CONFIDENTIAL

Santé publique Inserm

Institut national
de la santé et de la recherche médicale

Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers

CLINICAL STUDY PROTOCOL

EudraCT Number:	2015-001287-20 NCT#: 02453048
Study Identification Number:	C14-80
Short name:	BPZE1 Phase Ib
Phase:	Ib
Principal Clinical Investigator:	Nabil Al-Tawil, MD, PhD, Consultant physician Karolinska Trial Alliance, Phase I Unit Karolinska University Hospital SE-141 86 Stockholm, Sweden Phone: +46 8 58 58 58 84 Fax: +46 8 58 58 69 20 Email: nabil.al-tawil@karolinska.se
Medical Advisor and Safety Officer on behalf of Public Health Agency of Sweden	Jann Storsaeter, MD, PhD, Senior physician Public Health Agency of Sweden SE-171 82 Solna, Sweden Phone: +46 73 382 74 16 Email:jann.storsaeter@fhi.no ; jann.storsaeter@folkhalsomyndigheten.se
Principal Laboratory Investigator/ Swedish 'sponsor correspondent' with Swedish authorities	Rigmor Thorstensson, PhD, Assoc. Prof, Senior advisor Public Health Agency of Sweden SE-171 82 Solna, Sweden Phone: +46 70 256 26 70 Fax: +46 8 31 83 34 Email : rigmor.thorstensson@folkhalsomyndigheten.se
Sponsor:	Inserm - Biopark, Bâtiment A, Institut Santé Publique - Pôle Recherche Clinique (PRC) 8 rue de la Croix Jarry, 75013 Paris Phone:+33 1 44 23 60 41 Fax: +33 1 44 23 61 26
Scientific responsible INSERM:	Camille Locht, PhD, professor Inserm – Institut Pasteur de Lille 1, rue du Prof. Calmette F-59019 Lille Cedex, France Phone: +33 3 20 87 11 51; Fax: +33 3 20 87 11 58; Email: camille.locht@pasteur-lille.fr
Protocol Version:	**2.0**
Protocol Date:	**2015-09-01**

Table of contents

I. General information

I.1. CONTACT LIST

Sponsor, Investigators and Study Centre

Sponsor:
Inserm
Representative : Sonia Guegen, Deputy Director
Institut Santé Publique – Pôle Recherche Clinique
8 rue de la Croix Jarry
F-75013 Paris, France
Phone: +33 1 44 23 61 05
Fax: +33 1 44 23 67 10
Email: sonia.gueguen@inserm.fr

Principal Clinical Investigator
Nabil Al-Tawil, MD, MSc, PhD, Consultant physician
Karolinska Trial Alliance, Phase I Unit
Karolinska University Hospital
SE-141 86 Stockholm, Sweden
Phone: +46 8 58 58 58 84
Fax: +46 8 5858 69 20
Email: nabil.al-tawil@karolinska.se

Medical Advisor and Safety Officer on behalf of the Public Health Agency of Sweden
Jann Storsaeter, MD, PhD, Senior physician
Public Health Agency of Sweden
SE-171 82 Solna, Sweden
Phone: +46 73 382 74 16
Email : jann.storsaeter@fhi.no; jann.storsaeter@folkhalsomyndigheten.se

Principal Laboratory Investigator/Swedish 'sponsor's correspondent' with the Swedish authorities
Rigmor Thorstensson, PhD, Assoc. Prof, Senior advisor
Public Health Agency of Sweden
SE-171 82 Solna, Sweden
Phone: +46 70 256 26 70
Fax: +46 8 31 83 34
Email: rigmor.thorstensson@folkhalsomyndigheten.se

Legal partner with the Swedish authorities
Johan Carlson, M.D. PhD, Director General
Public Health Agency of Sweden
SE-171 82 Solna, Sweden
Phone: +46 10-205 20 00
Email: johan.carlson@folkhalsomyndigheten.se

Study Centre
Karolinska Trial Alliance, Phase I Unit
Karolinska University Hospital,
SE-141 86 Stockholm, Sweden

Scientific Responsible INSERM

Camille Locht, PhD, Professor
INSERM – Institut Pasteur de Lille
1, rue du Prof. Calmette
F-59019 Lille Cedex, France
Phone: +33 3 20 87 11 51;
Fax: +33 3 20 87 11 58;
Email: camille.locht@pasteur-lille.fr

Responsible for monitoring

Karolinska Trial Alliance, Support Unit
Karolinska University Hospital,
Norra Stationsgatan 67
SE-171 76 Stockholm, Sweden

Responsible for pharmacovigilance

Alpha Diallo, Chargé de Pharmacovigilance
ANRS
101 rue de Tolbiac
F-75013 Paris, France
Phone: +33 1 53 94 60 40
Email: pharmacovigilance@anrs.fr

Responsible for clinical trial methodology, data management and statistics

EUCLID Clinical Trials Platform, CIC 1401 (Pr. Geneviève Chêne, Dr Laura Richert)
Bordeaux University Hospital –Inserm - University of Bordeaux
ISPED
146 rue Léo Saignal
F-33076 Bordeaux cedex, France

