural log-transformed GMTs is greater than -0.693.

For serum IgG against diphtheria and tetanus, the non-interference objective will be tested as non-inferiority of BPZE1+Boostrix group compared to the Boostrix group for the proportion of subjects with antibody concentration ≥0.1 IU/mL.

The formal hypotheses is:

$$H_0: \pi_{BBP} - \pi_B \leq -0.1 \; vs. \; H_A: \pi_{BBP} - \pi_B > -0.1,$$

where π_{BBP} is the proportion of subjects with antibody (diphtheria or tetanus) concentration ≥0.1 IU/mL for the BPZE1+Boostrix group and π_B is the corresponding proportion for the Boostrix group.

The 95% CI for between group difference in the proportion of subjects with antibody concentration ≥0.1 IU/mL will be calculated using the Agresti-Caffo method. Non-inferiority, or non-interference, will be demonstrated if the lower limit of this 95% CI is greater than -0.1.

Sensitivity analysis for key secondary endpoints will be performed based on the Immunogenicity Analysis Set.

8.3.Secondary Endpoints Analyses

Unless otherwise specified, data will be summarized for each endpoint as described in Section 3.2. Listings will be provided for all data as collected.

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

Descriptive statistics (GMC/GMR [also known as GMT per protocol], 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 timepoint and by treatment groups. Mucosal S-IgA will also be normalized as [specific S-IgA]/[Total S-IgA (mg/mL)].

A non-inferiority hypothesis will be tested to demonstrate BPZE1 can adequately induce pertussis specific mucosal S-IgA responses at Day 29 against WCE (GMC/GMR and GMFR) compared with Boostrix. A two-sample t-test will be used to compare the natural log-transformed GMC/GMRs between BPZE1 and Boostrix groups. The non-inferiority margin is set at 2-fold decrease or -0.693 on natural log scale. Non-inferiority will be demonstrated if the lower limit of the 95% CI of group difference on natural log scale is greater than -0.693.

To test the hypothesis that BPZE1 can adequately induce pertussis-specific mucosal S-IgA responses at Day 29, Day 85, and Day 169/EOS against WCE, PRN, PT, and FHA compared with baseline by treatment group, separate paired t-tests will be used to compare the natural log-transformed GMC/GMR at Day 29, Day 85, Day 169/EOS with the natural log-transformed GMC/GMR at baseline within each of treatment groups. The corresponding 95% CIs will be calculated on the natural log scale, then both lower and upper limits will be exponentiated to obtain the 95% CIs on the original scale.

A non-inferiority hypothesis will be tested to demonstrate that BPZE1+Boostrix induction of mucosal S-IgA immunity is non-inferior to BPZE1 at Day 29 and Day 169/EOS. Separate ANCOVA models that include baseline S-IgA and treatment group as covariates will be used to compare the natural log-transformed GMCs/GMRs between BPZE1+Boostrix and BPZE1 groups. The non-inferiority objective will be demonstrated if the lower limit of the 95% CI of group difference on the natural log scale is greater than -0.693.

Subgroup analysis by age group (6-10 years and 11-17 years, inclusive, and 10 – 17 years [for requested analyses]), time since last aP vaccination prior to study entry (<median time, ≥median time), or other baseline demographics, and by baseline antibody level (by quantile) will be conducted to examine differential responses to induction of mucosal S-IgA at Day 29 for BPZE1

and BPZE1 + Boostrix groups. In addition to descriptive summaries, a forest plot of the corresponding 95% CIs will be generated.

Mucosal S-IgA seroconversion rate is defined as 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). Mucosal S-IgA seroconversion rates will be calculated by BPZE1, BPZE1 + Boostrix, and Boostrix groups on Day 29. The 95% CI will be calculated by using Agresti-Coull method. The likelihood ratio chi-square test will be used to compare seroconversion rates among treatment groups. Additional combinations of antibody response (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) may also be tested.

Graphics of the reverse cumulative distribution curves of each anti-pertussis mucosal antibody titer for mucosal S-IgA GMC/GMR will be generated for Day 29 by treatment group.

After natural logarithmic transformation, an ANCOVA model will be used to test the differences between treatment groups (BPZE1 vs Boostrix, BPZE1 + Boostrix vs Boostrix), with mucosal S-IgA against each of WCE, PT, PRN, FHA, and any additional anti-pertussis mucosal antibodies identified during assay development post vaccination at each of Days 29, 85, and 169/EOS as the dependent variable, vaccination as the treatment effect age, and baseline antibody value as covariates. The difference between treatment groups and the 95% CI for GMC/GMR on the natural log scale will be calculated.

