Study Number: IB-202P Protocol	Compound No.: BPZE1 Version: 5

Title:	A Phase 2b, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy Adults to Assess Protection Against Colonization Following Challenge with Virulent Wild-Type *Bordetella pertussis*

Effective Date: 06 March 2023

Short Title: Pertussis challenge study in adults vaccinated with BPZE1

[redacted]

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SPONSOR SIGNATURE PAGE

Sponsor Signatory:

INVESTIGATOR SIGNATURE PAGE

I agree to conduct this Study in accordance with the requirements of this document (the Clinical Study Protocol), the Study Reference Manual, and in accordance with the following:

- Declaration of Helsinki (revised version of Fortaleza, Brazil, 2013).
- The International Council on Harmonisation harmonised tripartite guideline regarding Good Clinical Practice (E6 R2, November 2016).
- The United Kingdom Statutory Instrument (UKSI) 2004 No. 1031 and UKSI 2006 No.1928
- All applicable local and global regulatory requirements
- Any amendments to these regulations

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ABBREVIATIONS

aPVs	Acellular pertussis vaccines
AE	Adverse event
AESI	Adverse event of special interest
BMI	Body mass index
B. pertussis	*Bordetella pertussis*
CFU	Colony forming unit
CI	Confidence interval
CRO	Contract research organisation
CSR	Clinical study report
DNT	Dermonecrotic toxin
DSMB	Data safety monitoring board
ECG	Electrocardiogram
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
EOS	End of study
FDA	Food and Drug Administration
FHA	Filamentous hemagglutinin
FIM2/3	Fimbriae types 2 and 3
FSH	Follicle stimulating hormone
GMC	Geometric mean concentration
GMFR	Geometric mean fold rise
GMP	Good manufacturing practice
GMT	Geometric mean titer
ICF	Informed consent form
ICH-GCP	International Council on Harmonisation Good Clinical Practice
IEC	Independent ethics committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
MAD	Mucosal atomization device
MedDRA	Medical dictionary for Regulatory Activities
NCS	Not clinically significant
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PRN	Pertactin
PT	Pertussis toxin
SAE	Serious adverse event
SAM	Synthetic absorptive matrix
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SAS	Statistical analysis system
SBA	Serum bactericidal assay
S-IgA	Secretory immunoglobulin A

SOP	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
TCT	Tracheal cytotoxin
TEAEs	Treatment-emergent adverse events
Th	T helper cell
TOPS	The Over-volunteering Prevention System
UK	United Kingdom
ULN	Upper limit of normal
VIS	Volunteer information sheet
WCE	Whole cell extract
WFI	Water for injection
WHO	World Health Organization

PROTOCOL SYNOPSIS

Study Information

Protocol Number:	IB-202P
Title:	A Phase 2b, Placebo-Controlled, Randomized Study of BPZE1 Intranasal Pertussis Vaccine in Healthy Adults to Assess Protection Against Colonization Following Challenge with Virulent Wild-Type *Bordetella pertussis*
Sponsor:	ILiAD Biotechnologies 4581 Weston Road, Suite 260 Weston, FL 33331
Study Phase:	2b
Study Sites:	Two centres in the UK
Product Indication:	Pertussis immunization booster via mucosal barrier in adults and adolescents (≥10 years old)

Rationale

The resurgence of *Bordetella pertussis*, despite high vaccination rates in childhood, is hypothesized to be linked to expanding use of acellular pertussis vaccines (aPVs), which do not prevent *B. pertussis* transmission or acquisition and induce anti-pertussis immunity of short duration. The availability of a non-injectable pertussis vaccine that affects colonisation, provides long-term protection, and has the potential to reduce or eliminate transmission would present a breakthrough in the prevention of *B. pertussis* disease.

Current acellular pertussis vaccine strategies do not induce mucosal immunity or prevent *B. pertussis* acquisition that fuels human-to-human transmission, which occurs during the initial 30-day period of *B. pertussis* infection. As humans are the only known natural reservoir for *B. pertussis*, targeting colonisation would provide a novel approach through mucosally-induced immunity to reduce the acquisition of *B. pertussis*, reduce the duration of infection, reduce transmission and therefore, ultimately reduce, and potentially eliminate, the *B. pertussis* reservoir in the population. Adolescents and adults are the largest reservoir of community *B. pertussis,* thus controlling transmission and outbreaks requires an approach that targets these groups. Recent developments in human challenge models for pertussis now allow a pathway forward to assess the degree of protection against early infection by way of colonisation assessments. Mucosally-induced immunity and protection from infection is expected to ultimately lead to control of transmission and reduction of epidemic cycles.

Intranasally administered BPZE1, a live attenuated pertussis vaccine, provides an opportunity to generate locally effective mucosal antibodies and T cell responses at the site of potential exposure. It mimics the route of entry of the wild-type pathogen and induces a broader immune response as measured by cellular, mucosal, and serum indices, compared to currently used aPVs. Inducing mucosal immunity with a live attenuated vaccine delivered through intranasal vaccination (e.g., BPZE1 vaccine) could halt pathogen progression from upper airway infection to toxin-mediated damage. With fewer upper airway infections, transmission will be curtailed, and herd immunity will more likely be achieved.

In a recent Phase 2b adult attenuated challenge model, subjects were vaccinated with either BPZE1 or an active control acellular pertussis vaccine (Boostrix™) and challenged 3 months later with BPZE1. The protective efficacy of BPZE1 vaccination against re-infection was 85% compared with Boostrix (10% and 70% colonisation rates following BPZE1 attenuated challenge, respectively).

This Phase 2b challenge study will investigate colonisation rates, immunologic response, and the safety of BPZE1 vaccination to potentially protect against colonising, virulent wild-type *B. pertussis* infection in healthy adults using a virulent challenge model. The comparator will be placebo. In addition, evidence of BPZE1-directed induction of mucosal immunity will be confirmed. The ability of BPZE1 to induce rapid and sustained anti-pertussis systemic immunity (Immunoglobulin A [IgA] and Immunoglobulin G [IgG]) along with functional and cellular immunity will also be assessed. This study will assist in determining an adequate sample size, appropriate procedures and focused objectives and endpoints for a future pivotal Phase 3 virulent challenge study.

Objectives and Endpoints

Primary	
Objective	**Endpoint**
To demonstrate that prior immunization with BPZE1 protects against colonisation as evidenced by a negative *B. pertussis* culture following virulent *B. pertussis* challenge 2–4 months after vaccination	The proportion of subjects by treatment group (BPZE1 and placebo) colonised on any day (Challenge Day 9, 11 or 14) following virulent challenge as determined by culture
Secondary	
Objectives	**Endpoints**
To demonstrate BPZE1 induction of anti-pertussis mucosal secretory IgA (S-IgA) antibody is improved from baseline to Day 28	The geometric mean fold rise (GMFR) of mucosal anti-pertussis S-IgA antibody (whole cell extract [WCE], filamentous hemagglutinin [FHA], pertactin [PRN], pertussis toxin [PT] and fimbriae types 2 and 3 [FIM2/3]) from baseline to Day 28 (BPZE1 and placebo).

	Secretory IgA to be normalized ([specific S-IgA]/[total S-IgA])
To demonstrate BPZE1 induction of systemic IgA is improved from baseline to Day 28	The GMFR of serum IgA antibody (WCE, FHA, PRN, PT and FIM2/3) from baseline to Day 28 (BPZE1 and placebo)
To demonstrate BPZE1 induction of systemic IgG is improved from baseline to Day 28	The GMFR of serum IgG antibody (WCE, FHA, PRN, PT and FIM2/3) from baseline to Day 28 (BPZE1 and placebo)
To assess reactogenicity profiles (by toxicity scoring) through 7 days following study vaccination	Occurrence and intensity of solicited AEs for nasal/respiratory and systemic reactogenicity through 7 days following vaccination by treatment group (BPZE1 and placebo)
To describe all unsolicited treatment-emergent adverse events (TEAEs) through 28 days following study vaccination and following virulent challenge	Occurrence and intensity of TEAEs through 28 days following study vaccination and following challenge by treatment group (BPZE1 and placebo)
To describe all unsolicited TEAEs related to vaccination from time of vaccination to challenge or related to challenge for 3 months after challenge	Occurrence and intensity of TEAEs related to vaccination from time of vaccination to challenge or related to challenge for 3 months after challenge by treatment group (BPZE1 and placebo)
To describe any adverse events (AEs) of special interest (AESI) and serious adverse events (SAEs) through end of study (EOS)	Occurrence, intensity, and relationship to study vaccine of AESIs and SAEs from vaccination through EOS by treatment group (BPZE1 and placebo)
Exploratory	
Objectives	**Endpoint**
To demonstrate prior immunization with BPZE1 reduces overall bacterial load following virulent *B. pertussis* challenge	Absolute counts (colony forming unit; CFU) in nasal wash samples on Challenge Days 9, 11 and 14 following virulent challenge by treatment group (BPZE1 and placebo)
To demonstrate BPZE1 induction of mucosal secretory immunity (S-IgA) and systemic immunity (IgA and IgG)	Geometric mean concentration (GMC)/geometric mean titre (GMT) of mucosal anti-pertussis S-IgA antibody, serum IgA and serum IgG antibody (WCE, FHA, PRN, PT and FIM2/3) throughout the study, by treatment group (BPZE1 and placebo). S-IgA to be normalized ([specific S-IgA]/[total S-IgA])

To demonstrate BPZE1 induction of immunity is similar to that observed following virulent challenge	The GMC/GMT and GMFR of mucosal anti-pertussis S-IgA antibody, serum IgA and serum IgG antibody (WCE, FHA, PRN, PT and FIM2/3) post-vaccination and post-challenge by treatment group (BPZE1 and placebo). S-IgA to be normalized ([specific S-IgA]/[total S-IgA])
To compare functional antibody response by serum bactericidal assay (SBA)	Proportion of subjects by treatment group (BPZE1 and placebo) with an increase in SBA 50% killing titre
Correlation and threshold analyses by individual and combination anti-pertussis IgG antibodies to SBA	Analysis (GMC/GMT) by treatment group (BPZE1 and placebo) for each *B. pertussis* strain studied by SBA and by anti-pertussis IgG antibody
To assess cytokine and T helper cell (Th)1/Th2/Th17 dominance response using peripheral blood mononuclear cells (PBMCs), whole blood and/or nasopharyngeal samples	Cell-mediated immunity using PBMCs, whole blood and/or nasopharyngeal samples, including but not limited to cytokines, Th1/Th17 and Th2 responses

Study Population

A suitable number of subjects (approximately 60) will be screened, randomized and vaccinated so that approximately 44 subjects (for a minimum of 20 evaluable subjects per arm with culture results during the challenge phase) are enrolled and challenged in the study. Subjects who withdraw, are withdrawn due to an unrelated AE as determined by the Investigator, are lost to follow-up prior to the challenge phase, or subjects who miss all 3 culture results (Challenge Days 9, 11 and 14) during the challenge phase may be replaced at the discretion of the Sponsor.

Inclusion Criteria

Subjects must meet all the following inclusion criteria to be enrolled in the study:

1. Is a male or female between 18–50 years of age, inclusive on Day 0
2. Able to correctly answer all questions in the questionnaire provided during the consent process to ensure understanding of the study
3. Is capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with protocol requirements
4. Is fully conversant in the English language
5. Female subjects must not be pregnant nor breast-feeding and meet one of the following criteria:

a. Female subjects must be post-menopausal (defined as 12 consecutive months with no menses without an alternative medical cause) or surgically sterile (i.e., hysterectomy, bilateral tubal ligation, or bilateral oophorectomy)

(NOTE: These procedures must be confirmed by physical examination, or by subject recall of specific date and hospital/facility of procedure, or by medical documentation of the procedure. A blood sample for testing follicle-stimulating hormone [FSH] level may be drawn to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy (when appropriate) after history of 12 months of no menses has been taken.)

OR

b. Female subjects of childbearing potential (defined as any female who has experienced menarche and is not yet in menopause) agree to true heterosexual abstinence (when this is in line with the preferred and usual lifestyle of the subject) from at least 21 days prior to enrolment through to 3 months after the virulent challenge or agree to consistently use any of the following methods of contraception from at least 21 days prior to enrolment through to 3 months after challenge:

- Condoms (male or female) with spermicide
- Diaphragm with spermicide
- Cervical cap with spermicide
- Intrauterine device
- Oral or patch contraceptives
- Other approved pharmaceutical contraceptive methods that are designed to inhibit ovulation and protect against pregnancy

(NOTE: Periodic abstinence [e.g., calendar, ovulation, symptom-thermal, post ovulation methods], declaration of abstinence for the duration of exposure to study vaccines, and withdrawal are not acceptable methods of contraception.)

6. Willing to refrain from any nasal sprays (including intranasal steroid sprays) and nasal washes not part of the study for 14 days prior to vaccination (Day 0) and for 28 days following vaccination and challenge
7. Is a non-smoker at the time of enrolment, has not smoked (or vaped) in the past 7 days prior to vaccination (including marijuana), and is willing not to smoke (or vape; including marijuana) from the time of vaccination throughout the in-unit challenge phase
8. Has a stable health status as assessed by the Investigator inclusive of medical history, vital sign assessments and physical examination

9. Has been sufficiently vaccinated (per site and local guidelines) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; proof of vaccination required) >14 days prior to study vaccination
10. Has access to a consistent and reliable means of telephone, text, and email contact, by personal mobile electronic device
11. Is able to understand and comply with planned study procedures including admission for virulent challenge for 17 days and willingness to take the curative antibiotic regimen (azithromycin after inoculation with *B. pertussis*)
12. Lives a reasonable distance from the clinical site(s) to be able to travel to and from the clinical site(s) for follow-up visits
13. Agrees to stay in contact with the clinical site for the duration of the study, has no current plans to move from the study area and provides updated contact information as necessary
14. Willing to provide written agreement to and abide by infection control rules from challenge until 1 week following completion of azithromycin eradication

Exclusion Criteria

Subjects must not be enrolled if they meet any of the following exclusion criteria:

1. Body mass index <17 kg/m^2 or >35 kg/m^2
2. History of being vaccinated against pertussis within 5 years of enrolment
3. History of never being vaccinated for pertussis in lifetime
4. A diagnosis of pertussis by laboratory confirmation or by physician diagnosis in the past 5 years
5. Serum anti-pertussis toxin IgG >20 IU/mL conducted locally during Screening
6. Serum anti-pertussis PRN IgG >30 IU/mL conducted locally during Screening
7. Previously inoculated with *B. pertussis* in a pertussis challenge study
8. Screening laboratory values outside of the local laboratory's normal ranges (chemistry or haematology excluding white blood cell differential) and clinically significant, except for documented Gilbert's syndrome with direct bilirubin ≤ upper limit of normal. (Laboratory samples may be repeated once during the screening period)
9. Positive test for human immunodeficiency virus, hepatitis B or hepatitis C at Screening
10. Use of illicit drugs (excluding marijuana), evidenced by urine toxicology at Screening or a history of drug/alcohol abuse within the past 2 years

11. Any chronic illness being treated actively with evidence of recent intervention for worsening or fluctuating symptoms such that a stable baseline of disease is not possible (at the discretion of the Investigator)
12. The subject has a history of active cancer (malignancy) in the last 10 years (exception is subjects with adequately treated non-melanomatous skin carcinoma, who may participate in the study)
13. Existing chronic disorders inclusive of lung (e.g., asthma, chronic obstructive pulmonary disease), kidney, heart (including screening QT interval corrected according to Fridericia's formula ≥ 440 msec on the 12-lead ECG), liver, diabetes, immunodeficiency (acquired or congenital), autoimmune (exception is Hashimoto's thyroid disease) or significant neurologic condition (including, but not limited to, facial paralysis or Bell's palsy). Note, well-controlled chronic disorders including hypertension and hypercholesterolemia are permitted
14. History of Guillain-Barré syndrome (genetic/congenital or acquired)
15. History of head trauma with potential of cribriform plate fracture within 1 year prior to Day 0
16. History of nasal or sinus surgery within 6 months or receipt of facial cosmetic fillers within 3 months prior to Day 0 or diagnosis of nasal polyps
17. Has taken immunosuppressive therapy or other immune-modifying drugs (including but not limited to systemic corticosteroids, biologics, and methotrexate) in the past 6 months, is on scheduled immunosuppressive therapy or is planning to start immunosuppressive therapy during the trial. For systemic corticosteroids this means prednisone or equivalent for 10 days or more. The use of corticosteroids (inhaled, topical, ophthalmologic or localized injections in joints) are permitted
18. Receipt of immunoglobulins or any blood products within 3 months prior to study vaccine administration or planned receipt during the study
19. The subject resides in a residence or has routine contact (face to face <2 meters) with persons with known immunodeficiency, including persons on immunosuppressant therapy from study vaccination to challenge and for 1 week after exiting the challenge unit
20. The subject resides in a residence, works regularly with, or has contact (face to face <2 meters) with, infants less than 1 year of age, partially immunised infants or pregnant women, adults >65 years of age who have not received a dose of acellular pertussis vaccine (e.g. Tdap) within the past 10 years, from study vaccination to challenge and for 1 week after exiting the challenge unit
21. Known hypersensitivity to any component of the study vaccine
22. Contraindications or allergic to azithromycin, erythromycin or other macrolide antibiotics

23. Taking medication that may interact with azithromycin (e.g., nelfinavir, warfarin, digoxin and phenytoin)
24. Inability to adhere to the protocol, visit schedule or sample collection needs (including housing in the challenge unit)
25. Any medical condition, including previous serious event due to a vaccination that, in the opinion of the Investigator, might interfere with the evaluation of the study objectives or might affect the safety of the individual (e.g., frequent migraines, major depression or history of recent suicidal attempt)
26. Participation in any other clinical trial for the testing of an unlicensed product during the previous 3 months or planned during the study conduct

(NOTE: 'Testing' is intended to mean receipt of an investigational product. Subjects in long term safety follow-up studies where unlicensed product has completed administration >3 months prior to this study enrolment are allowed. Allowance is given for subjects who have received a product under emergency use authorization if the product has been given >14 days before study enrolment.)

27. Subject is a first-degree relative of study team member

Additional Exclusion from Entry into Challenge Unit

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

28. Has received any form of immunotherapy determined to potentially affect the immune response or increase the risk of severe disease in a virulent challenge setting (as determined by medical consultation with Sponsor, when necessary)
29. Has a *B. pertussis* positive nasal sample (by culture) within 7 days of admission to challenge unit

Other Considerations for Temporarily Holding Vaccination or Entry into Challenge Unit

Subjects meeting any of the following criteria may have a planned study vaccination or challenge admission deferred to a later date, but these criteria are not exclusionary for study enrolment. Subjects may be enrolled on a return visit if they remain within 45 days of consenting. Otherwise, subjects must be re-consented and reassessed per the inclusion and exclusion criteria (i.e., re-enrolled). Subjects may be admitted for virulent challenge until Day 120 post-vaccination; if a subject is unable to be admitted for virulent challenge by Day 120 post-vaccination, the subject should continue to be followed-up for safety per the Schedule of Events.