I.2. GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
AEFI	Adverse Event Following Immunization
AR	Adverse Reaction
ANRS	Agence Nationale de Recherche sur le Sida
Bpm	Beats per minute
CIOMS	Council of International Organisation of Medicinal Sciences
CFU	Colony Forming Units
CRA	Clinical Research Associate
CRF	Case Report Form
DNT	Dermonecrotic Toxin
DSMB	Data Safety Monitoring Board
ECG	Electrocardiography
EMA	European Medicines Agency
FHA	Filamentous Hemagglutinin
FVFS	First Visit First Subject
FVLS	First Visit Last Subject
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
INSERM	"French Health and Medical Research National Institute"
IU/ml	International Unit per millilitre
LVLS	Last Visit Last Subject
ml	Milliliter
PBMC	Peripheral Blood Mononuclear Cells
PRN	Pertactin
PT	Pertussis Toxin
RBC	Red Blood Cells
RT	Room Temperature
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standardised Operational Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCT	Tracheal Cytotoxin
WBC	White Blood Cells

I.3. SYNOPSIS

Study title	Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers
Study Identification Number:	C14-80
Sponsor	INSERM

Investigational product	Live attenuated *Bordetella pertussis* BPZE1 strain or placebo
Active ingredient	Live attenuated *Bordetella pertussis* BPZE1 bacteria
Phase	Ib
Medical Advisor and Safety Officer on behalf of Public Health Agency of Sweden	Jann Storsaeter, MD, PhD, Senior physician 171 82 Solna Sweden Phone: +46 73 382 74 16 Email: jann.storsaeter@fhi.no; jann.storsaeter@folkhalsomyndigheten.se
Planned study period	First visit of the first subject: August 2015 First visit of the last subject: February 2016 Last visit of the last subject: September 2016
Objectives	**Primary objective** To assess the general safety and tolerability in the respiratory tract including specific adverse events such as cough/spasmodic cough, during the 4- 28 days after vaccination by a single ascending dose of the genetically modified *B. pertussis* strain in healthy adult volunteers. **Secondary objectives** To assess after vaccination by a single ascending dose of the genetically modified *B. pertussis* strain in healthy adult volunteers: 1. the safety and tolerability of the vaccine over 6 months post-vaccination 2. the frequency of colonization of the human respiratory tract by live attenuated *B. pertussis* strain and its magnitude (in terms of colony forming units) over 28-days post-vaccination. 3. the levels of serum IgG and IgA antibodies to 4 different *B. pertussis* antigens: pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3 over 6 months after vaccination. **Exploratory objectives** To assess the B and T cell immune responses to 4 different *B. pertussis* antigens (pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3) over 6 months after vaccination by : 1. the levels of IgG and IgA in nasopharyngeal aspirate. 2. the levels of cytokines after stimulation of PBMC with *B. pertussis* antigens and unrelated antigens. 3. the cytokine levels in nasopharyngeal aspirate.
Sample size	Total to be included: 54 (3 groups of different escalating doses – 12 active and 4 placebo per group + one group of 6)

Study title	Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers
Study Identification Number:	C14-80
Sponsor	INSERM

Study plan	A phase Ib dose-escalating study with consecutive dose groups and a randomized placebo control group in each dose group (Groups 1 – 3). The volunteers will be recruited in a step-wise fashion with 16 individuals in each of three groups. Group 1: 12 individuals will be vaccinated once intranasally with 10^7 cfu of the organism. 4 individuals will be randomized to the placebo (the diluent alone) group. Group 2: 12 individuals will be vaccinated once intranasally with 10^8 cfu of the organism. 4 individuals will be randomized to the placebo (the diluent alone) group. Group 3: 12 individuals will be vaccinated once intranasally with 10^9 cfu of the organism. 4 individuals will be randomized to the placebo (the diluent alone) group. Group 4: 6 individuals with high anti-PRN antibody levels will be vaccinated once intranasally with 10^9 cfu of the organism (open-label). For Groups 1 - 3, the administration of vaccine or diluent alone will be performed in a double-blind fashion with administration of coded vials. Group 4 is a non-blinded, open-label subgroup with the unique aim of collecting exploratory data on colonization and immune responses in subjects with pre-existing high PRN antibody levels. <u>Visit 1 (screening 1-6 weeks before vaccination)</u> The subjects will be informed and will provide written informed consent to participate in the study. Full physical examination, ECG, and vital signs. Blood samples for analysis of cell blood counts (haemoglobin, total and differential WBC, red blood cells (RBC), platelets), blood chemistry (potassium, calcium, sodium, creatinin, albumin, serum bilirubin, alkaline phosphatases, alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), glutamyltransferase (GT), high sensitive C-reactive protein, blood glucose, thyroid stimulating hormone (TSH), human chorionic gonadotropin (hCG) (females only), and antibodies against PT and PRN. Blood samples for analysis for infection with HIV, hepatitis B and C. Urine samples for dipstick analysis of pH, erythrocytes, leucocytes, protein, glucose, ketones and bacteria (nitrite) and the drugs cocaine, amphetamine, cannabis, morphine, benzodiazepines, and methylenedioxymethamphetamine. <u>Visit 2 (day 0, the day of vaccination)</u> Review inclusion/exclusion criteria. Limited physical examination and measure of vital signs. Blood cell counts (haemoglobin, total and differential WBC, RBC, platelets). Blood samples for immunological assays. Urine sample (females only) for pregnancy testing. Vaccinate subjects. The volunteer will stay at the study centre for 6 hours after administration of the vaccine. Information provided to the volunteer on how to fill in the Diary daily concerning adverse events. <u>Visit 3 (4±1 days after vaccination)</u>. Limited physical examination and measure of vital signs. Structured questionnaire concerning general and adverse events in the respiratory tract and a question about unsolicited adverse events. Nasopharyngeal aspiration for BPZE1 culture plating. Blood (in the high dose group 3 only) and nasopharyngeal samples for immunological assays. <u>Visit 4 (7±1 days after vaccination).</u> Limited physical examination and measure of vital signs. Structured questionnaire concerning general and adverse events in the respiratory tract and a question about unsolicited adverse events. Blood cell counts (haemoglobin, total and differential WBC, RBC, platelets). Nasopharyngeal aspiration for BPZE1 culture plating.