8.3.2. Analyses of Secondary Serum Immunogenicity (IgA and IgG) Endpoints

Descriptive responses (GMC [also known as GMT per protocol], GMFR, seroconversion) of IgA and IgG against WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies identified during assay development will be conducted at each time point and by treatment group.

To test the hypothesis that BPZE1 and BPZE1 + Boostrix can induce significantly greater serum immunity (IgA, IgG) at Day 29 than baseline, separate paired t-tests will be used to compare the natural log-transformed GMC at Day 29 with the natural log-transformed GMC at baseline within each treatment group. The corresponding 95% CIs will be calculated on the natural log scale, then both lower and upper limits will be exponentiated to get the 95% CIs on the original scale.

A non-inferiority hypothesis will be tested to demonstrate BPZE1 + Boostrix induction of serum IgA is non-inferior to Boostrix at Day 29. An ANCOVA model that includes baseline IgA and treatment group as covariates will be used to compare the natural log-transformed GMCs at Day 29 between BPZE1+Boostrix and Boostrix groups. The non-inferiority margin is set at a 2-fold decrease or -0.693 on natural log scale. Non-inferiority will be demonstrated if the lower limit of the 95% CI of group difference on natural log scale is greater than -0.693.

To test the hypotheses that BPZE1 and BPZE1 + Boostrix can induce significantly greater serum immunity (IgA, IgG) at Day 85 and Day 169/EOS than baseline, separate paired t-tests will be used to compare the natural log-transformed GMC at Day 85 and Day 169/EOS with the natural log-transformed GMC at baseline within each treatment group. The corresponding 95% CIs by treatment group and day will be calculated on the natural log scale, then both lower and upper limits will be exponentiated to get the 95% CIs on the original scale.

The non-inferiority hypothesis will be tested to demonstrate: 1) BPZE1 induction of serum IgA and IgG is non-inferior to Boostrix at Day 85 and Day 169/EOS; 2) BPZE1+ Boostrix induction

of serum IgA and IgG is non-inferior to Boostrix at Day 85 and Day 169/EOS. An ANCOVA model that includes baseline IgA or IgG and treatment group as covariates will be used to compare the natural log-transformed GMCs at Day 85 and Day 169/EOS between either BPZE1 or BPZE1+Boostrix and Boostrix groups. The non-inferiority margin is set at a 2-fold decrease or -0.693 on natural log scale. Non-inferiority will be demonstrated if the lower limit of the 95% CI of group difference on natural log scale is greater than -0.693.

Subgroup analysis by age group (6-10 years and 11-17 years, inclusive, and 10 – 17 years [for requested analyses]), time since last aP vaccination prior to study entry (<median time, ≥median time), or other baseline demographics, and by baseline antibody level (by quantile) will be conducted to examine differential responses to induction of serum immunity (IgA, IgG) at Day 29 by treatment group (BPZE1, BPZE1+Boostrix, and Boostrix). In addition to descriptive summaries, a forest plot of the corresponding 95% CIs will be generated for each subgroup.

To compare BPZE1 and BPZE1 + Boostrix induction of measurable functional antibody (subset of subjects) with Boostrix at Day 29, separate two-sample t-tests will be used to compare between treatment group differences in natural log-transformed GMC at Day 29. The corresponding 95% CIs by treatment group will be calculated on the natural log scale, then both lower and upper limits will be exponentiated to get the 95% CIs on the original scale.

Seroconversion rates with 95% CIs for IgA and IgG will be calculated at Days 29 and 85 by treatment group and day. The 95% CI will be calculated by using Agresti-Coull method. The likelihood ratio chi-square test will be used to compare seroconversion rate between treatment groups. Additional combinations of antibody response (WCE, PT, FHA, PRN, and any additional anti-pertussis antibodies) may also be tested.

Seropositive rates with 95% CIs for IgA and IgG based on Ward Criteria will be calculated at Day 29 by treatment group. The 95% CI will be calculated by using Agresti-Coull method. The likelihood ratio chi-square test will be used to compare seroconversion rate between treatment groups. Ward Criteria Seropositive is defined as increase 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) against pertussis toxin (PT), or increase 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) against 2 other pertussis antigens (pertactin [PRN], filamentous hemagglutinin [FHA], fimbriae serotype 2 and 3 [FIM2/3]).

Graphics of the reverse cumulative distribution curves of anti-pertussis antibody titers for IgA and IgG GMC will be generated for Day 29.