- Has recent symptoms or illness (documented by AE) that requires deferment of a virulent challenge due to confounding effects or new/recent disease onset such that a virulent challenge is not medically acceptable for safety reasons. Therefore, the Investigator will determine the appropriate allowance of time with the subject either

resolving recent illness or medical condition determined to be stable (although within the timelines noted above)

- The subject has, or reports, a body temperature of 100.4°F (38°C) or higher within the past 3 days. The subject may be vaccinated/challenged once the fever resolves and body temperature (subject measured) remains below 100.4°F (38°C) for at least 3 days
- The subject has, or reports, any infection, acute respiratory tract symptoms or rhinorrhoea within the past 3 days. Subject may be vaccinated/challenged once all symptoms have been resolved for at least 7 days
- The subject has used antibiotics (including systemic antibiotics, antibiotic lozenges and topical antibiotics) within 10 days prior to study vaccination or within 30 days prior to challenge
- Has a SARS-CoV-2 positive sample within 3 days prior to time of vaccination or challenge unit entry. Requires retest with negative results prior to vaccination or prior to entering challenge unit

Study Design

This is a randomised, double-blinded, placebo-controlled trial of BPZE1 that includes virulent *B. pertussis* challenge followed by a safety follow-up.

Consenting eligible subjects will receive a single dose of BPZE1 or placebo. 2–4 months later, they will be challenged with *B. pertussis* and admitted to a challenge unit. Subjects will remain in the challenge unit for a total of 17 days and 16 nights during which time they will be monitored closely. If a subject develops symptoms of pertussis (per Investigator discretion), antibiotic (azithromycin) will be started, and the subject will remain in the unit for 3 additional days of observation before discharge. If symptoms of pertussis do not develop, then subjects will receive antibiotic (azithromycin) from Days 14–16 of the challenge unit stay. Subjects will undergo safety follow-up for at least 6 months post-vaccination and at least 3 months post-challenge, for a total follow-up of 6–7 months post-vaccination.

The exact visits, and procedures to be performed at each visit, can be found in the Schedule of Events.

An independent Data Safety Monitoring Board (DSMB) will be established by the Sponsor prior to the study commencing. Refer to the DSMB charter for specific responsibilities.

The primary database lock will occur after the last subject's virulent challenge visit (Visit C16). The longer-term safety and durability responses at the end of the study will be included in the final database lock after all planned study procedures through EOS are complete.

Vaccination Phase

After signing the informed consent form (ICF) and meeting all inclusion and none of the exclusion criteria, a suitable number of eligible subjects (approximately 60) will be randomised 1:1 to receive BPZE1 (10^9 CFU) or placebo (formulation buffer) via an intranasal atomization device (Table 1) so that approximately 44 subjects are enrolled and challenged in the study for a minimum of 20 evaluable subjects per arm with culture results during the challenge phase. It is estimated that an approximate dropout rate of 10% during the challenge phase may occur, allowing for a minimum of 20 evaluable subjects in each arm.

Table 1 Dosing Scheme

Treatment Arms N=22[a]	Nasal Vaccination (Day 0)		*B. pertussis* Virulent Challenge (Day 60[c])
	BPZE1	Placebo[b]	
A	X	-	X
B	-	X	X

a. Evaluable subjects estimated at 20 per treatment arm with two additional to account for dropouts
b. Intranasal application of 0.4 mL per nostril (0.8 mL total volume delivered) formulation buffer placebo
c. Subject may enter virulent challenge unit between Days 60 to 120

Unblinded clinical staff will manage vaccine logistics and preparation, but they will not be involved in study-related assessments or have subject contact for data collection. Blinded individuals will administer the study vaccine.

On Day 0, nasal/respiratory and systemic reactogenicity will be assessed approximately 30 minutes after vaccination prior to release from clinical observation. Then, all subjects will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following study vaccination (retrospective of the highest value in the previous 24 hours). Both nasal/respiratory, and general systemic reactogenicity events including actual measurements as needed (i.e., body temperature) will be recorded. Trained clinical staff will review the information from the subject diary to ensure completeness and accuracy. A standard toxicity grade will be assigned to each reactogenicity event. Should subjects experience a reactogenicity event with a potential toxicity of Grade 3 at any time they should contact the site and be seen or referred to a qualified medical facility within 24 hours for further evaluation.

Challenge Phase

Between Days 60 and 120 after vaccination (to accommodate in-unit capacity) subjects will plan to enter the challenge unit, but they must first be reviewed to be confirmed suitable to proceed to virulent challenge (e.g., vitals, clinical exam, culture for *B. pertussis*, additional eligibility criteria). Once subjects are cleared for virulent challenge, a dose of the *B. pertussis* strain B1917 at 10^5 CFU dose will be administered while lying

supine and using the procedures established within the challenge unit. The inoculum will be instilled into each nostril with a Gilson pipette by droplet. Subjects will remain supine for 15 minutes. Subjects will remain in the unit for a total of 17 days for observation. Daily monitoring will occur including vital signs, physical exam (as needed), signs/symptoms of pertussis infection (e.g., catarrhal symptoms), and select clinical laboratory testing will occur on pre-specified days. Nasal washes will occur at the baseline of the challenge and on Challenge Days 9, 11, and 14, or prior to initiating antibiotics should persistent symptoms of pertussis (as per the Investigator's discretion) occur as well as after receiving antibiotics. If persistent symptoms suggestive of pertussis disease occur, then subjects will be started on azithromycin and remain in the unit for approximately 3 days from the initiation of antibiotics for observation before discharge. The remaining subjects will receive azithromycin daily from Day 14 of their challenge unit stay and will be discharged from the Challenge Unit on Day 16, after the third dose of azithromycin is administered. The 3-day course of azithromycin will be dosed at 500 mg orally once daily (as indicated by UK Health Security Agency for post-exposure prophylaxis for respiratory infections) and subjects must abide by stipulated infection control rules. If a *B. pertussis* culture collected on the last day of the in-unit stay is positive, the subject will return to the site to receive continued out-patient antibiotic treatment (azithromycin). Nasopharyngeal swabs, mucosal samples and blood samples for immunogenicity testing, cell-mediated immunity, and safety (IgA, IgG, functional and cell-mediated) will be collected as per the Schedule of Events.

Follow-up Phase

Subjects will be followed for safety approximately 6 months following vaccination or 3 months following challenge, whichever is longer. Adverse events will be collected as follows:

- All AEs will be collected through 28 days after study vaccination and 28 days after virulent challenge
- All AEs related either to vaccination or to challenge will be collected from time of vaccination to challenge or for 3 months after challenge, respectively
- All AESIs and SAEs will be monitored from signing of informed consent through EOS

Study Duration

The subjects will each be enrolled in the study for up to 8 months. This includes a screening phase of up to 30 days prior to enrolment, a vaccination phase lasting up to 120 days post-vaccination, a challenge phase of 17 days and a safety follow-up lasting until either 180 days post-vaccination or 90 days post-challenge (whichever is longer).

Study Assessments

Nasal wash samples will be evaluated by culture for *B. pertussis* colonization to determine the colonization status (Yes/No) and colonization burden (bacterial counts). Immune measurements will be conducted on serum (IgA and IgG) and nasal mucosal secretion samples (e.g., nasosorption; S-IgA) for anti-pertussis antibodies of WCE, PT, FHA, PRN, and FIM2/3. Serum bactericidal activity (50% killing titre) will be determined. Nasopharyngeal swab samples and blood samples (whole blood and PBMCs) will be collected to be evaluated for cell-mediated immunity. Additional testing for antibodies specific to *B. pertussis* may be performed at a later date as BPZE1 and virulent wild-type *B. pertussis* induce broad immune responses to many *B. pertussis* antigens, since they do not only contain the purified antigens found in Tdap booster vaccines (standard of care). Safety laboratory testing for study entry and additional testing during the challenge unit stay will be conducted according to the Schedule of Events and using FDA toxicity scoring parameters.

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

Safety assessments for all subjects will include the following: vital sign measurements (heart rate, systolic/diastolic blood pressure, temperature, respiratory rate, oxygen saturation), physical examinations (including weight, height, and derived BMI at baseline), solicited 7-day reactogenicity, and unsolicited AEs (including AESIs and SAEs). Past medical history and medications/vaccines will be recorded, as well as any vaccination that occurs outside of the trial during the trial period. Additional collection of data elements (vital signs, symptoms, significant physical exam findings during challenge unit stay) will also be included. Specific daily evaluations for pertussis-related symptoms will be collected.

Study Vaccination

- BPZE1, reconstituted with sterile water to provide 10^9 CFU per 0.8 mL; administered intranasally, with approximately 0.4 mL per nostril (0.8 mL total volume delivered), by mucosal atomization device (MAD)
- Intranasal placebo (lyophilized BPZE1 buffer reconstituted with sterile water); administered intranasally, with approximately 0.4 mL per nostril (0.8 mL total volume delivered), by MAD

Statistics

Sample Size

Table 3 depicts the power for the challenge study as computed under the following assumptions: two-sided significance level of 0.05, use of a likelihood ratio test for testing equality of two proportions, a sample size of 20 in each of the two groups, a colonisation rate of 60%, 70%, and 80% in the unvaccinated group and colonisation rates of 10%, 20%, 30%, and 40% in the vaccinated group.

Group sample sizes of 20 evaluable subjects in each group achieve a power of at least 90% to detect differences between colonisation rates of at least 50% in six scenarios. These scenarios include the following colonisation rates for the placebo versus BPZE1 groups, respectively: 60% versus 10%, 70% versus 10%, 70% versus 20%, 80% versus 10%, 80% versus 20% and 80% versus 30%, which are bolded in Table 3.

Table 3 Power for Sample Sizes of 20 in Each Group for Varying Colonisation Rates Based on a Likelihood Ratio Test

Colonisation Rate			
Placebo	**BPZE1**	**N/group**	**Power**
60%	**10%**	20	94%
	20%		75%
	30%		48%
	40%		24%
70%	**10%**	20	99%
	20%		92%
	30%		73%
	40%		48%
80%	**10%**	20	100%
	20%		99%
	30%		92%
	40%		75%

Table 4 includes results based on vaccine efficacy computed from the colonisation rates in each population, rather than comparing the two colonisation rates and testing the null hypothesis that they are equal in the two populations. The computations presented in the table below are based on a normal approximation. The table includes the required sample

size for a power of 80% and for lower bounds of vaccine efficacy of 0%, 10%, 20%, and 30%. For a colonisation rate of 70% in the placebo group and a lower bound of efficacy of 20%, the study will be powered for efficacy rates of 71% or higher. Focusing on an efficacy rate greater than 50% and a lower bound of 0% (sample sizes in **bold**), the study is well powered to detect efficacy rates of 57% or higher.

Table 4 Estimated Sample Size Based on Assumed Colonisation rates and Power of 80% and Lower Bounds of 0%, 10%, 20% and 30%

Colonisation rate		Sample size/group				
Control	Vaccine	Efficacy	LB 0%	LB 10%	LB 20%	LB 30%
60%	10%	0.833	**11**	**12**	**14**	**18**
	20%	0.667	**18**	22	29	42
	30%	0.500	33	47	75	149
	40%	0.333	77	141	390	5675
70%	10%	0.857	**8**	**9**	**10**	**12**
	20%	0.714	**12**	**14**	**18**	24
	30%	0.571	**19**	25	36	60
	40%	0.429	33	51	95	268
80%	10%	0.875	**6**	**6**	**7**	**9**
	20%	0.750	**8**	**9**	**12**	**16**
	30%	0.625	**12**	**15**	**20**	31
	40%	0.500	**18**	25	40	82

For the endpoint of absolute counts, the standard deviation was computed to be 2.2 for the natural log of the counts in the BPZE1 group and 4.2 for the natural log of the counts in the control group. Assuming a sample size of 20 evaluable subjects in each group, a two-sample t-test accounting for unequal variances, and a two-sided significance level of 0.05, the study has approximately 80% power to detect differences of the order of 3.1 units between the BPZE1 group and the control group on the natural log scale.

Statistical Methods

Statistical analysis will be performed using SAS (Statistical Analysis System) software Version 9.4 or later. Continuous variables will be summarized using the mean, standard deviation, median, first quartile, third quartile, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages, as

well as a 2-sided 95% confidence interval (CI) for proportions computed using a Agresti-Coull method. All statistical tests will be 2-sided at 0.05 significance level.

For the primary analysis of the between treatment groups difference in the proportion of subjects colonized on any day (Challenge Day 9, 11 or 14) following challenge, a likelihood ratio test and the corresponding 95% confidence interval will be computed with colonization defined as a positive result on any of the 3 days. Additional summaries for each of the days will be presented including the results of the likelihood ratio test and confidence interval.

Immunogenicity endpoints (GMC/GMT, GMFR, and/or seroconversion) will be summarized at baseline and each time point by treatment group. Reverse cumulative distribution curves, which are also known as survival curves, will be presented for each measure. Comparison of the BPZE1 and control groups will use a two-sample t-test. Confidence intervals will be back-transformed from the log scale to the original scale.

Exploratory analyses include analyses to assess whether colonization status has a relationship to pre-challenge mucosal immunity levels (by individual or by combination of anti-pertussis antibodies) or SBA activity. Analyses will include the GMCs/GMTs with 95% CIs and GMFRs with 95% CIs and p-values testing as per Statistical Analysis Plan (SAP). Pearson's and Spearman correlations and repeated measures analyses will be conducted to assess for correlations and for SBA and protection against colonization. For immunogenicity analysis, it is assumed that the natural log of the data is normally distributed. All statistical tests will be two-sided at 0.05 significance level and nominal p-values will be reported.

The SAP will provide additional details of all analyses.

1. INTRODUCTION

1.1. Background

Bordetella pertussis is a gram negative, aerobic coccobacillus that causes a non-invasive pathogenic respiratory tract infection (known commonly as 'pertussis' or 'whooping cough'). Since *B. pertussis* is endemic only to human beings and does not survive for a meaningful duration in the air or on surfaces, disease transmission typically occurs between infected humans and their close contacts via water droplets and aerosolized spray expelled by coughing, sneezing, talking, or breathing during the early infectious period. *Bordetella pertussis* induces a two-stage disease process which may last up to 3 months: a) the infectious stage where upper respiratory tract colonisation/ infection is established and symptoms manifest; and b) the toxin-mediated stage in which damage to the lower respiratory tract has occurred due to *B. pertussis*-specific toxins and is irreversible with available medical treatments. The infectious stage begins with non-specific catarrhal symptoms similar to other upper respiratory infections. Shortly after bacteria colonise the upper respiratory tract, physiological changes to the body (i.e., disease) have already begun with early catarrhal signs and symptoms often misconstrued as the "common cold" (e.g., viral upper respiratory tract infection), and going unrecognised/untested, resulting in significant delays in diagnosis. Even in the earliest period of infection, damage to epithelial cells and paralysis of the mucosal cilia, which mediates the clearance of mucus, has already started and toxin-mediated effects in the lower respiratory tract ensue [Babu *et al*, 2001; Mattoo *et al*, 2005]. Early use of antibiotics to treat pertussis is important, especially as the treatment is unlikely to help after 3 weeks of illness [Centers for Disease Control and Prevention].

Based on emerging evidence, a review by the Scientific Advisory Group for Emergencies through the World Health Organization (WHO) has led to the conclusion that resurgence of *B. pertussis* infections is potentially linked to the use of acellular pertussis vaccines (aPVs) and recent analysis by the Advisory Committee on Immunization Practices concurs [World Health Organisation, 2015; Liang, 2018]. Furthermore, current position and review papers raise the arguments that the combination of limited durability and unconstrained disease transmission are playing increasing roles in *B. pertussis* resurgence [Bolotin *et al,* 2015, Decker *et al*, 2021; Plotkin *et al,* 2014; Schwartz *et al*, 2016; Warfel and Edwards, 2015; Witt *et al*, 2013; Zerbo *et al*, 2019].

Multiple studies now support the notion that persistent epidemiological cycles of *B. pertussis* outbreaks are the result of aPVs not controlling the infectious period or disease transmission. Instead, aPVs exert their main protective effect on later-stage pertussis disease, which is protection against toxin-mediated damage, but with limited durability. With *B. pertussis* infectivity rates similar to that of other childhood diseases in the pre-vaccination era (e.g., polio, smallpox, measles) and greater than seasonal respiratory diseases such as influenza and respiratory syncytial virus, a focus on controlling the first stage of infection (e.g., colonising infection) is paramount to controlling recurrent epidemics [McGirr *et al*, 2013; Biggerstaff *et al*, 2014; Van Boven *et al*, 2020;

Kretzschmar *et al*, 2010]. The prolonged period and high bacterial burden of *B. pertussis* upper respiratory infection results in highly contagious individuals who are the source of person-to-person *B. pertussis* transmission, irrespective of aPV vaccination status. A systematic review has identified high levels of transmission in humans, including asymptomatic individuals. In 14 studies, 46.2% of household contacts testing positive for *B. pertussis* reported having mild/atypical pertussis, whereas in 24 studies, 55.6% of household contacts tested positive for *B. pertussis* but were reported to be asymptomatic [Craig *et al*, 2020]. The mean serial interval (average time between the time of symptom onset of a primary case [infector] and that of a secondary case [infectee]) in a systematic review was 22.8 days (95% CI 10–35 days) [Vink *et al*, 2014]. During colonising infections, when the disease transmission potential is at its highest, modelling has identified secondary attack rate estimates up to 80% [Althouse *et al*, 2015].

In 2015, due to increasing severity and extent of *B. pertussis* outbreaks, the National Institute for Allergy and Infectious Diseases added *B. pertussis* to the emerging infectious disease pathogen list [National Institute of Allergy and Infectious Diseases] and the Center for Disease Control and Prevention listed *B. pertussis* on the watch list for *Antibiotic Resistance Threats in the United States* [Centres for Disease Control and Prevention].