Study title	Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers
Study Identification Number:	C14-80
Sponsor	INSERM

	Blood and nasopharyngeal samples for immunological assays. Visit 5 (11±1 days after vaccination). Limited physical examination and measure of vital signs. Structured questionnaire concerning general and adverse events in the respiratory tract and a question about unsolicited adverse events. Nasopharyngeal aspiration for BPZE1 culture plating. Visit 6 (14±1 days after vaccination). Limited physical examination and measure of vital signs. Structured questionnaire concerning general and adverse events in the respiratory tract and a question about unsolicited adverse events. Blood cell counts (haemoglobin, total and differential WBC, RBC, platelets). Nasopharyngeal aspiration for BPZE1 culture plating. Blood and nasopharyngeal samples for immunological assays. Visit 7 (21 ±1 days after vaccination). Limited physical examination and measure of vital signs. Structured questionnaire concerning general and adverse events in the respiratory tract and a question about unsolicited adverse events. Nasopharyngeal aspiration for BPZE1 culture plating. Blood and nasopharyngeal samples for immunological assays. Visit 8 (28 (-1; + 7) days after vaccination). Limited physical examination and measure of vital signs. Structured questionnaire concerning general and adverse events in the respiratory tract and a question about unsolicited adverse events. Subject diary collected and filed. Blood cell counts (haemoglobin, total and differential WBC, RBC, platelets). Females only, human chorionic gonadotropin (hCG). Nasopharyngeal aspiration for BPZE1 culture plating; If positive culture for BPZE1 after 4 weeks a new nasopharyngeal sample should be collected 2-3 weeks later for culture. Blood and nasopharyngeal samples for immunological assays. Visit 8' (45 (-5; +10) days after vaccination). This visit will only be scheduled if BPZE1 was detected in the sample collected on the visit day 28. Limited physical examination and measure of vital signs. Questions concerning unsolicited adverse events. Nasopharyngeal aspiration for BPZE1 culture plating. Visit 9 (6±1 months after vaccination). Questions concerning unsolicited adverse events. Limited physical examination and measure of vital signs. Blood cell counts (haemoglobin, total and differential WBC, RBC, platelets). Blood and nasopharyngeal samples for immunological assays.
Inclusion criteria	Subject will be included in the study if he/she meets all the following criteria: 1. Healthy individual between 18 and 32 years of age, vaccinated or unvaccinated with acellular pertussis vaccine. 2. Female subject of child bearing potential must be willing to ensure to use one of the following methods of contraception from two weeks before vaccination till one month after vaccination to avoid pregnancy during the study: • Complete abstinence from penile-vaginal intercourse

Study title	Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers
Study Identification Number:	C14-80
Sponsor	INSERM

	• Double barrier method (male condom/spermicide, male condom/diaphragm diaphragm/spermicide) • Any intrauterine device (IUD) with published data showing that the expected failure rate is <1% per year • Approved hormonal contraception (except low-dose gestagen), which is not contraindicated to study vaccine administration 3. Informed consent form (ICF) signed by the subject. 4. Subject shall be able to attend all scheduled visits and to understand and comply with the study procedures.
Exclusion criteria	If any of the following criteria are met, the subject should not be included in the study: 1. Individual with PT and/or PRN serum IgG antibodies ≥20 International units/ml (IU/ml). NOTE! One control group with PRN serum IgG antibodies ≥ 20 IU/ml and PT serum IgG antibodies <20 IU/ml will be included. 2. Vaccinated with the study vaccine in the Child Innovac study (EudraCT number 2010-019936-11). 3. Pregnant or lactating women. Pregnancy not planned and to be avoided during the study by use of effective contraceptive methods. 4. Blood pressure after resting ≥ 150/90 mm Hg at screening. 5. Heart rate after resting ≥ 80 bpm at screening. 6. Respiratory rate after resting ≥ 20/minute at screening. 7. Unwillingness to refrain from the use of nicotine products from screening through day 28. 8. Use of narcotic drugs and/or a history of drug/alcohol abuse within the past 2 years prior to screening 9. The subject has donated blood or suffered from blood loss of at least 450 ml (1 unit of blood) within 60 days prior to screening or donated plasma within 14 days prior to screening. 10. Receipt of immunoglobulin, blood derived products, systemic corticosteroids or other immunosuppressant drugs within 90 days prior to day 0. 11. Asthma or other chronic respiratory problems. 12. Use of corticosteroids in the respiratory tract (e.g. nasal steroids, inhaled steroids) whitin 30 days prior to day 0. 13. Receipt of a vaccine within the last 30 days prior to day 0 or planned vaccination within the next 30 days after day 0. 14. Known hypersensitivity to any component of the study vaccine. 15. Current participation in any other clinical trial or participation (and during the whole study) in any clinical trial in the previous 3 months prior to day 0. 16. Inability to adhere to the protocol, including plans to move from the area. 17. Family (first degree) history of congenital or hereditary immunodeficiency. 18. Past or present infection with HIV, hepatitis B or C. 19. Chronic conditions requiring ongoing active medical interventions, such as diabetes mellitus or cardiovascular disease.