8.4.Analyses of Endpoints for the Optional Substudy

8.4.1. Analysis of Colonization Endpoints

Colonization summary tables will be produced based on the sample type (mid-turbinate swab and nasopharyngeal swab).

The analyses in this section of the colonization substudy will be based on the PP immunogenicity analysis set but include only subjects who are enrolled in the optional open-label substudy. A sensitivity analysis will be conducted using the Immunogenicity Analysis Set but include only subjects who are enrolled in the optional open-label substudy.

To assess if prior BPZE1 induced mucosal immunity substantially reduces colonization at Days 92 and 99, the proportion of subjects with colonization at Day 92 or Day 99 will be analyzed. The 2-sided 95% CI in the proportion of subjects with colonization at either Day 92 or Day 99 (*B. pertussis* culture or PCR) will be calculated using Agresti-Coull method and summarized by treatment groups (BPZE1, BPZE1 + Boostrix, BPZE1 and BPZE1 + Boostrix, Boostrix control). The likelihood ratio chi-square test will be used to compare the proportions between treatment groups.

Separate relative risks, which are defined as the ratio of two treatment group proportions, will be calculated for 1) BPZE1 vs. BPZE1 + Boostrix; 2) BPZE1 vs. Boostrix control; 3) BPZE1 + Boostrix vs. Boostrix control; 4) BPZE1 and BPZE1 + Boostrix vs. Boostrix control. The CI for relative risk will be computed using the likelihood ratio method.

The relative risk reduction (RRR) is defined as RRR (%) = (1 – RR) x 100. The CI for the RRR will be obtained by transforming the CI for the relative risk using (1 – UL, 1 – LL), where UL denotes the upper limit of the CI for the RR and LL denotes the lower limit of the CI for the RR.

The hypotheses for the primary endpoint are:

H0: RRR $\leq$0%

HI: RRR >0%

Rejection of the null hypothesis demonstrates a statistically significant vaccine effect, (i.e., LL of the CI >0%), and will be considered to meet the pre-specified study success criterion.

Colonization at Day 8 safety lead-in group will be analyzed to determine specimen type. Colonization at Day 29 or Day 85 will be analyzed for clearance of BPZE1.

8.4.2. Analysis of Mucosal Secretory Immunogenicity Endpoints

BPZE1, BPZE1 + Boostrix, BPZE1 and BPZE1 + Boostrix, and Boostrix control induction of mucosal S-IgA at Days 29 and 85 will be summarized by protection against colonization (Colonizers/Non-colonizers) and by treatment group and by timepoint. Separate two-sample t-tests will be used to compare the natural log-transformed GMRs at Days 29 and 85 between Colonizers and Non-colonizers by treatment group and by timepoint.

To test the hypothesis that BPZE1 and BPZE1 + Boostrix can induce mucosal S-IgA at Day 113 compared with Day 85, separate paired t-tests will be used to compare the natural log-transformed GMCs/GMRs at Day 113 with the natural log-transformed GMCs/GMRs at Day 85 within each treatment group. The corresponding 95% CIs will be calculated on the natural log scale, then both lower and upper limits will be exponentiated to get the 95% CIs on the original scale.

BPZE1, BPZE1 + Boostrix, or Boostrix control induction of mucosal S-IgA at Day 113 and Day 169/EOS will be summarized by participation in the substudy (Yes/No) and by treatment group and by timepoint. Separate ANCOVA models that include baseline S-IgA and an indicator of participation in the substudy (Yes/No) as covariates will be used to compare between subjects who participated in the substudy and subjects who did not participate in the substudy, by treatment group and by timepoint.

8.4.3. Analysis of Serum Immunogenicity Endpoints

To test the hypothesis that BPZE1 induction of serum immunity (IgA, IgG) at Day 113 compared with baseline, or Day 113 compared with Day 85, separate paired t-tests will be used to compare the natural log-transformed GMC at Day 113 with the natural log-transformed GMC at either baseline or Day 85 within each treatment group (BPZE1, BPZE1 + Boostrix, Boostrix). The corresponding 95% CIs will be calculated on the natural log scale, then both lower and upper limits will be exponentiated to get the 95% CIs on the original scale.

BPZE1, BPZE1 + Boostrix, and Boostrix induction of serum immunity (IgA, IgG) at Day 113 and Day 169/EOS will be summarized by participation in the substudy (Yes/No) and by treatment group and by timepoint. Separate two sample t-tests will be used to compare the natural log-transformed GMCs at Day 113 and Day 169/EOS between subjects who participated in the substudy and subjects who did not participate in the substudy (Day 169/EOS only), by treatment group and by timepoint.