For the next generation of pertussis vaccines, there is a need to combat both transmission and durability issues that currently contribute to epidemic outbreaks. To achieve this goal, next generation vaccines must ideally have durable protection at the mucosal barrier by (1) restricting *B. pertussis* acquisition and (2) achieving rapid clearance of the organism, thereby preventing (a) the long infectious period, (b) the later stage toxin-mediated damage and (c) halting transmission potential. BPZE1 has demonstrated the ability to prevent *B. pertussis* colonising infections, induce measurable mucosal secretory immunoglobulin A (S-IgA) responses, and induce systemic anti-pertussis antibodies after a single vaccination (both immunoglobulin A [IgA] and immunoglobulin G [IgG]). The demonstration of systemic anti-pertussis antibody responses is important in case *B. pertussis* should ever elude the mucosal barrier of protection established by BPZE1.

1.1.1. Name and Description of the Investigational Product

BPZE1 has been developed as a live attenuated pertussis vaccine candidate using genetic inactivation or removal of three major virulence factors from the *B. pertussis* Tohama I strain: pertussis toxin (PT), tracheal cytotoxin (TCT) and dermonecrotic toxin (DNT) [Mielcarek *et al*, 2006]. The pertussis toxin (*pt)* gene is modified by altering two codons, resulting in the replacement of Arg-9 with lysine and Glu-129 with glycine in the substrate binding and catalytic centre of the S1 subunit of PT, respectively. The S1 subunit mutations prevent adenosine diphosphate-ribosylation of G-proteins of the host cell that are involved in signal transduction processes. Both mutations separately abolish the enzymatic activity [Locht and Antoine, 1995]. The *ampG* gene of *B. pertussis* is replaced by the *ampG* gene of *Escherichia coli*. This gene substitution results in an enhanced internalization of the peptidoglycan breakdown products by the AmpG

transport protein, and the breakdown products are reused for the biosynthesis of the bacterial cell wall. The gene substitution results demonstrated an over 99% decrease in the excretion of peptidoglycan degradation product TCT by BPZE1 [Mielcarek *et al*, 2006]. The *B. pertussis dnt* gene (the structural gene coding for DNT), which shows strong homology with the *B. bronchiseptica* gene, is deleted. The deletion avoids cytoplasmic expression of the toxin in the BPZE1 strain. Dermonecrotic toxin causes necrotic lesions after intradermal injection in adult mice, and intravenous administration of microquantities is lethal in newborn mice [Locht *et al*, 1999]. Recently the cellular receptor of DNT was identified, which is mainly expressed on cells of the nervous system, implicating DNT as a potential neurotoxin [Teruya *et al*, 2020].

1.1.2. Name and Description of Challenge Agent

The PERISCOPE consortium, formed to facilitate the development and licensing of the next generation of improved pertussis vaccines, developed a *B. pertussis* human virulent wild-type challenge (virulent challenge) model to predict and evaluate vaccine efficacy [PERISCOPE project]. The human model included the use of the same *B. pertussis* isolate to be used in this study, strain B1917, which is representative of current isolates in Europe [de Graaf *et al*, 2020; Bart *et al*, 2015] and nearly genetically identical to the US clinical isolate D420. The strain, isolated in 2000 from a Dutch patient with *B. pertussis* disease, is characterised by *ptxP3-ptxA1-prn2-fim3-2, fim2-1* MLVA27, PFGE BpSR11 and expresses pertactin (PRN), pertussis toxin (PT) and filamentous hemagglutinin (FHA). This strain has been extensively characterised in the mouse model as well as by proteomics and transcriptomics and has a closed genome available. It is fully sensitive to azithromycin *in vitro*.

1.1.3. Non-clinical Studies

Direct evidence that aPVs do not alter the *B. pertussis* infectious period (e.g., colonisation of the upper airway) has been demonstrated in the non-human primate baboon challenge model. In this model, a full aPV series given to baboons failed to prevent upper respiratory tract colonisation from a virulent *B. pertussis* challenge (strain D420), and neither colony counts (burden) nor duration of colonisation was reduced compared to naïve baboons [Warfel *et al*, 2014]. In addition, when aPV-vaccinated baboons were challenged and then co-housed with unchallenged naïve baboons, transmission was observed up to 40 days later, despite infected baboons being asymptomatic [Warfel *et al*, 2014]. The baboon model that was established demonstrated that exposure to wild-type *B. pertussis* resulted in classical symptoms of pertussis and induced leukocytosis, lending credibility that this is a fitting disease model [Warfel *et al*, 2012]. Using the same virulent *B. pertussis* challenge model, when baboons were vaccinated with BPZE1 (10^9 or 10^{10} colony forming units [CFU]) and then subsequently challenged (1.5×10^{10} CFU of *B. pertussis,* strain D420), protection against both colonisation and disease was evident [Locht *et al*, 2017].

Further details on non-clinical studies can be found in the Investigator's Brochure [Investigator's Brochure 2021].

1.1.4. Clinical Studies

Success with the baboon model allowed for the next stage in virulent pertussis challenge: a controlled human challenge model. This model was established at University of Southampton, in the United Kingdom (UK) utilising an isolate of virulent *B. pertussis*, strain B1917. Healthy adults (age 18–45 years) were enrolled if they had not been vaccinated with *B. pertussis* vaccine within 5 years and had low anti-pertussis toxin IgG antibodies (≤20 IU/mL) and negative *B. pertussis* culture to ensure no recent community exposure. In this dose escalation study an optimal challenge dose of 10^5 CFU wild-type *B. pertussis*, the B1917 strain was shown to induce colonisation in 80% of subjects [de Graaf *et al*, 2020]. Some subjects with colonisation had increases in serum anti-pertussis toxin IgG levels corresponding to having been infected.

In addition to virulent challenge models, an attenuated human challenge model has been developed using BPZE1 (Tohama I derivative, parental strain BPSM) in the recent Phase 2b adult study conducted by ILiAD Biotechnologies [Keech, 2020a; Keech, 2020b]. The Phase 2b study corroborates what has been observed in the virulent challenge models: most individuals (70%) who received an aPV vaccination (Boostrix™) just 3 months earlier were unable to avert colonisation with BPZE1 attenuated challenge ($1x10^9$ CFU). In contrast, prior vaccination 3 months earlier with BPZE1 resulted in 90% protection against re-colonisation following attenuated challenge (estimated 85% efficacy versus aPVs). In the same study, BPZE1 demonstrated induction of anti-pertussis mucosal antibodies, which were not consistently evident following Boostrix vaccination. BPZE1 also induced systemic anti-pertussis antibodies with a balanced immunoglobulin (Ig)A:IgG response. More recently, in a post-hoc, proof-of-concept study, using a randomised convenience sample from the Phase 2b study, BPZE1 vaccination was shown to have similar protection in a serum bactericidal assay (SBA) as Boostrix using a *B. pertussis* PRN(+) strain; however, when tested in a *B. pertussis* PRN(-) strain, BPZE1 maintained protective antibody levels, whereas Boostrix did not [Keech *et al* 2021]. This suggests that BPZE1 induces a broad functional antibody response. Lastly, BPZE1 has been shown to be well-tolerated with acceptable solicited reactogenicity (systemic and nasal/mucosal), mainly mild in severity and of limited duration (mean ≤3 days). Prior Boostrix or BPZE1 vaccination followed by attenuated challenge with BPZE1 3 months later also resulted in no concerning safety findings.

Further details on clinical studies can be found in the Investigator's Brochure [Investigator's Brochure 2021].

1.1.5. Study Conduct

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the International Council on Harmonisation

harmonised tripartite guideline regarding Good Clinical Practice (ICH GCP), the protocol and all applicable regulations.

1.2. Rationale

The resurgence of *B. pertussis*, despite high vaccination rates, is hypothesized to be linked to the expanding use of aPVs, which do not prevent *B. pertussis* transmission or acquisition and induce immunity against pertussis disease of limited duration. The availability of a non-injectable pertussis vaccine that affects colonisation, provides prolonged protection, and has the potential to reduce or eliminate transmission would present a breakthrough in the prevention of colonising *B. pertussis* infections.

Current acellular pertussis vaccine strategies do not induce mucosal immunity, nor prevent *B. pertussis* acquisition that fuels human-to-human transmission, which occurs during the initial 30-day period of *B. pertussis* infection. As humans are the only known natural reservoir for *B. pertussis*, targeting colonisation would provide a novel approach through mucosally-induced immunity to reduce the acquisition of *B. pertussis*, reduce the duration of infection, ultimately reduce the *B. pertussis* reservoir in the population and potentially eradicate pertussis. Adolescents and adults are the largest reservoir of community *B. pertussis*; controlling transmission and outbreaks requires an approach that targets these groups. Recent developments in human challenge models for pertussis now allow a pathway forward to assess the degree of protection against early infection by way of colonisation assessments. Mucosally-induced immunity and protection from infection is expected to ultimately lead to control of transmission and reduction of epidemic cycles.

The intranasally administered BPZE1 live attenuated vaccine provides an opportunity to generate locally effective mucosal antibodies and T cell responses at the site of potential exposure, mimic the route of entry of the wild-type pathogen, and induces a broader immune response as measured by cellular, mucosal, and serum indices, compared to currently used aPVs. Inducing mucosal immunity with a live attenuated vaccine delivered through intranasal vaccination (e.g., BPZE1 vaccine) could halt pathogen progression from upper airway infection to toxin-mediated damage. With fewer upper airway infections, transmission will be curtailed, and herd immunity will more likely be achieved.

In a recent Phase 2b adult attenuated challenge model, subjects were vaccinated with either BPZE1 or an active control acellular pertussis vaccine (Boostrix™) and challenged 3 months later with BPZE1. The protective efficacy of BPZE1 vaccination against re-infection was 85% compared to Boostrix (10% and 70% colonisation rates following BPZE1 attenuated challenge, respectively).

This Phase 2b virulent challenge study will investigate colonisation rates, immunologic response and the safety of BPZE1 vaccination as well as looking at how BPZE1 potentially protects against colonising wild-type *B. pertussis* infection in healthy adults using a virulent challenge model. The comparator will be placebo. In addition, continued

evidence of BPZE1-directed induction of mucosal immunity will be confirmed. The ability of BPZE1 to induce rapid and sustained anti-pertussis systemic immunity (IgA and IgG) along with functional and cell-mediated immunity will be assessed. This study will assist in determining an adequate sample size, appropriate procedures and focused objectives and endpoints for a future pivotal Phase 3 virulent challenge study.

1.3. Potential Risks and Benefits to Human Subjects

1.3.1. Potential Risks

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

1.3.2. Risks of Study Participation

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

1.3.3. Risks Relating to BPZE1

The risks of BPZE1 administration are expected to be minimal and clinically manageable. *Bordetella pertussis* colonisation is strictly limited to respiratory epithelium without dissemination of the bacteria outside the respiratory tract, which also excludes systemic bacteraemia of the BPZE1 strain. Clearance of BPZE1 follows kinetics of wild-type clearance over the course of 28 days with most subjects clearing in the first 2 weeks (data on file). In the recent Phase 2b study, systemic and nasal/respiratory reactogenicity events were mainly none to mild in severity with mean duration of 3 days, consistent with the safety profile seen in the Phase 1 studies. A second dose of BPZE1 did not induce greater reactogenicity events. No Grade 3 reactogenicity events occurred without a secondary cause (unrelated to vaccination). Treatment-emergent AEs were mainly mild. There were no SAEs attributed to vaccination in any studies to date.

Bordetella pertussis is spread mainly via aerosol formed by coughing of infected persons. The coughing is induced by the TCT, which is more than 99% reduced in BPZE1. The BPZE1 strain is not expected to induce coughing (as demonstrated in two adult Phase 1 and two Phase 2 studies), and thus, transmission is highly unlikely. *Bordetella* spp. have fastidious growth requirements and have limited survival time outside the human body.

Bordetella pertussis has not been shown to be allergenic in any preclinical or clinical studies to date, nor have any of the excipients in the lyophilized formulation. BPZE1 has been shown to protect against airway inflammation induced by allergens or viral infections in a murine model [Cauchi *et al*, 2018; Kavanagh *et al*, 2010; Li *et al*, 2010; Li *et al*, 2012; Schnoeller *et al*, 2014]. BPZE1 has also been shown to protect against wild-type *B. pertussis* infection 3 hours after immunization in a murine model [Mielcarek *et al,*

2006]. However, there remains a theoretical risk of allergic reaction, as is present with any vaccine product and therefore subjects will remain in clinic for 30 minutes of observation following vaccination.

Either the BPZE1 vaccine or the placebo will be administered nasally via the intranasal mucosal atomization device (MAD) attached to a syringe to healthy adult volunteers under strictly controlled conditions. The MAD atomizes the liquid vaccine as it exits the syringe, and could induce local nasal reactogenicity such as sneezing, irritated nasal passage, cough, sore throat or in very rare cases, epistaxis. These reactogenicity parameters will be assessed daily for 7 days following study vaccination.

To minimize the risk of transmission, the subjects will stay at the study centre for at least 30 minutes after administration on Day 0. In addition, although no cross-contamination between subjects has been observed in the previous clinical studies of BPZE1, and no risk to the family members of study subjects been reported, as a precaution, subjects with frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc.), partially immunized infants, pregnant women, subjects who live in the same household as individuals with known immunodeficiency or individuals on immunosuppressant therapy, will be excluded from participation in the study.

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

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

1.3.4. Risks to the Environment or Potential for Interaction with Wild-type *B. pertussis* Strains

To avoid accidental exposure, actions should be taken to minimize generation of aerosols, since the bacterium is strictly a respiratory tract organism. The clinical staff members should wear eye-protective glasses and masks during the vaccination. Persons handling the BPZE1 bacteria should wear gloves and must wash their hands with a suitable disinfecting soap before touching their skin and eyes. Effective antibiotic treatment with azithromycin (or an appropriate antibiotic if the subject is allergic to azithromycin) should be given in case of accidental transmission to other humans.

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

The genetic modifications in BPZE1 strongly increase the *in vivo* safety:

- The double nucleotide mutation in the substrate binding and the active site of the PT results in a strong reduction of the enzyme activity
- The replacement of the *B. pertussis ampG* gene by the *E. coli ampG* gene results in an over 99% reduction in release of the TCT in the medium
- The DNT is not expressed in the BPZE1 strain
- BPZE1 is not invasive and has no selective advantage in the environment. The potential for exchange of genetic material is virtually non-existent since *B. pertussis* does not harbour plasmids or conjugative transposons. In addition, *B. pertussis* Tohama I (origin of BPZE1) does not harbour intact prophage genomes and is incapable of producing functional phage particles

Chronic carriage of *B. pertussis* has not been reported and is not expected. No cross-contamination between the subjects was observed in the previous Phase 1 or 2 clinical trials of BPZE1, nor was any risk to the family members of study subjects observed. In case of transmission to other humans, that is, those who are accidentally exposed, an efficient treatment against *B. pertussis* is commercially available and is based on administering erythromycin/azithromycin. BPZE1 has been shown to be as sensitive to erythromycin as wild-type *B. pertussis*.

Due to robust preclinical safety data, BPZE1 has been classified as a Biosafety Level 1 organism by French authorities République Française Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (French Ministry of Higher Education, Research and Innovation). Germany, Belgium, Spain, Sweden and the United States have accepted the Biosafety Level 1 rating for the purpose of manufacturing and clinical studies.

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

1.3.5. Risks Relating to the Challenge Agent

Approximately 60–120 days after inoculation with BPZE1, subjects will be admitted to the clinical research challenge unit and challenged with the *B. pertussis,* B1917 strain at 10^5 CFU. Subjects will remain in the unit for a total of 17 days for observation and daily monitoring. If persistent symptoms occur, then subjects will be started on azithromycin and remain in the unit for an additional 3 days for observation before discharge. The remaining subjects will receive azithromycin on Day 14 of their challenge unit stay and will be discharged from the Challenge Unit on Day 16 after the third dose of azithromycin is administered. The 3-day course of azithromycin will be dosed at 500 mg, by mouth, once daily (as indicated by UK Health Security Agency for post-exposure prophylaxis for respiratory infections) and subjects must abide by stipulated infection

control rules. If the *B. pertussis* culture collected on the last day of the in-unit stay is positive, the subject will return to the site to receive additional antibiotic treatment (azithromycin).

Potential risks of azithromycin include abdominal discomfort, nausea, vomiting, diarrhea, rash, liver dysfunction and in rare instances antibiotic-associated colitis, heart rhythm problems, pancreatitis, and severe skin/mucosal reactions. See current package insert for listing of risks due to use of azithromycin [Package insert 2020].

This challenge model has previously been used in a study in which 34 subjects were enrolled and approximately 80% of inoculated subjects were infected at the dose level to be used in this study. There were no serious adverse events (SAEs) during the course of the challenge study, no subjects received rescue-eradication therapy and no subjects withdrew due to study-related adverse events (AEs). Minor AEs were reported (specifically cough, rhinorrhoea and nasal congestion) but overall, controlled human pertussis infection was safe with no significant safety concerns in any subject [de Graaf *et al,* 2020].

The challenge element of the study appears to pose no environmental risk as no shedding of *B. pertussis* was detected in any of the systematically collected environmental samples (such as those taken from masks, air samples collected during aerosol-provoking procedures, bedroom air samples, contact cultures and fingertip cultures).

1.3.6. Known Potential Benefits

The benefits of the study lie primarily in the opportunities to science and humanity. No direct personal benefit from participation in the study can be guaranteed, as the vaccine may or may not confer protection in humans. It is possible, though, that taking part in this study will result in the subject having a degree of immunity to whooping cough, but we cannot be certain that the subject will benefit directly from this study. Subjects will receive information about their general health status during the study.

In summary, although there is no personal benefit to the subjects, the opportunities for science and potential future vaccines recipients and the low-risk nature of the study procedures mean that, in the opinion of the Sponsor, the risk-benefit profile is favourable for performing this study.

2. OBJECTIVES

2.1. Primary Objective

The primary objective is to demonstrate that prior immunization with BPZE1 protects against colonisation as evidenced by a negative *B. pertussis* culture following virulent *B. pertussis* challenge 2–4 months after vaccination.