Study title	Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers
Study Identification Number:	C14-80
Sponsor	INSERM

	20. Any autoimmune or immunodeficiency disease/condition (inherited or iatrogenic). 21. Any medical condition which, 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. evolving encephalopathy not attributable to another identifiable cause within 7 days of administration of a previous dose of any vaccine, hospitalization due to major depression or history of suicidal attempt. 22. Abnormal laboratory values outside the limit of normal values for the screening laboratory with clinical significance at the discretion of the investigator. 23. Person in frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc) or residence in the same household as persons with known immunodeficiency including persons on immunosuppressant therapy.
Temporary contra-indications to vaccination	Reschedule vaccination and visit 2 if • Febrile illness (oral temperature ≥ 38.0°C) at the day of vaccination. • Upper respiratory infection (URI), rhinitis and/or rhinoconjunctivitis.
Investigational product	Live attenuated *Bordetella pertussis* BPZE1 strain or placebo
Presentation	Vial
Dose	2 x 0.4 mL (0.4 mL per nostril)
Route	Nasal
Vaccination schedule	Drops administered by tuberculin syringes, one syringe containing 0.4 mL for each nostril at visit 2 (0.4 mL per nostril containing half the dose $5x10^6$; $5x10^7$; $5x10^8$ bacteria to give a total dose of 10^7; 10^8; 10^9, respectively.).
Duration of treatment:	Single administration at day 0
Excluded non-study vaccines/therapies during study	The following therapies should be avoided during the first month after vaccination • Antibacterial agents effective against *B pertussis*. • Immunoglobulin, corticosteroids for systemic use or for use in the respiratory tract (e.g. nasal steroids, inhaled steroids), other immunosuppressive agents or immunostimulating agents. • Pertussis vaccines. • Topical nasal therapies. **If it is necessary to give a medication or a vaccine, this shall be permitted for obvious reasons. All medications and vaccines will be documented in the case report form (CRF). The volunteer will be followed as pre-planned for adverse events. The immunological part of the study can also continue, but the results from the volunteer should be reported separately in the statistical analyses.** If the volunteer needs a medication or a vaccine more than one month after inclusion in the study this should be registered and both the principal investigator and the sponsor will decide whether the volunteer can continue in the study or not.

Study title	Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers
Study Identification Number:	C14-80
Sponsor	INSERM

Endpoints	**PRIMARY ENDPOINT**
	The primary safety endpoint will be the number and proportion of participants, per dose group and randomized allocation, with at least one of the following adverse events between Day 0 (Visit 2) and Day 28 (Visit 8)
	• Cough and spasmodic cough of grade 2 or higher.
	• Other respiratory tract AE related or possibly related to vaccination of grade 3 or higher.
	• Any other AE related or possibly related to vaccination of grade 3 or higher
	SECONDARY SAFETY ENDPOINTS
	Number and proportion of participants, per dose group and randomized allocation, with:
	• at least one episode of cough or spasmodic cough, described by grade, between Day 4 and Day 28
	• at least one solicited or unsolicited AEFI from respiratory tract between Day 0 and Day 28
	• at least one AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia) between Day 0 and Day 28
	• at least one other AEFI from the respiratory tract between Day 0 and Day 28
	• at least one episode of cough, described by grade, between Day 0 and Day 28
	• at least one episode of spasmodic cough, described by grade, between Day 0 and Day 28
	• at least one solicited systemic AEFI between Day 0 and Day 28 (fever, headache, tiredness)
	• at least one unsolicited AEFI between Day 0 and Day 28
	• at least one SAE related or possibly related to vaccination declared between D28 and the end of the trial
	Adverse events following immunization (AEFI) should be reported at study visits during the first 28 days of the concerned subject.
	Serious adverse events (SAE) should be reported immediately but no later than 24 hours after detection. AEFI which lead to difficulties in breathing (asthma, spasmodic cough, pneumonia) will be considered as serious and reported to the Data Safety Monitoring Board (DSMB) and the sponsor.
	Other secondary endpoints
	COLONIZATION OF THE BPZE1 STRAIN IN THE NASOPHARYNGEAL MUCOSA
	Per dose group and randomized allocation:
	• Number and proportion of participants who have been colonized by the modified B. pertussis strain BPZE1 at each post-vaccination visit
	• Number of visits during which bacteria are detected
	• Magnitude of bacterial colonization (number of cfu) per post-vaccination visit
	IMMUNOGENICITY
	Per dose group and randomized allocation:
	• IgG and IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in serum at visits 2, 4, 6, 7, 8 and 9
	  • Geometric mean levels
	  • Number and proportion of participants with positive responses, defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD (minimum level of detection) after vaccination
	Sera will be tested blindly for pertussis specific IgG antibodies in batch for each group. Antibody levels to PT, FHA, PRN, and fimbriae 2/3 will be expressed in IU/mL calibrated against reference antisera from the National Institute for Biological Standards and Controls.

Study title	Phase Ib (high dose), double-blind, single centre, dose-escalating, placebo-controlled, randomized study of a live attenuated *B. pertussis* strain given as a single intranasal dose to healthy adult volunteers
Study Identification Number:	C14-80
Sponsor	INSERM

	Exploratory endpoints
	• IgG and IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in nasopharyngeal aspirate at visits 2, 4, 6, 7, 8 and 9
	  • Geometric mean levels
	  • Number and proportion of participants with positive responses, defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD after vaccination
	• Median levels of cytokines in nasopharyngeal aspirate
	• Median levels of cytokines in cell culture supernatants after stimulation of PBMC with PT, FHA, PRN and unrelated antigen