BPZE1, BPZE1+Boostrix, BPZE1 and BPZE1+Boostrix, Boostrix control induction of serum immunity (IgA, IgG) at Days 29 and 85 will be summarized by protection against colonization (colonized/non-colonized) and by treatment group and by timepoint. Comparison between colonized subjects and non-colonized subjects will be made using separate two-sample t-tests by treatment group and by timepoint.

The number and proportion of subjects with ≥50% killing titer (or equivalent) at Day 113 compared to baseline and Day 29 will be summarized by treatment group and by timepoint. The 95% CI will be provided by using Agresti-Coull method. A McNemar test will be used to compare proportions between timepoints in each treatment groups (Day 113 vs baseline; Day 113 vs Day 29).

The number and proportion of subjects with a ⩾2x increase from baseline in SBA bactericidal titer (50% killing titer) will be summarized by treatment group, by timepoint and by strain. The 95% CIs for each treatment group will be computed using the Agresti-Coull method and the 95% CIs for the difference in proportions between groups (BPZE1 vs Boostrix, BPZE1 + Boostrix vs Boostrix) will be computed using Agresti Caffo method. A chi-square test will be performed to compare the proportion of subjects between groups (BPZE1 vs Boostrix, BPZE1 + Boostrix vs Boostrix) with a ⩾2x increase in SBA bactericidal titer and two-sided p-value will be provided.

Reverse cumulative distribution curves will be provided for SBA (PRN+ and PRN-) at baseline, Day 29 and Day 113.

9. Safety Analysis

Unless otherwise specified, safety analysis will be performed based on the Safety Analysis Set. Safety analysis for safety lead-in cohort will be performed based on the Safety Lead-in Analysis Set. All summaries and analyses will be presented by treatment groups (BPZE1, BPZE1 + Boostrix, Boostrix), age group, and overall for first study vaccination and following re-vaccination/attenuated challenge (substudy) separately.

9.1.Solicited Adverse Events

The number and percentage of subjects with any 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, age group and overall, and by timepoint.

Solicited reactogenicity events occurring from the time of study vaccination on Day 1 for 7 consecutive days starting the day following study vaccination, including local, nasal/respiratory reactions and systemic reactogenicity, will be summarized as presenting both the number and frequency, categorized by the severity grades according to respective reactogenicity grades (Table 9-1-1, Table 9-1-2, and Table 9-1-3).

Additionally, solicited AEs will be analyzed by determining the maximum severity through 7 days following study vaccination on Day 1 to summarize the frequency and percentage of subjects reporting each local, nasal/respiratory, or systemic event.

Redness measurements, swelling measurements, and oral temperature if subjects have fever for 7 days following vaccination will be summarized using descriptive statistics.

Duration through 7 days following study vaccination and duration through resolution following study vaccination will be summarized using descriptive statistics. Duration through 7 days is defined as days with the reactogenicity event onset through 7 days following vaccination per patient reported daily diary. Duration beyond 7 days is defined as days with the reactogenicity event continuing beyond 7 days as captured in adverse events.

Solicited AEs will be reported separately using the same methods above following study vaccination on Day 85 in the substudy.

Table 9-1-1 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 9-1-2 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 9-1-3 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 (e.g. IV hydration)
Diarrhoea	Transient or intermittent episodes of unformed stools OR increase of $\leq$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 $\geq$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.

9.2.Unsolicited Adverse Events

Unsolicited AEs will be coded according to Medical Dictionary for Regulatory Activities (MedDRA) latest version. Unless otherwise specified, unsolicited AEs will be summarized by System Organ Class (SOC), FDA Medical Query (FMQ) and Preferred Term (PT), with SOCs sorted in the alphabetical order and PTs within each SOC in descending order of subject incidence.

TEAE is defined as an AE that starts or an existing AE that worsens on or after the date of vaccination. AEs will be summarized by treatment groups, age group, and main study and substudy separately. In general AEs will be summarized from Day 1 to end of study for overall and subjects in substudy. AEs from Day 85 to end of study for subjects in substudy will be summarized separately. Between screening and Day 1, AEs will be collected if classified as SAEs or if considered related to study procedure or study involvement. All AEs regardless of causality will be recorded for 28 days after any vaccination/challenge and summarized. All AESI and SAE will be recorded and summarized from the time of vaccination to Day 169/EOS. All MAAEs occurring through 84 days following any vaccination (and through EOS) will be recorded and summarized.