2.2. Secondary Objectives

The secondary immunogenicity objectives are:

- To demonstrate BPZE1 induction of mucosal anti-pertussis secretory IgA (S-IgA) antibody is improved from baseline to Day 28
- To demonstrate BPZE1 induction of systemic IgA is improved from baseline to Day 28
- To demonstrate BPZE1 induction of systemic IgG is improved from baseline to Day 28

The secondary safety objectives are:

- To assess reactogenicity profiles (by toxicity scoring) through 7 days following study vaccination
- To describe all unsolicited treatment-emergent AEs (TEAEs) through 28 days following study vaccination and following virulent challenge
- To describe all unsolicited TEAEs related to vaccination from time of vaccination to challenge or related to challenge for 3 months after challenge
- To describe any AEs of special interest (AESI) and SAE through end of study (EOS)

2.3. Exploratory Objectives

The exploratory objectives are:

- To demonstrate prior immunization with BPZE1 reduces overall bacterial load following virulent *B. pertussis* challenge
- To demonstrate BPZE1 induction of mucosal secretory immunity (S-IgA) and systemic immunity (IgA and IgG)
- To demonstrate BPZE1 induction of immunity is similar to that observed following virulent challenge
- To compare functional antibody response by SBA
- Correlation and threshold analyses by individual and combination anti-pertussis IgG antibodies to SBA
- To assess cytokine and T helper cell (Th)1/Th2/Th17 dominance response using whole blood, peripheral blood mononuclear cells (PBMCs) and/or nasopharyngeal samples

3. ENDPOINTS

3.1. Primary Endpoint

The primary endpoint is the proportion of subjects by treatment group (BPZE1 and placebo) colonised on any day (Challenge Day 9, 11 or 14) following virulent challenge as determined by culture.

3.2. Secondary Endpoints

The secondary immunogenicity endpoints are:

- The geometric mean fold rise (GMFR) of mucosal anti-pertussis S-IgA antibody (whole cell extract [WCE], FHA, PRN, PT and fimbriae types 2 and 3 [FIM2/3]) from baseline to Day 28 (BPZE1 and placebo). Secretory IgA to be normalized ([specific S-IgA]/[total S-IgA])
- The GMFR of serum IgA antibody (WCE, FHA, PRN, PT and FIM2/3) from baseline to Day 28 (BPZE1 and placebo)
- The GMFR of serum IgG antibody (WCE, FHA, PRN, PT and FIM2/3) from baseline to Day 28 (BPZE1 and placebo)

The secondary safety endpoints are:

- Occurrence and intensity of solicited AEs for nasal/respiratory and systemic reactogenicity through 7 days following vaccination by treatment group (BPZE1 and placebo)
- Occurrence and intensity of TEAEs through 28 days following study vaccination and following challenge by treatment group (BPZE1 and placebo)
- Occurrence and intensity of TEAEs related to vaccination from time of vaccination to challenge or related to challenge for 3 months after challenge by treatment group (BPZE1 and placebo)
- Occurrence, intensity, and relationship to study vaccine of AESIs and SAEs from vaccination through EOS by treatment group (BPZE1 and placebo)

3.3. Exploratory Endpoints

The exploratory endpoints are:

- Absolute counts (CFU) in nasal wash samples on Challenge Days 9, 11 and 14 following virulent challenge by treatment group (BPZE1 and placebo)
- Geometric mean concentration (GMC)/geometric mean titre (GMT) of mucosal anti-pertussis S-IgA antibody, serum IgA and serum IgG antibody (WCE, FHA, PRN, PT

and FIM2/3) throughout the study by treatment group (BPZE1 and placebo). Secretory IgA to be normalized ([specific S-IgA]/[total S-IgA])

- The GMC/GMT and GMFR of mucosal anti-pertussis S-IgA antibody, serum IgA and serum IgG antibody (WCE, FHA, PRN, PT and FIM2/3) post-vaccination and post-challenge by treatment group (BPZE1 and placebo). S-IgA to be normalized ([specific S-IgA]/[total S-IgA])
- Proportion of subjects by treatment group (BPZE1 and placebo) with an increase in SBA 50% killing titre
- Analysis (GMC/GMT) by treatment group (BPZE1 and placebo) for each *B. pertussis* strain studied by SBA and by anti-pertussis IgG antibody
- Cell-mediated immunity using whole blood, PBMC and/or nasal pharyngeal samples, including but not limited to cytokines, Th1/Th17 and Th2 responses

4. STUDY DESIGN

4.1. Summary of Study Design

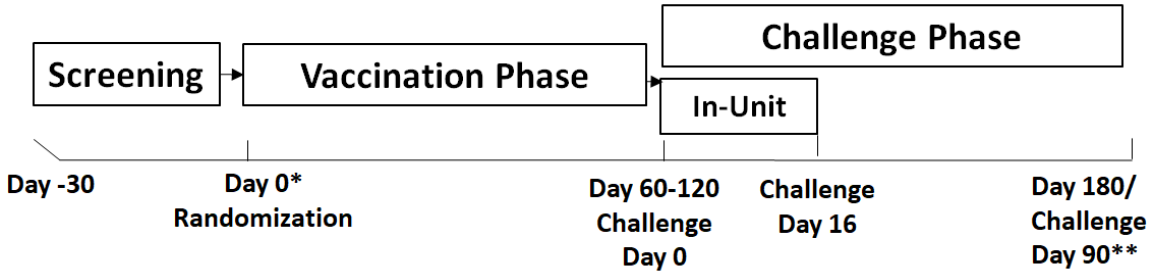
4.1.1. Study Design

This is a randomised, double-blinded, placebo-controlled trial of BPZE1 that includes virulent *B. pertussis* challenge followed by a safety follow-up.

Consenting, eligible subjects will receive a single dose of BPZE1 or placebo. 2–4 months later they will be challenged with *B. pertussis* and admitted to a challenge unit. Subjects will remain in the challenge unit for a total of 17 days and 16 nights during which time they will be monitored closely. If a subject develops symptoms of pertussis (per Investigator discretion), antibiotic (azithromycin) will be started and the subject will remain in the unit for 3 additional days of observation before discharge. If symptoms of pertussis do not develop, then subjects will receive antibiotic (azithromycin) from Days 14–16 of the challenge unit stay. Subjects will undergo safety follow-up for at least 6 months post-vaccination and at least 3 months post-challenge, for a total follow-up of 6–7 months.

A schematic diagram can be seen in Figure 1.

Figure 1 Schematic Diagram



* 1:1 randomization to BPZE1 or placebo

** Subjects will be followed up to 180 days after vaccination or 90 days after virulent challenge, whichever is longer.

The exact visits, and procedures to be performed at each visit, can be found in the Schedule of Events (Appendix 1: Schedule of Events).

An independent Data Safety Monitoring Board (DSMB) will be established by the Sponsor prior to the study commencing. Refer to the DSMB charter for specific responsibilities. Further information can be found in Section 14.1.

The primary database lock will occur after the last subject's virulent challenge visit (Visit C16). The longer-term safety and durability responses will be included in the final database lock after all planned study procedures through EOS are complete.

4.1.1.1. Vaccination Phase

After signing the informed consent form (ICF) and meeting all inclusion and none of the exclusion criteria, a suitable number of eligible subjects (approximately 60) will be randomized 1:1 to receive BPZE1 (10^9 CFU) or placebo (formulation buffer) via an intranasal MAD (Table 1). Approximately 44 subjects will be enrolled and challenged in the study for a minimum of 20 evaluable subjects per arm, with culture results during the challenge phase. It is estimated that an approximate dropout rate of 10% during the challenge may occur, thereby allowing for a minimum of 20 evaluable subjects in each arm.

Table 1 Dosing Scheme

Treatment Arms N=22[a]	Nasal Vaccination (Day 0)		*B. pertussis* Virulent Challenge (Day 60[c])
	BPZE1	Placebo[b]	
A	X	-	X
B	-	X	X

a. Evaluable subjects estimated at 20 per treatment arm with two additional to account for dropouts
b. Intranasal application of 0.4 mL per nostril (0.8 mL total volume delivered) formulation buffer placebo
c. Subject may enter virulent challenge unit between Days 60 to 120

Unblinded clinical staff will manage vaccine logistics and preparation, but they will not be involved in study-related assessments or have subject contact for data collection. Blinded individuals will administer the study vaccine.

On Day 0, nasal/respiratory and systemic reactogenicity will be assessed approximately 30 minutes after vaccination prior to release from clinical observation. Then, all subjects will record maximum daily reactogenicity in a subject diary for 7 consecutive days starting the day following study vaccination (retrospective of the highest value in the previous 24 hours). Both nasal/respiratory and general systemic reactogenicity events including actual measurements as needed (i.e., body temperature) will be recorded. Trained clinical staff will review the information from the subject diary to ensure completeness and accuracy. A standard Food and Drug Administration (FDA) toxicity grade will be assigned to each reactogenicity event (see Appendix 2: Table for Reactogenicity Grading). Should subjects experience a reactogenicity event with a potential toxicity of Grade 3 at any time they should contact the site and be seen or referred to a qualified medical facility within 24 hours for further evaluation.

4.1.1.2. Challenge Phase

Between Days 60 and 120 after vaccination (to accommodate challenge unit capacity) subjects will plan to enter the challenge unit, but they must first be confirmed suitable to proceed to virulent challenge (e.g., vitals, clinical exam, culture for *B. pertussis*, additional eligibility criteria). Once subjects are cleared for virulent challenge, a dose of the *B. pertussis* strain B1917 at 10^5 CFU will be administered while lying supine and using the procedures established within the challenge unit. The inoculum will be instilled into each nostril with a Gilson pipette by droplet. Subjects will remain supine for 15 minutes. Subjects will remain in the unit for a total of 17 days for observation. Daily monitoring will occur including vital signs, physical exam (as needed), signs/symptoms of pertussis infection (e.g., catarrhal symptoms), and select clinical laboratory testing will occur on pre-specified days (see Schedule of Events [Appendix 1: Schedule of Events] and Appendix 3: Table for Laboratory Grading for grading requirements). Nasal washes will occur at the baseline of the challenge and on Challenge Days 9, 11, and 14, or prior to initiating antibiotics should persistent symptoms of pertussis (as per the Investigator's discretion) occur as well as after receiving antibiotics. If persistent symptoms occur, then subjects will be started on azithromycin and remain in the unit for approximately 3 days from the initiation of antibiotics for observation before discharge. The remaining subjects will receive azithromycin daily from Day 14 of their challenge unit stay and will be discharged from the Challenge Unit on Day 16, after the third dose of azithromycin is administered. The 3-day course of azithromycin will be dosed at 500 mg orally once daily (as indicated by UK Health Security Agency for post-exposure prophylaxis for respiratory infections) and subjects must abide by stipulated infection control rules. If a *B. pertussis* culture collected on the last day of the in-unit stay is positive, the subject will return to the site to receive continued out-patient antibiotic treatment (azithromycin). Nasopharyngeal swabs, mucosal samples and blood samples for immunogenicity testing,

cell-mediated immunity, and safety (IgA, IgG, functional and cell- mediated) will be collected as per the Schedule of Events (Appendix 1: Schedule of Events).

4.1.1.3. Follow-up Phase

Subjects will be followed for safety for 6 months following vaccination or 3 months following challenge, whichever is longer. Adverse events will be collected as follows:

- All AEs will be collected through 28 days after study vaccination and 28 days after virulent challenge
- All AEs related either to vaccination or to challenge will be collected from time of vaccination to challenge or for 3 months after challenge, respectively
- All AESIs and SAEs will be monitored from signing of informed consent through EOS

4.1.2. Randomisation and Blinding

This is a double-blind study. Neither the subject nor the Investigator/site staff will know what study vaccine the subject is receiving with the exception of site staff involved in the preparation of the vaccination. These members of staff will take no further part in the study.

4.1.3. Duration of Subject Participation

The subjects will each be enrolled in the study for up to 8 months. This includes a screening phase of up to 30 days prior to enrolment, a vaccination phase lasting up to 120 days post-vaccination, a challenge phase of 17 days and a safety follow-up lasting until either 180 days post-vaccination or 90 days post-challenge (whichever is longer).

4.2. Stopping Rules

4.2.1. Study Stopping Rules/Vaccination or Challenge Pause Rules

There are no study specific stopping rules.

Further enrolment and study vaccinations will be paused for DSMB review/ recommendation if any of the following are reported:

- Any subject experiences ulceration, abscess, or necrosis in the nose that is considered related to study vaccination, through 28 days following study vaccination
- Any subject experiences severe respiratory symptoms of laryngospasm, shortness of breath or wheezing (grade 3 or greater) within 3 days of study vaccination

- Any subject experiences an SAE considered related to study vaccination from the time of the vaccination or related to challenge from the time of the challenge through to the subject's last study visit
- Suspected pertussis disease for which early antibiotic therapy is given to >20% of subjects administered virulent challenge

Grading scales for solicited reactogenicity events are included in Appendix 2: Table for Reactogenicity Grading.

If any of the vaccination pause rules are met following any subject receipt of study vaccination, then this study will not continue with the remaining enrolments or future study vaccinations or admissions to the challenge unit without a review by the DSMB and a recommendation from the DSMB to proceed (with or without alteration of protocol). Vaccination windows and challenge windows will be adjusted for any such pause to allow subjects to remain within the expected schedule of events. All events that classify for vaccination or challenge pause rules will be entered as AEs.

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

4.2.2. Individual Stopping Rules

There are no individual stopping rules.

Situations in which a subject may be withdrawn are outlined in Section 11.2.

5. STUDY POPULATION

5.1. Number of Subjects

A suitable number of subjects (approximately 60) will be screened, randomized and vaccinated so that approximately 44 subjects (for a minimum of 20 evaluable subjects per arm with culture results during the challenge phase) are enrolled and challenged in the study.

Subjects who withdraw, are withdrawn due to an unrelated AE as determined by the Investigator or are lost to follow-up prior to the challenge phase, or subjects who miss all 3 culture results (Challenge Days 9, 11 and 14) during the challenge phase, may be replaced at the discretion of the Sponsor.

5.2. Eligibility Criteria

5.2.1. Inclusion Criteria

Subjects must meet all the following criteria in order to be enrolled in the study:

1. Is a male or female between 18–50 years of age, inclusive on Day 0
2. Able to correctly answer all questions in the questionnaire provided during the consent process to ensure understanding of the study
3. Is capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with protocol requirements
4. Is fully conversant in the English language
5. Female subjects must not be pregnant nor breast-feeding and meet one of the following criteria:

    a. Female subjects must be post-menopausal (defined as 12 consecutive months with no menses without an alternative medical cause) or surgically sterile (i.e., hysterectomy, bilateral tubal ligation, or bilateral oophorectomy)

    (NOTE: These procedures must be confirmed by physical examination, or by subject recall of specific date and hospital/facility of procedure, or by medical documentation of said procedure. A blood sample for testing follicle-stimulating hormone [FSH] level may be drawn to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy (when appropriate) after history of 12 months of no menses has been taken.)

    OR

    b. Female subjects of childbearing potential (defined as any female who has experienced menarche and is not yet in menopause) agree to true heterosexual abstinence (when this is in line with the preferred and usual lifestyle of the subject) from at least 21 days prior to enrolment through to 3 months after the virulent challenge or agree to consistently use any of the following methods of contraception from at least 21 days prior to enrolment through to 3 months after challenge:

- Condoms (male or female) with spermicide
- Diaphragm with spermicide
- Cervical cap with spermicide
- Intrauterine device
- Oral or patch contraceptives
- Other approved pharmaceutical contraceptive methods that are designed to inhibit ovulation and protect against pregnancy

(NOTE: Periodic abstinence [e.g., calendar, ovulation, symptom-thermal, post-ovulation methods], declaration of abstinence for the duration of exposure to study vaccines, and withdrawal are not acceptable methods of contraception.)

6. Willing to refrain from any nasal sprays (including intranasal steroid sprays) and nasal washes not part of the study for 14 days prior to vaccination (Day 0) and for 28 days following vaccination and challenge
7. Is a non-smoker at the time of enrolment, has not smoked (or vaped) in the past 7 days prior to vaccination (including marijuana), and is willing not to smoke (or vape; including marijuana) from the time of vaccination throughout the in-patient challenge phase
8. Has a stable health status as assessed by the Investigator inclusive of medical history, vital sign assessments and physical examination
9. Has been sufficiently vaccinated (per site and local guidelines) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; proof of vaccination required) >14 days prior to study vaccination
10. Has access to a consistent and reliable means of telephone, text, and email contact, by personal mobile electronic device
11. Is able to understand and comply with planned study procedures including admission for virulent challenge for 17 days and willingness to take the curative antibiotic regimen (azithromycin after inoculation with *B. pertussis*)
12. Lives a reasonable distance from the clinical site(s) to be able to travel to and from the clinical site(s) for follow-up visits
13. Agrees to stay in contact with the clinical site for the duration of the study, has no current plans to move from the study area and provides updated contact information as necessary
14. Willing to provide written agreement to and abide by infection control rules from challenge until 1 week following completion of azithromycin eradication

5.2.2. Exclusion Criteria

Subjects must not be enrolled if they meet any of the following criteria:

1. Body mass index <17 kg/m^2 or >35 kg/m^2
2. History of being vaccinated against pertussis within 5 years of enrolment
3. History of never being vaccinated for pertussis in lifetime
4. A diagnosis of pertussis by laboratory confirmation or by physician diagnosis in the past 5 years
5. Serum anti-pertussis toxin IgG >20 IU/mL conducted locally during Screening
6. Serum anti-pertussis PRN IgG >30 IU/mL conducted locally during Screening
7. Previously inoculated with *B. pertussis* in a pertussis challenge study
8. Screening laboratory values outside of the local laboratory's normal ranges (chemistry or haematology excluding white blood cell differential) and clinically

significant, except for documented Gilbert's syndrome with direct bilirubin ≤ upper limit of normal. (Laboratory samples may be repeated once during the screening period)

9. Positive test for human immunodeficiency virus, hepatitis B or hepatitis C at Screening
10. Use of illicit drugs (excluding marijuana), evidenced by urine toxicology at Screening or a history of drug/alcohol abuse within the past 2 years
11. Any chronic illness being treated actively with evidence of recent intervention for worsening or fluctuating symptoms such that a stable baseline of disease is not possible (at the discretion of the Investigator)
12. The subject has a history of active cancer (malignancy) in the last 10 years (exception is subjects with adequately treated non-melanomatous skin carcinoma, who may participate in the study)
13. Existing chronic disorders inclusive of lung (e.g., asthma, chronic obstructive pulmonary disease), kidney, heart (including screening QT interval corrected according to Fridericia's formula ≥440 msec on the 12-lead ECG), liver, diabetes, immunodeficiency (acquired or congenital), autoimmune (exception is Hashimoto's thyroid disease) or significant neurologic condition (including, but not limited to, facial paralysis or Bell's palsy). Note, well-controlled chronic disorders including hypertension and hypercholesterolemia are permitted
14. History of Guillain-Barré syndrome (genetic/congenital or acquired)
15. History of head trauma with potential of cribriform plate fracture within 1 year prior to Day 0
16. History of nasal or sinus surgery within 6 months or receipt of facial cosmetic fillers within 3 months prior to Day 0 or diagnosis of nasal polyps
17. Has taken immunosuppressive therapy or other immune-modifying drugs (including but not limited to systemic corticosteroids, biologics and methotrexate) in the past 6 months, is on scheduled immunosuppressive therapy or is planning to start immunosuppressive therapy during the trial. For systemic corticosteroids this means prednisone or equivalent for 10 days or more. The use of corticosteroids (inhaled, topical, ophthalmologic, or localized injections in joints) are permitted
18. Receipt of immunoglobulins or any blood products within 3 months prior to study vaccine administration or planned receipt during the study
19. The subject resides in a residence or has routine contact (face to face <2 meters) with persons with known immunodeficiency including persons on immunosuppressant therapy, from study vaccination to challenge and for 1 week after exiting the challenge unit
20. The subject resides in a residence, works regularly with, or has contact (face to face <2 meters), with infants less than 1 year of age, partially immunised infants or

pregnant women, adults >65 years of age who have not received a dose of acellular pertussis vaccine (e.g. Tdap) within the past 10 years from study vaccination to challenge and for 1 week after exiting the challenge unit

21. Known hypersensitivity to any component of the study vaccine
22. Contraindications or allergic to azithromycin, erythromycin or other macrolide antibiotics
23. Taking medication that may interact with azithromycin (e.g., nelfinavir, warfarin, digoxin and phenytoin)
24. Inability to adhere to the protocol, visit schedule or sample collection needs (including housing in the challenge unit)
25. Any medical condition, including previous serious event due to a vaccination that, in the opinion of the Investigator, might interfere with the evaluation of the study objectives or might affect the safety of the individual (e.g., frequent migraines, major depression or history of recent suicidal attempt)
26. Participation in any other clinical trial for the testing of an unlicensed product during the previous 3 months or planned during the study conduct

(NOTE: 'Testing' is intended to mean receipt of an investigational product. Subjects in long term safety follow-up studies where unlicensed product has completed administration >3 months prior to this study enrolment are allowed. Allowance is given for subjects who have received a product under emergency use authorization if the product has been given >14 days before study enrolment.)