Statistical analyses	All participants having received at least one nasal injection will be included in the analysis (modified intention to treat population). All primary analyses will be performed per dose group and randomized allocation (for arms receiving active vaccination) and for the pooled placebo arms (unless there is evidence for a time trend in placebo observations between groups). **Primary safety endpoint** • Described per group and randomized allocation, in modified ITT population, with participants with missing follow-up data considered event-free in primary analysis • Displaying numbers, percentages and 95% confidence intervals. • Each individual component of the primary endpoint will also be described **Secondary endpoints** Secondary endpoints will be described per group and randomized allocation, displaying numbers, percentages and 95% confidence intervals (qualitative variables); medians and interquartile intervals (quantitative variables) or geometric mean and 95% confidence interval (antibody titers). Number, time of occurrence and duration of AEFIs will also be described. Cough, spasmodic cough and any symptom from the respiratory tract will be described for each of the vaccine groups and the pooled placebo group, and for the groups of colonized and non-colonized individuals. Pre- and post- vaccination serum IgG and IgA antibody endpoints will be described per group and randomized allocation. Within group comparisons may be done using appropriate tests for paired measurements. Cough, spasmodic cough and any symptom from the respiratory tract, as well as antibody titers, will be described for each of the vaccine groups and the pooled placebo group, and for the groups of colonized and non-colonized individuals. **Exploratory endpoints** • Pre- and post- vaccination nasopharyngeal IgG and IgA antibody endpoints will be described per group and randomized allocation. • The median of cytokines levels in nasopharyngeal aspirate will be displayed for pre- and post-vaccination samples in each vaccine group. • The median levels of T cell responses by measuring cytokines after stimulation with PT, FHA, PRN and unrelated antigen will be displayed for pre- and post-vaccination samples in each vaccine group. Intra-group comparisons by statistical tests for paired samples will be done for each dose group and randomized allocation. In addition, a description of cough and spasmodic cough episodes per groups of colonized versus non-colonized individuals will be performed.

I.4. PROCEDURAL FLOW-CHART

Study Visit	1	2	3	4	5	6	7	8	8'	9
	1-6 weeks prior to visit 2	Day 0	Day 4±1	Day 7±1	Day 11±1	Day 14±1	Day 21±1	Day 28 -1; +7	Day 45 -5; +10	Month 6±1
Administrative Requirements:										
Recruitment, Medical record number, Screen form,	x									
Information and study consent[a]	x									
Demographic information (age, gender, ethnicity)	x									
Vaccination:		x								
Clinical Requirements:										
Complete medical history	x									
Physical examination include. vital signs[b]	x	x	x	x	x	x	x	x	x	x
Interim History/Diary (AEFI documentation, concomitant medication)[c,d]		x	x	x	x	x	x	x	x	x
Randomization		x								
Safety Labs:										
ECG	x									
Hematology[e]	x	x		x		x		x		x
Blood chemistry[f]	x									
Screening for HIV, hepatitis B and C[g]	x									
Serum pertussis toxin and pertactin IgG antibodies	x									
Urine analysis[h]	x	X*						X*		
Nasopharyngeal aspirate for BPZE1 culture and quantitative colony counts			x	x	x	x	x	x[i]	x[i]	
Immunogenicity Labs and Sample Archive:										
Binding pertussis serum antibodies		x		x		x	x	x		x
Binding pertussis nasopharyngeal antibodies			x	x		x	x	x		x
Nasopharyngeal cytokines			x	x	x	x	x	x		x
Lymphoproliferative response		x	x[j]			x		x		x
Cryo-preserved cells		x				x		x		x
TOTAL BLOOD VOLUME APPROXIMATELY (ML)	23	109	16[j]	8.5		109	4.5	109		109

[a] The ICF must be signed by the subject and investigator before any study specific procedure.

[b] At Visit 1, full physical examination (e.g. inspection of tonsils, palpation of glands, auscultation of pulm, measuring height and weight) including ECG and vital signs (oral temperature, blood pressure, heart rate, respiratory rate). For the following visits, physical exam will be restricted to general appearance, heart, lung, skin, nose, throat and vital signs.

[c] Adverse events following immunization (AEFI) occurring within 2 hours post-vaccination have to be recorded at the study centre and AEFI occurring from Day 0 to Day 28 have to be recorded in the Diary. **If a vaccine-related AEFI is not resolved by Day 28, it must be followed up until resolution or stabilization.**

[d] Serious adverse events (SAE) occurring from Visit 1 (screening) to Visit 9 have to be recorded at the study site. If an SAE is not resolved at the last visit of the concerned subject, it must be followed up until resolution or stabilization.

[e] Hematology (haemoglobin, total and differential WBC, RBC, platelets).

[f] Blood chemistry (potassium, calcium, sodium, creatinin, albumin, serum bilirubin, alkaline phosphatases, alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), glutamyltransferase (GT), high sensitive C-reactive protein, blood glucose, thyreoidea stimulating hormone (TSH), human chorionic gonadotropin (hCG), females only.

[g] Including HIV1/HIV2, HBsAg, anti-HBc and anti-HCV.

[h] Dipstick analysis of pH, erythrocytes, leucocytes, protein, glucose, ketones and bacteria (nitrite) and the drugs cocaine, amphetamine, cannabis, morphine, benzodiazepines, and methylenedioxymethamphetamine. *Urine dipstick pregnancy test is to be done at visit 2 before randomization (if positive exclude) and at visit 8.

[i] If positive culture after 4 weeks a new nasopharyngeal sample should be collected 2-4 weeks later for culture.

[j] Only high dose group 3.

II. Important medical procedures to be followed by the investigator

II.1. Eye exposure

The Principle Investigator (PI) or delegate at the clinical trial centre will see all staff and any volunteer who accidentally gets droplets of the vaccine in the eye. The principal investigator or delegate will see all non-trivial cases. Equipment for eye washing will be available. Protective goggles (included in universal precautions) will be worn by subject and staff when administering the vaccine.