9.2.1. Incidence of Adverse Events

An overview summary of the following TEAE categories will be provided:

- Any TEAE
- Any TEAE through 28 Days Following Study Vaccination Day 1
- Any TEAE related to vaccination
- Any TEAE related to MAD
- Any serious TEAE
- Any serious TEAE related to vaccination
- Any serious TEAE related to MAD
- Any AESI
- Any AESI Related to Vaccination
- Any MAAE
- Any MAAE Related to Vaccination
- Any MAAE Related to MAD
- Any TEAE leading to study discontinuation
- Any TEAE leading to death

9.2.2. Relationship of Adverse Events to Vaccination

The relationship of vaccination to TEAE will be characterized using the following 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.

Missing relationship will be counted as related to study vaccination.

All TEAEs through 28 days following first study vaccination and following re-vaccination/attenuated challenge (substudy) will be summarized by relationship to study vaccination, and by treatment group, age group and overall.

9.2.3. Severity of Adverse Event

The severity 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.

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

All AEs through 28 days following first study vaccination and following re-vaccination/attenuated challenge (substudy) will be summarized by SOC/FMQ/PT and severity to study vaccination, and by treatment group.

9.2.4. 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.

Serious TEAEs will be summarized by SOC/PT, and by severity and relationship separately. 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 to be related to BPZE1 will be classified as a SUSAR.

A by-subject listing will be provided.

9.2.5. 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. All MAAEs will be summarized as number and percentage by SOC and PT, and by treatment groups, cross tabulated with severity and relationship separately.

A by-subject listing will be provided.

9.2.6. 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.

Subjects with SARS-CoV-2 infections as well as AESIs associated with COVID-19 infection or SARs-CoV-2 vaccine-related events will be summarized as number and percentage by SOC and PT, and by treatment groups and time since study vaccination, by severity and relationship separately.

A by-subject listing will be provided.

9.2.7. Adverse Events Leading to Study Discontinuation

TEAEs leading to study discontinuation will be summarized by SOC and PT and by treatment group.

A by-subject listing will be provided.

9.2.8. Death

TEAEs leading to death will be provided in a by-subject listing.

9.3.Clinical Laboratory Evaluations

9.3.1. 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.

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

9.4.Vital Sign Measurements

Vital sign measurements will include body temperature (oral, tympanic, or noncontact) and heart rate and will be collected at screening, Day 1 before vaccination and then at 30 (+30) minutes after the last study vaccination on Day 1 for all subjects, Day 85 before vaccination and then at 30 (+30) minutes after the last study vaccination for subjects in substudy, and any unscheduled visits.

If temperature is to be taken orally, the subject must not eat or drink anything hot or cold within 10 minutes prior to measurement. Temperature 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).

Vital sign measurements will be summarized using descriptive statistics (mean, standard deviation, median, min, max) for reported values and change from baseline at each scheduled visit. Subjects in optional substudy will be summarized separately.

A by-subject listing will be provided.

9.5.Physical Examination

A targeted physical examination at Screening and Day 1will 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.

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

A by-subject listing will be provided for all physical examination data.

10. Interim Analysis

An interim database analysis 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, CRO, laboratories) will remain blinded to subject assignment until EOS or end of sample testing, depending on the activities of the associated entities.

11. Changes in the Planned Analysis

To support a rationale for immunobridging to adults, additional analyses for the age group of 10 – 17 years was added to the SAP prior to the sponsor being unblinded to subject-level data.

12. References

Locht C, Papin JF, Lecher S, et al. Live attenuated pertussis vaccine BPZE1 protects baboons against *Bordetella pertussis* disease and infection. J Infect Dis. 2017;216(1):117-24.

Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci USA. 2014;111(2):787-92.

13. Appendices

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]**	**SSV 3**	**SSV 4**	**SSV 5**	**SSV 6**	**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	

	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]**	**SSV 3**	**SSV 4**	**SSV 5**	**SSV 6**	**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**
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					
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

	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]**	**SSV 3**	**SSV 4**	**SSV 5**	**SSV 6**	**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**
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 **Error! Reference source not found.**) to any reactogenicity event. The diary will be reviewed following the first visit after vaccination for all subjects.

[u] Subjects <u>not</u> in the safety lead-in cohort will be randomly assigned (1:1) to provide mid-turbinate/nasopharyngeal samples on <u>either</u> 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.