27. Subject is a first-degree relative of study team member

Additional Exclusion from Entry into Challenge Unit

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

28. Has received any form of immunotherapy determined to potentially affect the immune response or increase the risk of severe disease in a virulent challenge setting (as determined by medical consultation with Sponsor, when necessary)
29. Has a *B. pertussis* positive nasal sample (by culture) within 7 days of admission to challenge unit

Other Considerations for Temporarily Holding Vaccination or Entry into Challenge Unit

Subjects meeting any of the following criteria may have a planned study vaccination or challenge admission deferred to a later date, but these criteria are not exclusionary for study enrolment. Subjects may be enrolled on a return visit if they remain within 45 days of consenting. Otherwise, subjects must be re-consented and reassessed per the inclusion and exclusion criteria (i.e., re-enrolled). Subjects may be admitted for virulent challenge until Day 120 post-vaccination; if a subject is unable to be admitted for virulent challenge

by Day 120 post-vaccination, the subject should continue to be followed-up for safety per the Schedule of Events.

- Has recent symptoms or illness (documented by AE) that requires deferment of a virulent challenge due to confounding effects or new/recent disease onset such that a virulent challenge is not medically acceptable for safety reasons. Therefore, the Investigator will determine the appropriate allowance of time with the subject either resolving recent illness or medical condition determined to be stable (although within the timelines noted above)
- The subject has, or reports, a body temperature of 100.4°F (38°C) or higher within the past 3 days. The subject may be vaccinated/challenged once the fever resolves and body temperature (subject measured) remains below 100.4°F (38°C) for at least 3 days
- The subject has, or reports, any infection, acute respiratory tract symptoms or rhinorrhoea within the past 3 days. Subject may be vaccinated/challenged once all symptoms have been resolved for at least 7 days
- The subject used antibiotics (including systemic antibiotics, antibiotic lozenges and topical antibiotics) within 10 days prior to study vaccination or within 30 days prior to challenge
- Has a SARS-CoV-2 positive sample within 3 days prior to time of vaccination or challenge unit entry. Requires retest with negative results prior to vaccination or prior to entering challenge unit

6. STUDY ASSESSMENTS AND PROCEDURES

Study procedures will be performed at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). No procedures should be performed prior to the subject providing written informed consent.

6.1. Screening Procedures

6.1.1. Volunteer Information Sheet/Pre-Consent Questionnaire/Informed Consent Form/The Overvolunteering Prevention System Registration

A volunteer information sheet (VIS) will be available on the study website and may be given to potential subjects before the screening visit. The VIS will include all risks and safety measures that are involved in the study.

Individuals who have expressed an interest in taking part in the study will be invited to attend a screening session after a short telephone screening. During the screening visit the study will be explained. If the subject has any questions they can be addressed during this visit.

After their understanding of the study aims and level of involvement required has been established and if they wish to participate, subjects will be asked to complete a questionnaire testing their understanding of the challenge phase of the trial. Subjects who fail to answer all questions correctly on their first attempt will be allowed to re-take the questionnaire following further discussion with the investigator. If the subject is not able to answer all questions correctly within three attempts, he/she will be asked to read the VIS again and come back at least 24 hours later for a screening visit and repeat the pre-consent questionnaire.

After the questionnaire has been completed and any questions answered, a written informed consent in compliance with regulatory authority regulations shall be obtained from each subject before entering the study or performing any non-routine procedure that involves risk to the subject. Both the VIS and ICF will be reviewed and approved by the sponsor and site(s), prior to independent ethics committee (IEC) review and approval. If the VIS and ICF are revised during the course of the study, the all active participating subjects must sign the revised forms unless advised by the sponsor, and subject to approval by the IEC.

Once the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give written informed consent by signing and dating the ICF. The authorised person obtaining the informed consent will also sign and date the ICF.

The ICF/VIS will contain separate sections that address the use of remaining mandatory samples for exploratory research (if applicable) and explain/address the exploratory research portion of the study. Subject medical records must state that written informed consent was obtained.

The investigator shall retain the signed original ICF(s) and provide copies of the signed original form(s) to the subject.

As part of the informed consent process, subjects will also consent to registration in The Overvolunteering Prevention Systems (TOPS) database (www.tops.org.uk). Registration in TOPS is required to help ensure the health and safety of the subjects during the study, by tracking prior study participation, timing of recent sample collections and/or potential medication interactions.

6.1.2. Inclusion and Exclusion Criteria

Inclusion and exclusion criteria will be reviewed for each potential subject. Eligibility will be documented in the electronic case report form (eCRF).

6.1.3. Demographic and Baseline Data

The following data will be collected at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events).

- Age/date of birth
- Sex
- Race/ethnicity
- Weight
- Height
- Body mass index (electronically derived from weight and height)

6.1.4. Medical History

The following data will be collected from the subjects at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). General practitioner's records are not required; however, any available site medical records and/or histories should be reviewed before randomization.

- Prior medical conditions
- Ongoing medical conditions
- Surgeries
- Significant, non-screening, procedures (e.g., endoscopy, uterine ablation, lithotripsy)

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

6.2. Efficacy Procedures

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

6.2.1. Nasopharyngeal Swabs and Nasal Washes

Nasopharyngeal swabs and nasal washes should be collected at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events) with the nasopharyngeal swab(s) (up to two) collected before the nasal wash.

Nasopharyngeal swabs will be used to test for cell-mediated immunity. Nasal washes will be used for *B. pertussis* colonisation by culture and PCR testing.

Further details on sampling, processing, and shipping procedures can be found in the study laboratory manual.

6.2.2. Nasal Mucosal Secretion Samples

Secretion samples should be collected at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). Samples will be used for testing levels of immunogenicity.

Further details on sampling, processing, and shipping procedures can be found in the study laboratory manual.

6.2.3. Blood Samples

6.2.3.1. Serum Immunogenicity

Samples for serum immunogenicity should be collected at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). The processed samples will be shipped to an analytical laboratory where the tests (including enzyme-linked immunosorbent assay [ELISA] and SBA) will be performed.

Further details on sampling, processing, and shipping procedures can be found in the study laboratory manual.

6.2.3.2. Whole Blood and Peripheral Blood Mononuclear Cell Samples

Blood samples for analysis of whole blood and PBMCs should be collected at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). The samples will be used to test for cell mediated immunity at a central laboratory.

Further details on sampling, processing, and shipping procedures can be found in the study laboratory manual.

6.3. Safety Procedures

6.3.1. Infection Control Rules

Subjects will be asked to adhere to infection control measures to limit possible onward transmission of *B. pertussis*. Subjects must sign the infection control agreement during Screening and again prior to the virulent challenge (Appendix 1: Schedule of Events).

Infection control rules are in place beginning with the virulent challenge and ending one week after discharge from the challenge unit facility:

During admission:

- Volunteers must wear a surgical mask unless alone in their own personal room or bathroom facilities
- Volunteers should wash their hands before leaving their room
- Volunteers must not enter the rooms of other volunteers.
- Volunteers must not have any contact with high risk of transmission with any individuals – such contact includes:
    a. Intimate/sexual contact
    b. Contact that may involve transfer of respiratory secretions e.g., kissing
    c. Sharing cutlery or drinking vessels
- The volunteer may receive a maximum of two guests at a time between 8.00 and 22.00, who must wear masks covering nose and mouth while in close proximity to the volunteer and must adhere to strict infection control procedures. These guests must not be:
    - Unimmunised or partially immunised children and infants aged <1 year
    - Pregnant women >32 weeks who have not received pertussis vaccination at least a week prior to contact
    - Immunosuppressed or frail individuals
    - Healthcare workers regularly working with vulnerable individuals as above
- Volunteers are allowed to leave the challenge unit during daytime for a maximum of 2 hours twice a day during daytime with the agreement of the study team

While outside the challenge unit and for 1 week following discharge:

- Volunteers must wash their hands before leaving the challenge unit or their home
- Volunteers must be contactable by mobile phone, which has the study emergency phone number programmed in, and contact the clinical study team if they have any symptoms suggestive of early pertussis disease.
- Volunteers must be able to return to the challenge unit within 120 minutes
- Volunteers must avoid crowded social environments
- Volunteers must wear a surgical mask during any face to face contact with other individuals
- Volunteers must avoid contact with:
    - Unimmunised or partially immunised children and infants aged <1 year

- Pregnant women >32 weeks who have not received pertussis vaccination at least a week prior to contact
- Immunosuppressed or frail individuals
- Healthcare workers regularly working with vulnerable individuals as above

- Volunteers must not have any contact with high risk of transmission with any individuals – such contact includes:
  - Bedroom sharing
  - Intimate/sexual contact
  - Contact that may involve transfer of respiratory secretions e.g., kissing
  - Sharing cutlery or drinking vessels

For safety, all the subjects will receive antibiotic eradication treatment with a macrolide (azithromycin) after the virulent challenge.

6.3.2. Vital Signs

Vital signs (heart rate, systolic/diastolic blood pressure, temperature, respiratory rate, oxygen saturation) will be assessed at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events), pre-vaccination and pre-challenge and approximately 30 minutes following vaccination and challenge, respectively. During the challenge unit stay, vital signs will be collected at least twice daily (once in the morning and once in the evening). Additional vital signs may be performed at any time point at the Investigator's discretion.

Subjects should rest in a supine position for 10 minutes before the vital signs are assessed.

Any vital signs with results falling outside normal ranges may be repeated at the discretion of the Investigator. After randomisation, if any results falling outside the normal ranges are deemed clinically significant by the Investigator or appropriately qualified designee, they should be recorded as an AE/SAE.

6.3.3. Physical Examination

A full or targeted physical examination will be performed at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). During the full physical examination, the following areas will be assessed by the Investigator or medically-qualified designee: general appearance; eyes, ears, nose and throat, head and neck; chest and lungs; cardiovascular; abdomen; musculoskeletal; lymphatic; dermatologic; neurologic and extremities.

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

6.3.4. Laboratory Assessments

Samples for safety laboratory tests should be taken at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). Additional samples may be collected at the Investigator's discretion. Any clinically significant results and all Grade 3 or higher results should be discussed with the CRO.

A single repeat sample will be permitted during the screening process to allow inclusion.

6.3.4.1. Haematology

For safety, haematology will be assessed during the study.

- Total blood count consisting of:
  - Haemoglobin concentration
  - Platelets
  - White blood cells
  - Neutrophils
  - Lymphocytes
  - Monocytes
  - Eosinophils
  - Basophils

Blood samples will be analysed locally and samples will be destroyed following database lock (at the latest). Further details on processing can be found in the study laboratory manual.

6.3.4.2. Clinical Chemistry

For safety, the following parameters will be assessed during the study:

- Sodium
- Potassium
- Urea
- Creatinine
- Total protein
- Albumin
- Total bilirubin
- Alanine aminotransferase

- Alkaline phosphatase
- Random glucose
- C-reactive protein

Blood samples will be analysed locally and samples will be destroyed following database lock (at the latest). Further details on processing can be found in the study laboratory manual.

6.3.4.3. Additional Laboratory Parameters

The following parameters will be assessed, at a local or central laboratory, at specified time points, depending on the parameter (see Laboratory Manual):

- Serology
  - Human immunodeficiency virus
  - Hepatitis B
  - Hepatitis C
  - Anti-PT antibodies (local laboratory results to be used for screening purposes. Central laboratory results to be used for analyses)
  - Anti-PRN antibodies (local laboratory results to be used for screening purposes. Central laboratory results to be used for analyses)
- Urine toxicity screen
- Severe acute respiratory syndrome coronavirus 2 testing (SARS-CoV-2 test for active infection (an approved local test accepted by public health authorities and readily available) will be administered to subjects within 72 hours prior to vaccination and challenge unit admission. Should the subject have a positive SARS-CoV-2 test result, regardless of symptomatology, then vaccination/admission to the challenge unit will be temporarily held until the subject has a negative test result)
- Respiratory PCR panel

6.3.5. Twelve-Lead Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be performed at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events). Additional ECGs may be performed at any time point at the Investigator's discretion. QT interval corrected according to Fridericia's formula will be calculated. The ECG measurements will be made with the subject in a semi-supine position and having rested in this position for at least 10 minutes before each time point.

Any ECG with results falling outside the normal ranges may be repeated at the discretion of the Investigator. If any results falling outside the normal ranges are deemed not

clinically significant by the Investigator, or appropriately qualified designee, this should be clearly stated on the hard copies of the ECG and signed and dated by the Investigator. If an ECG trace indicates an abnormality that is measured by the equipment but is deemed normal by the Investigator, this should be clearly stated on the ECG trace as normal and signed and dated by the Investigator or appropriately qualified designee. If the ECG trace indicates an abnormality that is present but deemed as not clinically significant by the Investigator or appropriately qualified designee, then this should be clearly stated on the ECG trace as "NCS" and signed and dated by the Investigator or appropriately qualified designee. After randomisation, any results falling outside the normal ranges and deemed clinically significant by the Investigator or appropriately qualified designee should be recorded in the electronic case report form (eCRF) as an AE/SAE.

6.3.6. Pregnancy Testing

Serum and/or urine will be collected from female subjects of childbearing potential at the times outlined in the Schedule of Events (Appendix 1: Schedule of Events) to enable pregnancy tests to be performed. Serum and urine samples will be analysed locally, and samples will be destroyed following analysis. A positive urine pregnancy test during the study will be confirmed by a serum test.

Female subjects who are identified as being pregnant during the study will be withdrawn from further participation in study vaccination and/or challenge, but they will continue to attend safety follow-up visits.

6.3.7. Post-Vaccination Evaluation

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

1. Obtain vital signs
2. Assess for reactogenicity based on nasal/respiratory and systemic (including oral temperature) FDA toxicity grading scales

Immediate reactogenicity (including grade any actual measurements) will be recorded at 30 minutes following study vaccination. 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). A thermometer for measuring body temperature (oral or tympanic) will also be distributed to subjects. The use of anti-pyrogenic medication will also be specifically queried by the subject diary during the 7 days following study vaccination.

Nasal/respiratory and general systemic reactogenicity events will be recorded, including start and stop dates and actual measurements of body temperature. Trained clinical staff will review the information from the subject diary with the subjects at the following visit and apply a standard toxicity grade (refer to Appendix 2: Table for Reactogenicity

Grading) to any reactogenicity event. Subjects will be instructed that should they have reactogenicity with a potential toxicity Grade 3 at any time, they should contact the site on the same day and be seen or referred to a qualified medical facility within 24 hours. Any discrepancies between the Investigator- and the subject reported outcomes of reactogenicity will be noted in the source documents prior to entry into the clinical database.

6.3.8. Post-Challenge Evaluation

During the in-unit period the volunteers will be reviewed at least twice per day (once in the morning and once in the evening) following a standardised checklist including body temperature, respiratory rate, heart rate, and early symptoms of pertussis: rhinorrhoea, nasal congestion, epistaxis, sneezing, ear pain, eye pain, sore throat, cough, dyspnoea, feeling generally unwell, tiredness and headache. Actions to be taken when symptoms of early *B. pertussis* disease are suspected are included in Appendix 5: Process to be followed in cases of suspected symptomatic *Bordetella pertussis* infection. All AEs are to be reported in the eCRF.

6.3.9. Azithromycin

If persistent symptoms suggestive of pertussis disease occur, then subjects will be started on azithromycin and remain in the unit for approximately 3 days from the initiation of antibiotics for observation before discharge. The remaining subjects will receive azithromycin daily from Day 14 of their challenge unit stay and will be discharged from the Challenge Unit on Day 16 after the third dose of azithromycin is administered. The 3-day course of azithromycin will be dosed at 500 mg orally once daily (as indicated by UK Health Security Agency for post-exposure prophylaxis for respiratory infections) and subjects must abide by stipulated infection control rules. If a *B. pertussis* culture collected on the last day of the in-unit stay is positive, the subject will return to the site to receive continued out-patient antibiotic treatment (azithromycin).

7. LIFESTYLE AND/OR DIETARY RESTRICTIONS

The following lifestyle and dietary restrictions apply throughout the study:

- Subjects should not use drugs of abuse unless prescribed by a physician (e.g., benzodiazepines)
- Subjects should not consume more than 14 units of alcohol per week. A unit is defined as one shot (25 mL) of spirits, half a pint (236 mL) of standard-strength beer or 1 small glass (125 mL) of wine
- Subjects should not consume alcohol within 24 hours prior to receiving study vaccination or receiving virulent challenge, and throughout the challenge in-unit stay
- Subjects should not smoke or vape from 1 week prior to vaccination until after the end of the in-house challenge phase

- Subjects should refrain from any nasal sprays and washes not part of the study for 28 days following vaccination and the challenge phase
- Female subjects who are of child-bearing potential should observe the contraceptive guidelines, outlined in the inclusion criteria, from 21 days prior to enrolment until 3 months after the challenge.