Accidental exposure of health care staff should be reported according to the routine at the Karolinska Trial Alliance, Karolinska University Hospital.

II.2. Stopping criteria for dose escalation/Continuation of study

In this study adverse events following immunization (AEFI) are defined according to the current definition generally used in vaccine pharmacovigilance (Report of CIOMS/WHO Working Group on vaccine pharmacovigilance 2012) as: "*any untoward medical occurrence which follows immunization and which does not necessarily have a causal relationship with the usage of the vaccine. The adverse event may be any unfavourable or unintended sign, abnormal laboratory finding, symptom or disease*."

An AEFI is considered serious if it results in death, is life-threatening, requires in-patient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.

All SAE will be reported in due time by the investigator to the sponsor with copy to the trial PI. Then the PI or the sponsor will request a DSMB meeting. The DSMB should give advice to the sponsor about stopping the study (temporarily or permanently) or continuing the study. The following criteria will be considered:

- The study may be stopped if a volunteer within the period 0-28 days after vaccination suffers from clinical whooping cough without laboratory demonstration of wild-type *B. pertussis*. In such a case it must be suspected that the symptoms are caused by BPZE1.
- The study may be stopped if a volunteer has a SAE which is considered by the DSMB to be most probably caused by BPZE1.

II.3. Method of blinding and breaking the study blind

The 16 subjects in each dosage group study will be vaccinated in a double-blind manner (active vaccine vs. placebo). That means that all trial participants and all site staff involved in participant recruitment and follow-up will be blinded to treatment assignment. Vaccine and placebo are not distinguishable at the 10^7 dose level when thawed. For higher dose levels (10^8, 10^9), where vaccine and placebo may be distinguishable when thawed, observer-blinding will be established, by the fact that the vaccine will be prepared and administered by a study nurse not involved in any other trial procedures

Sponsor staff and laboratory staff involved in review/analysis of data are unaware of the treatment assignments. The 6 volunteers with high PRN antibodies in the control group (group 4) will be vaccinated with active vaccine (no blinding, open label).

The information if a vial contains vaccine or placebo will only be available at the statistics center (EUCLID), Lund University Hospital pharmacy (for emergency unblinding), at the manufacturer's site (Q Biologicals) and at the sponsor pharmacovigilance site.

Since access to results of the cultivation of BPZE1 from the nasopharynx samples and the antibody determination may lead to treatment unblinding, these results will be strictly controlled within the respective laboratory. The access of these results outside the laboratory will be contingent on having a previous access to the randomization list and therefore will only be authorized after the completion of

the study, with the exception a statistician in order to perform an interim analysis after 28-days follow-up. At the end of the trial, the study will be unblinded after all safety and laboratory results are monitored and the data bases are locked.

EXCEPTIONS!

1) If any subject has detectable vaccine strain bacteria in the nasopharyngeal sample collected 28 (-1; +7) days after vaccination. Under such a circumstance the laboratory has to inform the investigator, who should arrange for an extra visit for collection of a new nasopharyngeal sample for control of bacterial presence.
2) In case of a medical event that investigator/physician in charge of the participant feels cannot be treated without knowing the identity of the study treatment, an emergency unblinding procedure is in place at the Lund University Hospital pharmacy (available 24 hours 7 days a week). If there is a possibility the investigator or delegate should be contacted before the code is broken. The investigator/physician in charge sends a fax of request to the pharmacy, which breaks the code by opening the individual sealed envelope, fills in the unblinding form and faxes the completed form to the investigator/physician in charge. The sponsor should be informed within 24 hours of any unblinding. As far as possible all examinations during study follow-up must be performed on all subjects who received the investigational product.

Unblinded interim reports for the DSMB will be prepared before escalation to the next dose by the trial statistician. An additional interim analysis will be performed when culture results are available from twenty-eight days after vaccination of the high dose group 3. For the latter, another statistician will get access to the randomization list for groups 1-3 to provide a summary of safety and colonization data to the Sponsor/ILiAD for use in discussion with regulatory agencies about a Phase II study. The interim analysis will provide no information about individual volunteers that could reveal the vial codes and the statistician performing the interim analysis will not be involved in any other trial procedure or in the final statistical analyses.

III. Introduction

III.1. Background

Before introduction of general vaccination, pertussis was a typical childhood disease. The incidence in children has been drastically reduced with vaccination of infants and booster doses to school children, but *B. pertussis* continues to circulate even in populations with high vaccination coverage of infants and children. Pertussis in adolescents and adults is increasing, and from these age groups the organism may spread to neonates and infants too young to be vaccinated. It is estimated that 16 million cases of whooping cough occur globally each year with 95% of the cases found in developing countries (WHO, Pertussis vaccines: WHO position paper. Wkly Epidemiol Rec 2010;85:385-400). This number is most likely underestimated because studies have shown that pertussis is an underdiagnosed disease. Severe and fatal cases are found in infants and patients with severe underlying diseases. Close to 200,000 children are estimated to die of pertussis each year, including children in developed countries. Pertussis is one of the few infectious diseases with a high incidence also in countries with high vaccination coverage. Thus, even in countries with high vaccination coverage and 1 – 2 booster doses the herd immunity achieved does not seem to protect infants from exposure. This emphasizes the need for a new global vaccine strategy with a high degree of individual protection of neonates through e.g. cocooning, maternal immunization or neonatal immunization.