8. INVESTIGATIONAL PRODUCT

8.1. Dosage and Administration

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

The Sponsor will ensure the investigational vaccine and placebo (lyophilised buffer) is properly labelled and distributed to the clinical site(s).

The supplies which will be used for vaccination in the study are outlined in Table 2.

Table 2 Study Investigational Medicinal Products

Product	Dosage and Route of Administration
BPZE1	2 x 0.4 mL (0.4 mL per nostril containing half the dose (5 x 10^8 CFU) bacteria to give a total dose of 10^9 CFU) administered intranasally via a MAD
Placebo	2 x 0.4 mL (0.4 mL per nostril) reconstituted lyophilized buffer administered intranasally via a MAD

CFU=colony-forming units; MAD=mucosal atomization device

The intranasal placebo consists of lyophilised buffer diluted with sterile water for injection (WFI), which are the same constituents in the same quantities as the BPZE1 investigational vaccine, except for the attenuated *B. pertussis* cells.

Each vaccination with BPZE1 or placebo will utilise a single vial/syringe of product for each single use (1:1 assignment for study vaccine accountability).

Further details on administration are provided in the Pharmacy/Challenge Manual.

8.2. Dose Rationale

BPZE1 in a liquid formulation has been studied at various doses from 10^3 CFU to 10^9 CFU in two Phase 1 clinical studies in Sweden with no vaccine-related SAEs and comparable general AEs to placebo controls [Jahnmatz *et al*, 2020; Thorstensson *et al* 2014]. A total of 78 healthy subjects have received intranasally administered BPZE1 in a

liquid formulation. The Phase 1b study demonstrated the ability for a 0.4 mL (400 µL) per nostril dose of 10^7, 10^8, and 10^9 CFU BPZE1 to achieve transient nasopharyngeal colonization in >80% of subjects. Furthermore, 92% of the subjects in the 10^7 and 10^8 CFU/dose groups and 100% of the 10^9 CFU group had a positive serological response (immunoglobulin G [IgG] or immunoglobulin A [IgA]) to any of the four *B. pertussis* antigens tested, and 100% of the 10^9 CFU subjects had a positive serological response (IgG or IgA) to two or more of the antigens tested. The 10^9 CFU dosage appears to be the most promising to elicit the needed immune response in the majority of adults.

The Phase 2a and 2b studies assessed the 10^9 CFU dose of BPZE1 and showed that this dosage is safe and well-tolerated.

8.3. Maintaining the Blind

This is a double-blind study.

The site(s) being used will identify an unblinded individual(s) who will be provided with a randomisation list. This will be used to allocate the subjects to either BPZE1 or placebo. To maintain the blind, unblinded individuals will be involved in vaccine logistics and preparation. The unblinded team will not be involved in any study-related assessments or have subject contact for data collection following study vaccination. Blinded individuals will administer the study vaccine with further details in the Pharmacy/Challenge Manual.

Code break envelopes will be provided to the site(s) so that they can unblind an individual subject in the event of a medical emergency. Where possible they should discuss the situation with the Sponsor's medical monitor prior to unblinding; however, the subject's safety must always come first. Reasons for treatment unblinding must be clearly explained and justified in the eCRF. The date on which the code was broken together with the identity of the person responsible must also be documented.

8.4. Treatment Assignment

Subjects will be assigned to a study vaccine based on the order in which they are enrolled. This allocation will be performed by an unblinded individual at the site who will be independent of blinded staff determining eligibility of subjects.

Subjects will be randomised in a 1:1 ratio to receive a single dose of either BPZE1 (10^9 CFU) or placebo.

8.5. Packaging and Labelling

Vaccine will be provided to the sites in an open-label format. The labels will contain all information required to meet the applicable local regulatory requirements. The labels applied following dispensing will be blinded.

Further information on the study vaccine packaging, labelling, and dispensing are included in the Pharmacy/Challenge Manual.

8.6. Preparation

The dose of BPZE1 or placebo will be administered intranasally. A total of 0.8 mL of vaccine will be withdrawn for administration. Following reconstitution, the MAD will be attached to the syringe, and 0.4 mL volume will be administered to each nostril. In the case of placebo nasal vaccination, a similar volume of WFI will be reconstituted into a vial containing only lyophilised buffer, followed by 0.4 mL volume administered into each nostril with the vaccinator. The mucosal atomiser device provides a uniform, controlled delivery, which allows the vaccinator to accurately deliver 0.4 mL of vaccine to the initial nostril and then administer the remaining 0.4 mL to the opposite nostril.

Further details on preparation are provided in the Pharmacy/Challenge Manual.

8.7. Handling and Storage

BPZE1 and the placebo must be stored in a secure area (e.g., locked room or locked refrigerator), protected from light and moisture as required by manufacturer. The vaccine will be shipped from the Sponsor's European contract manufacturing organization to the CRO's repository and distribution centre and should be stored at -20°C or below prior to shipping to the clinical sites, where the vaccine will be stored at 8°C or below. Reconstituted BPZE1 should not be kept at room temperature prior to vaccination for more than 4 hours.

A detailed description regarding the storage and handling the study vaccine will be in the Pharmacy/Challenge Manual.

8.8. Product Accountability and Assessment of Compliance

In accordance with International Council on Harmonisation Good Clinical Practice (ICH-GCP), the study centre will account for all supplies of study vaccine. Details of receipt, storage, assembly, and return will be recorded. The unit of accountability will be one vial.

All unused supplies will either be destroyed or returned to the study Sponsor at the end of the study in accordance with instructions by the Sponsor.

All study vaccines will be administered by site staff. If the complete vaccination is not administered as planned this will be documented in the eCRF.

8.9. Treatment of Investigational Product Overdose

An overdose is any dose of study treatment given to a subject or taken by a subject that exceeds the dose described in the protocol. If the subject receives an overdose of the

study vaccine, then the Sponsor should be notified, in writing, within 24 hours of the Investigator becoming aware. This will be classified as a protocol violation.

The Investigator will treat the overdose according to their clinical judgment depending on the extent of the overdose and the clinical signs and symptoms exhibited by the subject. In case of any AEs associated with the overdose, these should be reported on relevant AE/SAE sections in the eCRF. BPZE1 is a live attenuated *B. pertussis* bacterium and is susceptible to erythromycin/azithromycin antibiotic treatment.

8.10. Occupational Safety

To avoid accidental exposure actions should be taken to minimize generation of aerosols, since the bacterium is strictly a respiratory tract organism. Clinical staff members performing reconstitution should wear eye-protective glasses, gloves and masks, and clean surfaces prior to and after performing reconstitution activities with 70% ethanol solution. Persons handling the BPZE1 bacteria for vaccination should wear gloves, goggles and face mask, and must wash their hands with a suitable disinfecting soap after administration. Effective antibiotic treatment with azithromycin could be given in case of accidental transmission to other humans, however, BPZE1 is an attenuated live organism and is not pathogenic.

The Material Safety Data Sheets will be made available where required by local regulations.

9. CHALLENGE AGENT

9.1. Manufacturing of the *Bordetella pertussis* Inoculum

The inoculum is prepared to good manufacturing practice (GMP) standard in licensed cGMP facilities and using a process free of animal-derived products. The identity and purity of the cell bank is confirmed, in addition to any other quality specifications agreed with the consortium and needed for compliance with regulatory requirements. There will be no culture of the challenge inoculum at the clinical site, other than to assess the dose and purity of the inoculum after inoculation and quality assessment.

More information regarding the challenge agent will be in the Pharmacy/Challenge Manual.

9.2. Quality Assessment of the Inoculum

Each vial used for inoculation will be thawed and tested for culture on *Bordetella* selective medium (charcoal blood agar with cephalexin) for determination and viable counts of *B. pertussis* and on a Plate Count Agar to assess the purity of the inoculum. Culture identity will be confirmed by visual appearance of colonies and Gram stain. Full molecular identification using for example, PCR and Matrix Assisted Laser Desorption

Ionisation Time-of-Flight Mass Spectrometry may be used to identify isolates to species level. UK standards for identification of *Bordetella* species [UK Standard for Microbiology Investigations] will be followed.

9.3. Transport of the Inoculum

The inoculum is transferred to the site under temperature-monitored conditions. For transport within the site, containers of inoculum are placed into secondary containers, which are transported in leak and shock resistant transport boxes with secured lids.

9.4. Storage of the *Bordetella pertussis* Inoculum

The cell banks are stored at -80°C (±20°C) in a locked, dedicated, temperature monitored freezer at the site.

9.5. Dilution of the Inoculum

One vial of inoculum will be removed from the freezer, thawed, and diluted to the required inoculum dose following the challenge specific standard operating procedures (SOP). Dilutions will be carried out by one staff member and checked by another member. The inoculum will be administered to the volunteer within an hour of removal from the freezer following the specific SOP: Administering the *B. pertussis* inoculum to healthy volunteers.

9.6. Administration of the Challenge Agent

Once subjects are cleared for virulent challenge of a dose of the *B. pertussis* B1917 strain at 10^5 CFU, the dose will be administered while lying supine and using the procedures established within the challenge unit. The inoculum will be instilled into each nostril with a Gilson pipette by droplet. Subjects will remain supine for 15 minutes.

9.7. Monitoring of the *Bordetella pertussis* Dose Given to the Subject

After the inoculum is administered, the tube residuum will be cultured for 5 days on *Bordetella* selective medium (charcoal blood agar with cephalexin) for isolation and purity-checking and viable counts of *B. pertussis* will be measured using a standard dilution technique. If the inoculum CFU count measured is more than five times the inoculum CFU count intended for the volunteer, they will be given oral azithromycin 500 mg once a day for 3 days, observed for an additional 48 hours in-unit, and excluded from the study. Safety follow-up in this case of overdosing will take place at Days 14 and 28.

9.8. Disposal of the Inoculum

All materials used during the challenge, including any remaining inoculum will be disposed of following site policy.

10. CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

All medications (including over the counter products, herbals, vitamins, supplements, and vaccinations) taken from 7 days prior to Screening until the EOS visit should be recorded. Information recorded should include the generic name, dose, route of administration, frequency, date started and stopped, and reason the medication was taken. Intake will include whether there was knowledge of past pertussis vaccination and (when available) the year of receipt. Use of new medication(s) should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

10.1. Permitted Medications

Subjects are permitted to take paracetamol at doses up to 4 g per day. The use of anti-pyrogenic medication will be specifically queried by diary during the 7 days following study vaccination.

Female subjects of childbearing potential are permitted to use contraception (e.g., contraceptive pill, contraceptive patch).

All subjects who are challenged should take azithromycin orally, once daily, for a minimum of three doses prior to being discharged from the challenge unit.

10.2. Prohibited Medications

Immunosuppressive therapy or other immune-modifying drugs (including but not limited to systemic corticosteroids, biologics and methotrexate) during the trial is prohibited. Corticosteroids (inhaled, topical, ophthalmologic or localized injections in joints) are permitted.

Antibiotics are not permitted during the challenge unit stay with the exception of azithromycin, as described in Section 6.3.9.

Medications mentioned in the inclusion/exclusion criteria are restricted as outlined in those criteria.

11. SUBJECT COMPLETION AND WITHDRAWAL

11.1. Subject Completion

A subject is classified as having completed the study when he/she completes the EOS visit.

11.2. Subject Withdrawal

Subjects may withdraw/discontinue from the study any time and for any reason without prejudice to their future medical care by the Investigator or at the study centre. The reason for subject withdrawal/discontinuation will be recorded.

A subject may be withdrawn from treatment or challenge for any of the following reasons:

- Withdrawal of consent to continue in the study (a subject may withdraw his or her consent at any time)
- The study Investigator or Sponsor, for any reason, decides the subject should be withdrawn from the treatment
- Adverse events, which cannot be tolerated by the subject
- Pregnancy
- Significant noncompliance with the protocol, as determined by the medical monitor(s)
- Sponsor decides to discontinue the subject's participation in the study or to terminate the study

If a subject is withdrawn from the study, where possible, he/she should continue to attend the safety follow-up visits so that data can be collected for the Intention-to-Treat Population. If subjects will not agree to this, then the subject should complete the EOS visit.

11.3. Treatment after the End of the Study

No treatment/vaccination will be provided after the EOS.

11.4. Screen Failures

Information on screening failures will be collected.

12. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

12.1. Definitions

12.1.1. Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal

relationship with this treatment. An AE can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

12.1.2. Treatment-Emergent Adverse Event

A TEAE is an AE that occurs after the subject has been vaccinated.

12.1.3. Adverse Events of Special Interest

The following AEs are classified as AESIs and will be collected on a unique eCRF through to the EOS:

- Adverse events related to infection with or vaccination for SARS-CoV-2

12.1.4. Serious Adverse Events

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity

OR

- Is a congenital anomaly/birth defect

Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not reach the above definition but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an accident and emergency department or at home treatment for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

12.1.5. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are SAEs, classified as related, which are not specified as expected in the approved Investigator's Brochure.

12.2. Safety Reporting

12.2.1. Reporting of Adverse Events

Adverse events will be collected as follows:

- All AEs will be collected through 28 days after study vaccination and 28 days after virulent challenge. Note that between screening (signing of ICF) and Day 0, AEs will be collected only if classified as serious or if considered related to study procedure or study involvement. Any event that results in vaccination delay (e.g., temporary exclusions due to acute upper respiratory symptoms/illness or fever, or investigator determined medical condition) during screening or during study conduct will also be recorded as an AE. Should any reactogenicity event extend beyond 7 days following vaccination, it will be recorded as an AE with the same start date as the reactogenicity event and followed until considered resolved
- All AEs related either to vaccination or to challenge will be collected from time of vaccination to challenge and for 3 months after challenge, respectively

Adverse events will be recorded in the eCRF. The CRO should be notified within 24 hours if the AE leads to withdrawal of the subject.

Any medical condition that is present at the time that the subject is screened will be considered as baseline (recorded as medical history) and not reported as an AE; however, if the severity of any pre-existing medical condition increases, it will be recorded as an AE. Adverse events must be graded for severity and assessed for relationship to study vaccination. Adverse events characterised as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

All subjects will record maximum daily reactogenicity/solicited AEs in the subject diary starting for the subsequent 7 days after vaccination. Subjects will be instructed that should they have a reactogenicity event with a potential toxicity Grade 3 at any time after vaccination, they should contact the site on the same day and be seen or referred to a qualified medical facility.

12.2.2. Reporting of Serious Adverse Events and Adverse Events of Special Interest

All AESIs and SAEs will be monitored from signing of informed consent through to the EOS visit.

All SAEs/AESIs occurring during the clinical study must be reported to the appropriate CRO contact person by investigational staff within 24 hours of their knowledge of the event.

Information regarding SAEs/AESIs will be transmitted to the CRO using the SAE Form, which must be completed and signed by a member of the investigational staff and transmitted to the CRO within 24 hours of knowledge of the event.

All SAEs/AESIs that have not resolved by the EOS, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilises
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study vaccine/challenge or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)
- Thirty days have elapsed after the EOS (last subject, last visit)

Any event requiring hospitalisation (or prolongation of hospitalisation) that occurs during the course of a subject's participation in a clinical study must be reported as an SAE, except hospitalisations for the following:

- Social reasons in the absence of an AE
- Surgery or procedure planned before entry into the study (must be documented in the source documents and eCRF)

The Sponsor will promptly evaluate any new SUSARs and expeditiously communicate possible new safety findings to investigators, institutional review boards or independent ethics committees, and applicable health authorities based on applicable legislation. Any SAE considered to be related to BPZE1 will be classified as a SUSAR. The Investigator (or Sponsor where required) must report these events to the appropriate IEC that approved the protocol unless otherwise required and documented by the IEC.

12.3. Classification of Adverse Events

12.3.1.1. Assessment of Severity

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

- Mild (Grade 1): these events require minimal or no treatment and do not interfere with the subject’s daily activities

- Moderate (Grade 2): these events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with normal functioning
- Severe (Grade 3): these events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating

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

Laboratory assessments and vital signs will be classified according to the FDA severity scales see Appendix 3: Table for Laboratory Grading and Appendix 4: Table for Vital Signs Grading.

12.3.1.2. Assessment of Causality

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

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

- Related to vaccination: 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 to vaccination: there is not a reasonable possibility that the administration of the study vaccination caused the event
- Related to challenge agent: there is a reasonable possibility that the challenge agent caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study vaccination and the AE
- Not related to challenge agent: there is not a reasonable possibility that the administration of the challenge agent caused the event

12.4. Pregnancy

Pregnancy is not regarded as an AE unless there is a suspicion that an investigational vaccine may have interfered with the effectiveness of a contraceptive medication. Any pregnancy that occurs during study participation must be reported using a clinical study pregnancy form. To ensure subject safety, each pregnancy must be reported to the Sponsor within 2 weeks of learning of its occurrence. If pregnancy occurs, neither further vaccination nor challenge will occur. The pregnancy must be followed-up to determine

outcome (including spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) and status of mother and child, even if the subject is discontinued from the study. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous miscarriages must be reported as an SAE.

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

13. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Further information on the data analysis and statistical considerations is provided in the SAP.

13.1. Study Design Considerations

13.1.1. Sample Size Assumptions

Table 3 depicts the power for the challenge study as computed under the following assumptions: two-sided significance level of 0.05, use of a likelihood ratio test for testing equality of two proportions, a sample size of 20 in each of the two groups, a colonisation rate of 60%, 70%, and 80% in the unvaccinated group and colonisation rates of 10%, 20%, 30%, and 40% in the vaccinated group.

Group sample sizes of 20 evaluable subjects in each group achieve a power of at least 90% to detect differences between colonisation rates of at least 50% in six scenarios. These scenarios include the following colonisation rates for the placebo and BPZE1 groups, respectively: 60% versus 10%, 70% versus 10%, 70% versus 20%, 80% versus 10%, 80% versus 20% and 80% versus 30%, which are bolded in Table 3.

Table 3 Power for Sample Sizes of 20 in Each Group for Varying Colonisation Rates Based on a Likelihood Ratio Test

Colonisation Rate			
Placebo	**BPZE1**	**N/group**	**Power**
60%	**10%**	20	94%
	20%		75%
	30%		48%
	40%		24%
70%	**10%**	20	99%
	20%		92%
	30%		73%
	40%		48%
80%	**10%**	20	100%
	20%		99%
	30%		92%
	40%		75%

Table 4 includes results based on vaccine efficacy computed from the colonisation rates in each population, rather than comparing the two colonisation rates and testing the null hypothesis that they are equal in the two populations. The computations presented in the table below are based on a normal approximation. The table includes the required sample size for a power of 80% and for lower bounds of vaccine efficacy of 0%, 10%, 20%, and 30%. For a colonisation rate of 70% in the placebo group and a lower bound of efficacy of 20%, the study will be powered for efficacy rates of 71% or higher. Focusing on an efficacy rate greater than 50% and a lower bound of 0% (sample sizes in **bold**), the study is well powered to detect efficacy rates of 57% or higher.

Table 4 Estimated Sample Size Based on Assumed Colonisation rates and Power of 80% and Lower Bounds of 0%, 10%, 20% and 30%

Colonisation rate		Sample size/group				
Control	Vaccine	Efficacy	LB 0%	LB 10%	LB 20%	LB 30%
60%	10%	0.833	**11**	**12**	**14**	**18**
	20%	0.667	**18**	22	29	42
	30%	0.500	33	47	75	149
	40%	0.333	77	141	390	5675
70%	10%	0.857	**8**	**9**	**10**	**12**
	20%	0.714	**12**	**14**	**18**	24
	30%	0.571	**19**	25	36	60
	40%	0.429	33	51	95	268
80%	10%	0.875	**6**	**6**	**7**	**9**
	20%	0.750	**8**	**9**	**12**	**16**
	30%	0.625	**12**	**15**	**20**	31
	40%	0.500	**18**	25	40	82

For the endpoint of absolute counts, the standard deviation in the BPZE1 group was computed to be 2.2 for the natural log of the counts and 4.2 for the natural log of the counts in the control group. Assuming a sample size of 20 evaluable subjects in each group, a two-sample t-test accounting for unequal variances, and a two-sided significance level of 0.05,the study has approximately 80% power to detect differences of the order of 3.1 units between the BPZE1 group and the control group on the natural log scale.

13.1.2. Sample Size Re-estimation

Sample size re-estimation will not be performed in this study.

13.1.3. Study Stopping Criteria

There are no study-specific stopping criteria.

13.2. Data Analysis Considerations

13.2.1. Analysis Populations

The ‘Intention-to-treat’ and ‘Safety’ Populations will both be defined as all subjects randomised to treatment who are vaccinated.

The ‘modified intent-to-treat’ (mITT) analysis population will be defined as all subjects randomized to treatment who are vaccinated, challenged, and have at least one culture result at Challenge Day 9, 11, or 14. Subjects will be classified according to the randomized treatment group.

The ‘Per-Protocol’ Population is defined as all subjects who are included in mITT population and completed the study with no major protocol deviations.

13.2.2. Treatment Comparisons

Comparisons will be made between the two treatment arms: BPZE1 and placebo.

13.2.3. Interim Analysis

No interim analysis will be performed.

13.2.4. Key Elements of Analysis Plan

Statistical analysis will be performed using SAS software Version 9.4 or later.

13.2.4.1. Efficacy Analyses

Statistical analysis will be performed using SAS software Version 9.4 or later. Continuous variables will be summarized using the mean, standard deviation, median, first quartile, third quartile, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages, as well as a 2-sided 95% confidence interval (CI) for proportions computed using the Agresti-Coull method. All statistical tests will be 2-sided at 0.05 significance level.

For the primary analysis of the between treatment groups difference in the proportion of subjects colonized on any day (Challenge Day 9, 11 or 14) following challenge, a likelihood ratio test and the corresponding 95% confidence interval will be computed with colonization defined as a positive result on any of the 3 days. Primary estimand is described in the SAP. Additional summaries for each of the days will be presented including the results of the likelihood ratio test and associated confidence intervals.

Immunogenicity endpoints (GMC/GMT, GMFR, and/or seroconversion) will be summarized at baseline and each time point by treatment groups. Reverse cumulative distribution curves, which are also known as survival curves, will be presented for each measure. Comparison of the BPZE1 and control groups will use a two-sample t-test. Confidence intervals will be back-transformed from the log scale to the original scale.

Exploratory analyses include analyses to assess whether colonization status has a relationship to pre-challenge mucosal immunity levels (by individual or by combination of anti-pertussis antibodies) or SBA activity. Analyses will include the GMCs/GMTs with 95% CIs and GMFRs with 95% CIs and p-values testing as per the SAP. Pearson’s

and Spearman correlations and repeated measures analyses will be conducted to assess for correlations and for SBA and protection against colonization. For immunogenicity analysis, it is assumed that the natural log of the data is normally distributed. All statistical tests will be 2-sided at 0.05 significance level and nominal p-values will be reported.

The SAP will provide additional details of all analyses.

13.2.4.2. Safety Analyses

All safety analyses will be performed using the safety population. Safety and tolerability will be assessed by clinical review of all safety parameters including AEs, laboratory values, and vital signs. The safety analyses will include results collected from randomization through Day 28 post-challenge as well as EOS. All safety presentations will be presented by treatment group.

13.2.4.2.1. Adverse Events

The verbatim terms used in the eCRF by Investigators to identify AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.1 or above. All reported TEAEs will be included in the analysis. For each AE, the percentage of subjects who experience at least one occurrence of the given event will be summarised by treatment group. Summaries of any AESI will also be included as part of the safety analysis.

Summaries, listings, datasets, or subject narratives may be provided, as appropriate, for those subjects who die, who discontinue treatment due to an AE or who experience an AESI or a serious AE.

13.2.4.2.2. Concomitant Medication

The verbatim terms used in the eCRF by the Investigators to identify concomitant medication will be coded using the WHO Drug Dictionary. A listing of the medications taken will be provided.

13.2.4.2.3. Clinical Laboratory Tests

Laboratory data will be summarised by type of laboratory test. Reference ranges and markedly abnormal results (specified in the SAP) will be used in the summary of laboratory data. Descriptive statistics will be calculated for each laboratory parameter at baseline and at each scheduled time point and boxplots will be created. Changes from baseline results will be presented in pre- versus post-treatment cross-tabulations (with classes for below, within, and above normal ranges). A listing of subjects with any laboratory results outside the reference ranges will be provided. A listing of subjects with values meeting FDA toxicity criteria (Appendix 3: Table for Laboratory Grading) will be summarised.

13.2.4.2.4. Vital Signs

Descriptive statistics of temperature, pulse rate, blood pressure (systolic and diastolic) values, and changes from baseline will be summarised at each scheduled time point. The percentage of subjects with values meeting FDA toxicity criteria (Appendix 4: Table for Vital Signs Grading) will be summarised.

13.2.5. Missing, Unused and Spurious Data

Missing data will be accounted for in all summaries by time, with reasons provided where possible (e.g., discontinuation). If any data are excluded from analyses the reason for their exclusion will be documented in the clinical study report (CSR).

13.2.6. Reporting Deviations from the Statistical Plan

Any deviations from the planned analyses will be described and justified in the final CSR.

14. STUDY ADMINISTRATION

14.1. Independent Data Safety Monitoring Board

Safety oversight will be conducted by an independent DSMB. The DSMB members will be separate and independent of study personnel participating in this study and should not have scientific, financial, or other conflict of interest related to this study. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this study.

The DSMB will convene prior to study initiation, if a pause is triggered, or upon the request of the medical monitor and/or sponsor. The medical monitor and sponsor are empowered to request a review by the DSMB for any safety reason.

The DSMB will operate under the rules of a sponsor-approved charter that will be approved at the organizational meeting of the DSMB. Procedures for DSMB reviews/meetings will be defined in the charter. The DSMB will review applicable data to include, but not limited to subject's clinical course or safety results (e.g., AEs, reactogenicity). Reports may include enrolment and demographic information, medical history, concomitant medications, physical assessments, and solicited and unsolicited AE/SAEs. Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by the sponsor. The DSMB may receive data in aggregate and presented by treatment group.

The DSMB will review grouped and unblinded data in the closed session only. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of continuing, modifying or terminating the study.

Further information on the composition, meeting schedule and responsibilities of the DSMB can be found in the DSMB Charter.

14.2. Regulatory and Ethical Considerations, Including the Informed Consent Process

Before initiation of the study site(s), the Sponsor, or their representatives, will obtain approval from the appropriate regulatory agency to conduct the study in accordance with ICH-GCP and applicable country-specific regulatory requirements.

The study will be conducted in accordance with all applicable regulatory requirements such as ICH-GCP, all applicable subject privacy requirements, and the ethical principles that are outlined in the Declaration of Helsinki 2013, including, but not limited to:

- An IEC review and approval of study protocol and any subsequent amendments and all ICFs or other information given to the subject
- Subject informed consent
- Investigator reporting requirements

The Sponsor will provide full details of the above procedures, either verbally, in writing, or both.

Written informed consent must be obtained from each subject before participation in the study. Written informed consent will be collected following a review of the volunteer information sheet by the potential subject and a discussion between the subject and the Investigator or suitably qualified designee.

The Investigator will cooperate with all regulatory inspections and will notify the Sponsor as soon as they are aware of an inspection which may involve this study. With the exception of statutory regulatory authority inspections, the Sponsor will be consulted in the event of inspection of the clinical site(s) by an outside authority before the Inspectors are permitted access to any of the study records or the study areas.

14.3. Study Monitoring

In accordance with applicable regulations, ICH-GCP, the monitoring plan and the Sponsor's and/or delegate procedures, monitors will contact the site before the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and the Sponsor's requirements. When reviewing data collection procedures, the discussion will include identification, agreement, and documentation of data items for which the CRF will serve as the source document.

The Sponsor and/or delegated monitors will visit the Investigator and study site(s) at periodic intervals (including remote monitoring activities), in addition to maintaining necessary telephone and letter contact to ensure that:

- The data are authentic, accurate and complete
- The subject's safety and rights are being protected
- The study is conducted in accordance with the currently approved protocol and any other study agreements, ICH-GCP and all applicable regulatory requirements

14.3.1. Access to Source Data

The Investigator and the head of the medical institution (where applicable) agrees to allow the monitor, Sponsor-appointed auditors, and regulatory inspectors direct access to all relevant documents.

14.3.2. Data Handling and Record Keeping

Following closure of the study, the Investigator or head of the medical institution (where applicable) must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible when needed (e.g., for a Sponsor audit or regulatory inspection) and must be available for review in conjunction with assessment of the facility, supporting systems, and relevant site staff.

Where permitted by local laws/regulations or institutional policy, some or all of the records may be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution must be exercised before such action is taken. The Investigator must ensure that all reproductions are legible and are a true and accurate copy of the original. In addition, they must meet accessibility and retrieval standards, including regeneration of a hard copy, if required. The Investigator must also ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for creating the reproductions.

The Sponsor will inform the Investigator of the time period for retaining the site records in order to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by local laws/regulations, the Sponsor SOPs and/or institutional requirements.

The Investigator must notify the Sponsor of any changes in the archival arrangements, including, but not limited to archival of records at an off-site facility or transfer of ownership of the records in the event that the Investigator is no longer associated with the site.

14.4. Data Management

For this study, subject data will be collected using an eCRF and combined with data provided from other sources in a validated data system.

Management of clinical data will be performed in accordance with the applicable Sponsor/Sponsor's representative standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data. Adverse events and concomitant medications terms will be coded using the MedDRA and the WHO Drug Dictionary, respectively.

When using electronic trial data handling and/or remote electronic trial data systems, the Sponsor or designee will:

a. Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance (i.e., validation)

b. Maintain SOPs for using these systems

c. Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail)

d. Maintain a security system that prevents unauthorised access to the data

e. Maintain a list of the individuals who are authorised to make data changes

f. Maintain adequate backup of the data

g. Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing)

Training on the use of the electronic data collection system will be provided to all relevant study site staff.

14.5. Provision of Study Results and Information to Investigators

Where required by applicable regulatory requirements, an Investigator signatory will be identified for the approval of the clinical study report. The Investigator will be provided

reasonable access to statistical tables, figures and relevant reports and will have the opportunity to review the complete study results at a mutually agreeable location.

The Sponsor will also provide the Investigator with the full summary of the study results. The Investigator is encouraged to share the summary results with the study subjects, as appropriate.

14.6. Insurance, Indemnity and Finance

The Sponsor maintains appropriate insurance coverage for clinical studies and will follow applicable local compensation laws.

The Sponsor will indemnify all Investigators participating in this study against future claims by study subjects; the terms of this will be detailed within a separate letter of indemnification. The indemnity will only apply where all study procedures have been carried out according to this protocol.

The financial aspects of the study are addressed in a separate agreement.

14.7. Publishing

The Sponsor retains ownership of all data. When the study is complete the sponsor shall arrange the development of a CSR. All proposed publications based on this study must be approved by the Sponsor.

The study will be registered in an international registry(ies) and the results will be loaded into the registry(ies) at the end of the study.

15. REFERENCES

Althouse BM, Scarpino SV. Asymptomatic transmission and the resurgence of Bordetella pertussis. BMC Med 2015; 13:1-2.

Babu MM, Bhargavi J, Saund RS, Singh SK. Virulence factors of Bordetella pertussis. Curr Sci 2001; 25:1512-22

Bart M, van der Heide H, Zeddeman, A *et al*. Complete genome sequences of 11 *Bordetella pertussis* strains representing the pandemic ptxP3 lineage. Gen Announc 2015; 3: e01394-15

Biggerstaff M, Cauchemez S, Reed C, Gambhir M, Finelli L. Estimates of the reproduction number for seasonal, pandemic, and zoonotic influenza: a systematic review of the literature. BMC Infect Dis 2014; 14:1-20.

Bolotin S, Harvill ET, Crowcroft NS. What to do about pertussis vaccines? Linking what we know about pertussis vaccine effectiveness, immunology and disease transmission to create a better vaccine. Pathog Dis 2015; 1:73

Campbell PT, McCaw JM, McIntyre P, McVernon J. Defining long-term drivers of pertussis resurgence, and optimal vaccine control strategies. Vaccine 2015; 33:5794-800.

Cauchi S, Locht C. Non-specific Effects of Live Attenuated Pertussis Vaccine Against Heterologous Infectious and Inflammatory Diseases. Front Immunol. 2018 Dec 7; 9:2872

Centers for Disease Control and Prevention. Pertussis (Whooping Cough). August 7 2017. https://www.cdc.gov/pertussis/about/diagnosis-treatment.html

Centres for Disease Control and Prevention. Antibiotic / Antimicrobal Resistance. March 2 2021. https://www.cdc.gov/drugresistance/biggest-threats.html

Craig R, Kunkel E, Crowcroft NS *et al.* Asymptomatic infection and transmission of pertussis in households: a systematic review. Clin Infect Dis 2020; 70:152-61.

Decker M, Edwards K. Pertussis (whooping cough). JID 2021;224(Supl4): S310-20

de Graaf H, Ibrahim M, Hill AR, *et al.* Controlled human infection with Bordetella pertussis induces asymptomatic, immunizing colonisation. Clin Infect Dis 2020; 71:403-11.

Feunou P, Mielcarek N, Locht C. Reciprocal interference of maternal and infant immunization in protection against pertussis. Vaccine. 2016; 34:1062–1069.

Investigator's Brochure for BPZE1. June 2021

Jahnmatz M, Richert L, Al-Tawil N, *et al*. Safety and immunogenicity of the live attenuated intranasal pertussis vaccine BPZE1: a phase 1b, double-blind, randomised, placebo-controlled dose-escalation study. Lancet Infect Dis. 2020; 20:1290-1301

Kavanagh H, Noone, C, Cahill E *et al.* Attenuated Bordetella pertussis vaccine strain BPZE1 modulates allergen-induced immunity and prevents allergic pulmonary pathology in a murine model. Clin Exp Allergy. 2010 Jun; 40:933-41

Keech C, Solovay K, Thalen M, Rubin K. A phase 2 clinical trial assessing the effect of BPZE1, a novel live attenuated pertussis vaccine in healthy adults. Presented at the World Vaccine Congress 2020a.

Keech C, Solvay K, Rubin K. Assessment of safety and immunology responses of BPZE1, an intranasal live attenuated pertussis vaccine in health adults. A phase 2 multi-center placebo-controlled randomised study. Poster presented at ESPID 2020b.

Keech C, Gorringe A, Cavelle B *et al*. Live attenuated vaccine BPZE1 but not Boostrix™ induces serum bactericidal activity (SBA) against pertactin (Prn)-negative *Bordetella pertussis*. Original title: Serum bactericidal activity induced by live attenuated pertussis vaccine BPZE1 is comparable to Boostrix™. Poster presented at IDSA for ID Week 2021 conference. Open Forum Infectious Diseases, 2021 Dec; 8 (1), S684.

Kretzschmar M, Teunis PF, Pebody RG. Incidence and reproduction numbers of pertussis: estimates from serological and social contact data in five European countries. PloS Med 2010; 7:e1000291.

Liang J, Tiwari T, Moro P. *et al*.Prevention of Pertussis, Tetanus, and Diphtheria with Vaccines in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR. 2018; 67:1–44.

Li R, Lim A, Phoon MC *et al*. Attenuated Bordetella pertussis protects against highly pathogenic influenza A viruses by dampening the cytokine storm. J Virol. 2010 Jul; 84:7105-13

Li R, Cheng C, Chong SZ, *et al*. Attenuated Bordetella pertussis BPZE1 protects against allergic airway inflammation and contact dermatitis in mouse models. Allergy. 2012 Oct; 67:1250-8.

Locht C, Antoine R. A proposed mechanism of ADP-ribosylation catalysed by the pertussis toxin S1 subunit. Biochimie 1995; 77:333-40.

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; 216:117-24.

Locht C. Molecular aspects of Bordetella pertussis pathogenesis. Int Microbiol 1999; 2:137-44.

Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. Clinical Microbiol Rev 2005; 18:326-82.

McGirr AA, Tuite AR, Fisman DN. Estimation of the underlying burden of pertussis in adolescents and adults in Southern Ontario, Canada. PloS One 2013; 8:e83850.

Mielcarek N, Debrie AS, Raze D. Live attenuated *B. pertussis* as a single-dose nasal vaccine against whooping cough. PloS pathog 2006; 2:e65.

National Institute of Allergy and Infectious Diseases. NIAID Emerging Infectious Diseases/Pathogens. July 26 2018. https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens

Package insert azithromycin revised in 2020 https://www.medicines.org.uk/emc/files/pil.1073.pdf accessed 24/Jan/22

PERISCOPE project. https://periscope-project.eu/ accessed on 27/Jun/21

Plotkin SA. The pertussis problem. Clin Infect Dis 2014; 58:830-3.

Schnoeller C, Roux X, Sawant D, *et al*. Attenuated *Bordetella pertussis* vaccine protects against respiratory syncytial virus disease via an IL-17-dependent mechanism. Am J Respir Crit Care Med. 2014 Jan 15; 189(2):194-202

Schwartz KL, Kwong JC, Deeks SL. Effectiveness of pertussis vaccination and duration of immunity. CMAJ 2016; 188:E399-406.