A genetically modified strain of *B. pertussis* has been constructed (BPZE1) (Mielcarek et al., Live attenuated B. pertussis as a single-dose nasal vaccine against whooping cough. PLoS Pathog 2006;2:e65). In this strain three toxins have been inactivated or deleted. The gene for pertussis toxin, one of the major virulence factors and protective antigens, has been modified so that a nontoxic protein with preserved immunological properties is produced by the organism. This toxoïd is similar to the genetically modified toxin which had a high efficacy in a double-blind placebo-controlled trial in Italy (Greco D et al. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. N Engl J Med 1996;334:341-8). The gene for dermonecrotic toxin (DNT) has been deleted, so this toxin is not expressed by BPZE1, and a replacement of the ampG gene of *B. pertussis* by that of E. coli has decreased the production of tracheal cytotoxin (TCT) to background levels.

The BPZE1 strain therefore has the potential for being a candidate for a safe nasal vaccine against pertussis, because the three major toxins are inactivated or deleted.

III.2. Results from the first in man study of BPZE1 and study rationale

In a phase I single center, dose-escalating, placebo-controlled study the genetically modified *B. pertussis* strain (BPZE1) was given as a single intranasal dose to healthy adult male volunteers between 21 and 28 years old (Appendix 1).

Since this was the first trial in man it was designed as a dose-escalation study to find an optimal dose inducing immune responses, and still being safe. The low (10^3 cfu), medium (10^5 cfu) and high doses (10^7 cfu) used were based on the results of the pre-clinical studies in mice.

The 48 randomized volunteers, 12 in each dose group and in one placebo group, adhered strictly to the study protocol with only one deviation. Unrelated to the vaccine the first vaccinated subject died 7 weeks after vaccination and did not leave the last sample at 6±1months. Thus evaluation of safety, the ability of the vaccine strain to colonize the nasopharyngeal mucosa and the induction of immune responses was performed for all 48 subjects. Out of totally 385 planned follow up visits according to the protocol only information from one visit was missing.

The study was a double-blind controlled study. Without a placebo group it would not be possible to evaluate if general or upper respiratory tract symptoms appeared in increased frequency among the vaccine recipients. A placebo group would also reduce the risk of observer bias if the volunteers shared experience of symptoms or among the investigators when evaluating the data.

The 12 subjects in the placebo group were evenly distributed over the whole study period to enable evaluation of apparent differences in the frequency of adverse events or immunogenicity over time.

The results of the study showed that the frequency of symptoms in the respiratory tract and general symptoms (rhinorrhea, sneezing, nasal congestion, headache and tiredness) was high during the first two weeks, but there was no difference between the placebo group and the different dose group or the subjects colonized by the bacteria. Cough was neither more common in the high dose group nor in the subjects colonized with the vaccine strain BPZE1 as compared to the placebo group or the low and medium dose groups. No cases of cough were reported during week 3 and 4, which would have been expected if the vaccine strain BPZE1 had similarity with wild type *B. pertussis*.

This study showed that the colonization was dose-dependent - one culture positive in each of the low and medium dose group and 5 out of 12 in the high dose group. The colonization was detected between day 4 and 28 after vaccination and the time-frame was similar to that of wild-type *B. pertussis*.

All randomized subjects had detectable antibodies against at least one pertussis antigen pre-vaccination. After vaccination only the 7 subjects with culture-verified colonization of the nasopharyngeal mucosa showed increased antibody levels. The antibodies against several of the pertussis antigens appeared from day 14 or 28 and remained high during the 6 months of follow up. Thus there was a strong correlation between colonization and development of immune responses.

Interestingly, the pre-vaccination FHA, PRN and fimbriae 2/3 antibody levels were significantly higher in the culture negative subjects vaccinated with the high dose. Although the numbers were small this may explain the low frequency (5/12) of colonization. This needs to be considered in future clinical trials in child and adult populations, since very few individuals are naïve with regard to exposure to pertussis antigens either because of vaccination or infection.

The results of this study raised questions how a higher degree of colonization can be achieved for example by using a higher vaccine dose or another method for application.

In summary this proof-of-concept study showed that the genetically modified strain of *B. pertussis* BPZE1 in humans can colonize the nasopharyngeal mucosa without eliciting any symptoms of mild or severe whooping cough and is otherwise safe and immunogenic. In future trials it will be crucial to ascertain that administration of BPZE1 also in a dosage sufficient to colonize greater than 70 percent of vaccinees still does not cause symptoms of mild or severe whooping cough.

III.3. BENEFIT/RISK AND ETHICAL ASSESSMENT

General advantage of a modified live vaccine given intranasally over currently available injectable acellular pertussis vaccines. Effective vaccines are needed to protect young infants (from 0 to 6 months, today the most vulnerable age group). Ultimately, the goal of the BPZE1 program is to create a neonatal vaccine that can protect infants shortly after birth with a novel, single-dose pertussis vaccine, but this will be a long pathway that will require a step-wise progression from adults. An intermediate strategy for protecting young infants would be to reduce child exposure to adults that have nasopharyngeal colonization with *B. pertussis*. The critical aim is to protect infants in the most vulnerable age group, prior to the regular vaccination schedule using current vaccines. Interim data from a preclinical study in a baboon model designed by the FDA has demonstrated that BPZE1 vaccinated baboons reduced the overall *B. pertussis* burden (i.e. total *B. pertussis* colonization over time) compared to historical aPV baboon results (Warfel et al, "Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model," PNAS, Jan 2014) by more than 99.8% in a matching protocol. The magnitude of treatment effect appears substantial with the potential for a large reduction in risk of *B. pertussis* colonizing infections in human adults that are transmittable to infants. If the less than 0.2% *B. pertussis* burden seen in the BPZE1 vaccinated baboons is predictive of relative vaccine performance in humans, BPZE1 will invariably lead to a substantial reduction in the opportunity for adult-to-infant transmission. If BPZE1 can rapidly reduce *B. pertussis* colonization of the nasopharynx (both burden and duration) in adult humans after challenge as observed in the juvenile baboon, the potential for adult BPZE1 vaccination to reverse the escalating rates of Pertussis is credible and an opportunity exists to decrease global mortality rates caused by *B. pertussis*.