Teruya S, Hiramatsu Y, Nakamura K. Bordetella dermonecrotic toxin is a neurotropic virulence factor that uses CaV3. 1 as the cell surface receptor. Mbio 2020; 11:e03146-19.

Thorstensson R, Trollfors B, Al-Tawil N, *et al.* A phase I clinical study of a live attenuated Bordetella pertussis vaccine—BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. PloS One. 2014 Jan 8; 9(1):e83449

UK Standard for Microbiology Investigations. Investigations of whooping cough. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/372648/B_6i8.pdf

Van Boven M, Teirlinck AC, Meijer A. Estimating transmission parameters for respiratory syncytial virus and predicting the impact of maternal and pediatric vaccination. J Infect Dis 2020; 222:S688-94.

Vink MA, Bootsma MC, Wallinga J. Serial intervals of respiratory infectious diseases: a systematic review and analysis. Am Journal Epidemiol 2014; 180:865-75.

Warfel JM, Beren J, Kelly VK, Lee G, Merkel TJ. Nonhuman primate model of pertussis. Infect Immun. 2012;80: 1530-6.

Warfel JM, Beren J, Merkel TJ. Airborne transmission of Bordetella pertussis. J Infect Dis 2012; 206:902-6.

Warfel JM, Edwards KM. Pertussis vaccines and the challenge of inducing durable immunity. Curr Opin Immunol 2015; 35:48-54.

Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. PNAS 2014; 111:787-92.

Witt MA, Arias L, Katz PH, Truong ET, Witt DJ. Reduced risk of pertussis among persons ever vaccinated with whole cell pertussis vaccine compared to recipients of acellular pertussis vaccines in a large US cohort. Clin Infect Dis 2013; 56(9):1248-54.

World Health Organisation. Pertussis vaccines: WHO Position paper – August 2015. WHO Weekly epidemiological record. 2015; 90:433–460.

Zerbo O, Bartlett J, Goddard K, Fireman B, Lewis E, Klein NP. Acellular pertussis vaccine effectiveness over time. Pediatrics 2019; 144.

Modified based on Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (fda.gov). Accessed 26 April 2022.Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases, National Institutes of Health, US Department of Health and Human Services. Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events. July 2017 [cited26 April 2022]. Available from: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (fda.gov). Accessed April 26, 2022.

16. APPENDICES PROVIDED FOR STUDY IB-202P

16.1. Appendix 1: Schedule of Events

		Vaccination Phase				Challenge Phase							Safety Follow-up	
Study visit	**Screening**	**1**	**2**	**3**	**4/C-1[a]**	**C0**	**C1-6[b]**	**C7**	**C8, C10, C12 & C13[b]**	**C9, C11 & C14[b]**	**C15**	**C16**	**5**	**6**
Days Relative to Vaccination[c]	**-30 to -7**	**0**	**7**	**28**	**52**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**180/EOS[q]**
Days Relative to Challenge					**-7[r]**	**0**	**1-6**	**7**	**8, 10, 12, and 13**	**9, 11 and 14**	**15**	**16**	**28**	**90[q]**
Window Allowance[c]	**30**	**NA**	**+3**	**+7**	**+60**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**+7**	**+30**
VIS/Informed consent/TOPS registration	X													
Inclusion/exclusion criteria	X	X				X								
Infection control agreement	X					X								
Demographic and baseline data	X													
Medical history	X	X												
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X[d]	X	X		X[d]	X	X	X	X	X	X	X	X
Physical examination	X[e]	X	X	X		X[e]	X	X	X	X	X	X	X	X
Serology and anti-PT and anti-PRN Ab (local and central lab)	X													
Safety laboratory samples	X				X			X		X (C9 and C14 only)				
Respiratory PCR panel						X[p]								
Urine toxicity screen	X				X									
Serum pregnancy test (FCBP only)	X													

		Vaccination Phase				Challenge Phase							Safety Follow-up	
Study visit	**Screening**	**1**	**2**	**3**	**4/C-1[a]**	**C0**	**C1-6[b]**	**C7**	**C8, C10, C12 & C13[b]**	**C9, C11 & C14[b]**	**C15**	**C16**	**5**	**6**
Days Relative to Vaccination[c]	**-30 to -7**	**0**	**7**	**28**	**52**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**180/EOS[q]**
Days Relative to Challenge					**-7[r]**	**0**	**1-6**	**7**	**8, 10, 12, and 13**	**9, 11 and 14**	**15**	**16**	**28**	**90[q]**
Window Allowance[c]	**30**	**NA**	**+3**	**+7**	**+60**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**+7**	**+30**
Urine pregnancy test (FCBP only)		X				X								
12-lead electrocardiogram	X													
SARS-CoV-2 test for active infection[f]		X				X								
Randomisation		X												
Vaccination		X												
Challenge						X								
Subject diary dispensing[g]		X												
Reactogenicity/Solicited AEs[g]		X[g,h]	X											
Post-challenge checklist						X	X	X	X	X	X	X		
Subject diary or checklist reviewed[i]			X			X	X	X	X	X	X	X		
Azithromycin										X (C14 only)[j]	X	X[j]		
Nasal wash for *B. pertussis* colonisation by culture ± PCR					X					X		X[k]	X	
Nasopharyngeal swab(s) for cell-mediated immunity	X		X	X	X					X (C9 only)			X	
Nasal mucosal secretion sample (SAM) for immunogenicity test	X			X	X								X	X

		Vaccination Phase				Challenge Phase							Safety Follow-up	
Study visit	**Screening**	**1**	**2**	**3**	**4/C-1[a]**	**C0**	**C1-6[b]**	**C7**	**C8, C10, C12 & C13[b]**	**C9, C11 & C14[b]**	**C15**	**C16**	**5**	**6**
Days Relative to Vaccination[c]	**-30 to -7**	**0**	**7**	**28**	**52**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**180/EOS[q]**
Days Relative to Challenge					**-7[r]**	**0**	**1-6**	**7**	**8, 10, 12, and 13**	**9, 11 and 14**	**15**	**16**	**28**	**90[q]**
Window Allowance[c]	**30**	**NA**	**+3**	**+7**	**+60**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**+7**	**+30**
Serum for immunogenicity (including ELISA and SBA assays)		X[l]		X		X[l]							X	X
Blood sampling for cellular-mediated immunity		X[l]	X	X		X[l]		X					X	
All unsolicited AEs[m]		X	X	X		X	X	X	X	X	X	X	X	
TEAEs related to vaccination[n]		X	X	X	X									
TEAE related to challenge[n]						X	X	X	X	X	X	X	X	X
AESIs and SAEs[o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X

AE=adverse event; AESI=adverse event of special interest; ELISA=enzyme-linked immunosorbent assay; EOS=end of study; FCBP=female(s) of childbearing potential; PCR=polymerase chain reaction; SAE=serious adverse event; SBA=serum bactericidal assay; TEAE=treatment emergent adverse event

a. Initiate screening for eligibility for the challenge phase; admit to challenge unit on Study visit C0
b. Each day has the same assessments, unless specified
c. Days relative to vaccination are only estimates as the window allowances are not inclusive. Subjects will remain in the study until either 6 months after vaccination or 3 months after challenge, whichever is longer. If a study pause occurs, the visits/windows will be adjusted to allow for subjects to continue without protocol deviation.
d. Pre-vaccination and approximately 30 minutes following vaccination and challenge; additional vital sign measurements to be taken per challenge unit as needed
e. Full physical examination including height and weight at Screening and Study Visit C0. Symptom-directed (targeted) physical examination at all other scheduled time points. Interim physical examinations will be performed at the discretion of the Investigator, if necessary
f. 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 negative test results within 72 hours prior to vaccination and admission to the challenge unit. Repeat testing is allowed on multiple occasions, if needed

g. All subjects will record maximum daily reactogenicity/solicited AEs in the subject diary for the subsequent 7 days after vaccination. Subjects will be instructed that should they have reactogenicity with a potential toxicity grade 3 at any time after vaccination, they should contact the site on the same day and be seen or referred to a qualified medical facility
h. Immediate reactogenicity will be assessed 30 minutes after the study vaccination, prior to release from clinical observation
i. Site staff reviews the information from the subject diary/checklist with the subjects to confirm accuracy. For reactogenicity and post-challenge checklist, the appropriate FDA toxicity grading should be applied. If any reactogenicity event extended beyond 7 days post-vaccination and is deemed clinically significant by AE classification, then it should be entered as an AE (graded by AE criteria) with the same start date as the reactogenicity event and followed to resolution. All AEs identified by the post-challenge checklist should be entered as an AE
j. Prior to Study visit C14, azithromycin may be initiated if subjects have symptoms consistent with pertussis, although assessments listed for C14 should be carried out prior to starting azithromycin. On Study visit C14, all subjects who have not yet started azithromycin should begin a 3-day course of azithromycin 500 mg per day after all study samples have been collected; the third dose of azithromycin must be taken prior to the subject exiting the challenge unit on Study visit C16
k. Subjects with positive culture or PCR sampled on C16 will be required to return to the site to receive additional antibiotic treatment
l. Collect prior to vaccination/challenge
m. All unsolicited AEs to be collected from time of vaccination to 28 days after vaccination and from time of challenge to 28 days after challenge
n. Treatment-related AEs due to vaccination and due to challenge to be collected from time of vaccination to challenge and then for 3 months after challenge, respectively
o. All AESIs and SAEs to be collected from time of signed informed consent to EOS
p. Maybe performed at the discretion of the Investigator throughout the challenge phase
q. This visit will occur at least 6 months post-vaccination or at least 3 months post-challenge
r. Visit may occur up to 8 days before C0 or as close to C0 as possible to allow availability of test results.

16.2. Appendix 2: Table for Reactogenicity Grading

Nasal/Respiratory/Systemic Reactogenicity Grading			
Reaction	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Runny nose	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort/prevents daily activity or seeks medical care
Stuffy nose/congestion	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with breathing from nose	Unable to breathe through nose or seeks medical care
Nasal pain/irritation	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort that prevents daily activity or seeks medical care
Epistaxis	Total duration of all episodes in a 24-hour period <30 minutes	Total duration of all episodes in a 24-hour period >30 minutes	Any bleeding that required visit for medical encounter
Sneezing	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort; prevents daily activity
Sinus pressure/pain	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort that prevents daily activity or seeks medical care
Sore/irritated throat	Noticeable but does not interfere with eating or drinking	Moderate discomfort that interferes with eating or drinking	Significant discomfort that prevents eating or drinking or seeks medical care
Cough	Noticeable but does not interfere with daily activity or sleeping	Frequent cough that interferes with daily activity or sleeping	Prevents daily activity, prevents sleep, or seeks medical care
Shortness of breath/wheezing	Noticeable but does not interfere with daily activity	Moderate discomfort that interferes with daily activity	Significant discomfort/ prevents daily activity or seeks medical encounter
Fever	100.4–101.1°F (38.0–38.4°C)	101.2–102.0°F (38.5–38.9°C)	>102°F (≥39.0°C)
Fatigue (tiredness)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise (general unwell feeling)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

Nasal/Respiratory/Systemic Reactogenicity Grading			
Reaction	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Myalgia (body aches/muscular pain)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (joint pain)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Rash/hypersensitivity	Pruritus OR local rash	Diffuse rash	Diffuse rash with blisters or mouth ulcerations, anaphylaxis, or angioedema

Source: Modified based on Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (fda.gov). Accessed 26 April 2022.Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases, National Institutes of Health, US Department of Health and Human Services. Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events. July 2017 [cited26 April 2022]. Available from: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

16.3. Appendix 3: Table for Laboratory Grading

Serum[a]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**[b]
Glucose – hyperglycemia Random (mg/dL; mmol/L)	110–125 (6.1–<7.0)	126–200 (7.0–11.1)	>200 (>11.1)	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen (mg/dL; mmol/L)	23–26 (8.2–9.5)	27–31 (9.6–11.1)	>31 (>11.1)	Requires dialysis
Creatinine (mg/dL; umol/L)	1.5–1.7 (133–154)	1.8–2.0 (155–180)	2.1–2.5 (181–225)	>2.5 (>225) or requires dialysis
Albumin – hypoalbuminemia (g/dL; g/L)	2.8–3.1 (28–31)	2.5–2.7 (25–27)	<2.5 (<25)	–
Total Protein – hypoproteinemia (g/dL; g/L)	5.5–6.0 (55–60)	5.0–5.4 (50–54)	<5.0 (<50)	–
Liver Function Tests – ALT, AST increase by factor	1.1–2.5 x ULN	2.6–5.0 x ULN	5.1–10 x ULN	>10 x ULN
Bilirubin – when accompanied by any increase in liver function test; increase by factor	1.1–1.25 x ULN	1.26–1.5 x ULN	1.51–1.75 x ULN	> 1.75 x ULN
Bilirubin – when liver function test is normal; increase by factor	1.1–1.5 x ULN	1.6–2.0 x ULN	2.0-3.0 x ULN	> 3.0 x ULN
Haematology[a]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**[b]
Haemoglobin (Female) (g/dL; g/L)	11.0–12.0 (110–120)	9.5–10.9 (95–109)	8.0–9.4 (80–94)	<8.0 (<80)
Haemoglobin (Male) (g/dL; g/L)	12.5–13.5 (125–135)	10.5–12.4 (105–124)	8.5–10.4 (85–104)	<8.5 (<85)
WBC elevation (cell/mm^3; $x10^9$/L)	10,800–15,000 (10.8–15)	15,001–20,000 (>15–20)	20,001–25,000 (>20–25)	>25,000 (>25)
WBC suppression (cell/mm^3; $x10^9$/L)	2500–3500 (2.5–3.5)	1500–2499 (1.5–<2.5)	1000–1499 (1.0–<1.5)	<1,000 (<1.0)
Platelets Decreased (cell/mm^3; $x10^9$/L)	125,000–140,000 (125–140)	100,000–124,000 (100–124)	25,000–99,000 (25–99)	<25,000 (<25)

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

a. The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate
b. The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125–129 mEq/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value

Note: Screening laboratory samples with toxicity scores greater than 1 are exclusion criteria for vaccination enrolment. Laboratory sampling while in the challenge unit will comply with challenge unit standard protocol with the proviso that concurrent evidence of illness requires a daily complete blood count with differential to be conducted.

Source: Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (fda.gov). Accessed April 26, 2022.

16.4. Appendix 4: Table for Vital Signs Grading

Vital signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life threatening (Grade 4)
Fever (°C)	38.0–38.4	38.5–38.0	>39.0	>40.0
Tachycardia (bpm)	101–115	116–130	>130	Emergency room visit or hospitalised for arrhythmia
Bradycardia (bpm)	50–54	45–49	<45	Emergency room visit or hospitalised for arrhythmia
Hypertension (systolic) mmHg	141–150	151–155	>155	Emergency room visit or hospitalised for malignant hypertension
Hypertension (diastolic) mmHg	91–95	96–100	>100	Emergency room visit or hospitalised for malignant hypertension
Hypotension (systolic) mmHg	85–89	80–84	<80	Emergency room visit or hospitalised for hypotensive shock

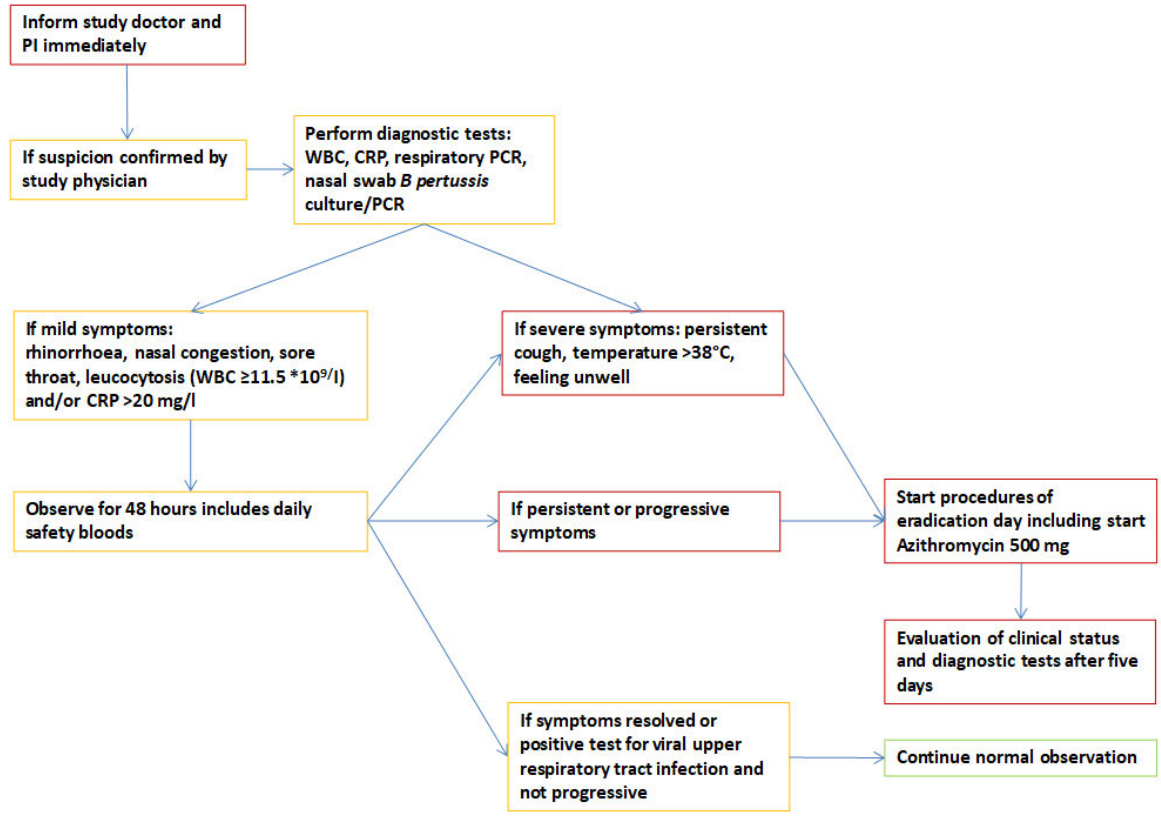
Bpm=beats per minute

a. Subject should be at rest for all vital sign measurements
b. Oral temperature; no recent hot or cold beverages or smoking
c. When resting heart rate is between 60 to 100 bpm. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes

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

Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (fda.gov). Accessed April 26, 2022.

16.5. Appendix 5: Process to be followed in cases of suspected symptomatic *Bordetella pertussis* infection



CRP: C-reactive protein; PCR: Polymerase chain reaction; PI: Principal Investigator; WBC : White blood cell