After an adult BPZE1 vaccine demonstrates safety and efficacy, the eventual goal is to replace current vaccination schedules by a single BPZE1 neonatal vaccination. An interim step would be to add a first nasal vaccination to protect very early in life prior to the current immunization schedule. Alternatively the

vaccine may be used as a booster vaccine. Early immune imprinting by infection or vaccination has a long-term impact on immunity. Current vaccination with acellular pertussis vaccines has been shown to bias the immune responses towards Th2 type responses, or to delay the Th1 type responses in children, even towards unrelated antigens and polyclonal immune stimulation. Nasal vaccination with BPZE1 is expected to promote the development of Th1 type responses, as natural infection with *B. pertussis* induces strong levels of IFN-γ towards *B. pertussis* antigens in very young children. The induction of Th1 type responses by nasal vaccination with BPZE1 has already been shown in infant mouse models. In addition to Th1 type responses nasal vaccination with BPZE1 also induces antibodies against the major protective antigens. It is therefore expected that both protective immune parameters (antibodies and Th1 type responses) will be induced in infants vaccinated with BPZE1.

Pertussis is a world-wide disease, present both in developing countries and in the developed world. The development of an effective nasal vaccine against this disease might thus have a planet-wide impact. Because of the global importance of the disease, the market of the product developed through the ILiAD-INSERM concerted action project is essentially worldwide.

Environment risk assessment of the live attenuated pertussis vaccine BPZE1

Detailed information about the genetically modified organism BPZE1 is available in the Environment risk assessment (Appendix 2 Environment risk assessment of vaccine used in the BPZE1 Phase 1b 1/Miljöriskanalys för vaccin ingående i BPZE1 Phase 1b).

The attenuated strain of *Bordetella pertussis* named BPZE1 was engineered by eliminating or genetically detoxifing three *B. pertussis* toxins, PT, DNT and TCT.

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

- No lethal effect of BPZE1 was observed in mice, even after nasal administration of 10^6 cfu. The histological analysis data showed a decreased colonization and proliferation power of the BPZE1 cells in the trachea and lungs and no weight reduction was observed after nasal administration of the BPZE1-strain.
- Dissemination of BPZE1 was not observed in mice with severe immunodeficiency.
- No cell toxicity of the pneumocyte and macrophage cell lines was observed after incubation with the BPZE1-strain (in vitro safety test).
- 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 95% reduction in release of the TCT in the medium.
- The DNT is not expressed in the BPZE1 strain.

Based on these data the BPZE1 has been classified as a Biosafety level 1 organism by the French authorities (Appendix 3).

There is no known animal vector or reservoir for *B. pertussis*. The genetic modifications (replacement of the *ampG* gene, deletion of the DNT and the mutations of the PT) are not expected to alter the host range of *B. pertussis* BPZE1 compared to the wild type *B. pertussis*.

The BPZE1 is bacteria not invasive and has no selective advantage in the environment. The potential for exchange of genetic material is virtually inexistent, since *B. pertussis* does not harbor plasmids or conjugative transposons. In addition, *B. pertussis* Tohama I (background used for the BPZE1 strain) does not harbor intact prophage genomes and is therefore incapable of producing functional phage particles.

No cross-contamination between the volunteers was observed in the previous phase 1 clinical trial of BPZE1.

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

Volunteers risk assessment of the live attenuated pertussis vaccine BPZE1.

B. pertussis colonization is strictly limited to respiratory epithelium without dissemination of the bacteria outside the respiratory tract, which also excludes systemic bacteraemia of the BPZE1 strain.

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

B pertussis has not been shown to be allergenic in any preclinical or clinical studies to date. On the contrary the BPZE1 has been demonstrated to protect against airway inflammations induced by allergens or viral infections in a mouse model. The BPZE1 has also been shown to protect against infection with wild type *B pertussis* infection already 3 hours after immunization in a mouse model (Mielcarek et al., Live attenuated B. pertussis as a single-dose nasal vaccine against whooping cough. PLoS Pathog 2006;2:e65).

The BPZE1 vaccine or placebo will be administered nasally via tuberculin syringes to healthy adult volunteers under strictly controlled conditions at the Karolinska Trial Alliance phase I unit. To minimize the risk of transmission the volunteers will stay at the study centre for 6 hours after administration of the vaccine. In addition volunteers with frequent contact with infants below one year of age or individuals with immunodeficiency will be excluded from participation in the study. The attenuated BPZE1 bacteria colonize the upper respiratory tract similarly to the wild-type *B. pertussis*. Colonization of live organism will be followed in nasopharyngeal aspirates performed at various intervals from the day of administration until the end of the study. Chronic carriage of *B. pertussis* has not been reported and is therefore not expected.

To avoid accidental exposure actions should be taken to minimize generation of aerosols, since the bacteria is strictly a respiratory tract organism. The volunteers and the staff members should wear eye-protective glasses 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 e.g. erythromycin will be given in case of accidental transmission to other humans.

Female mice when vaccinated with BPZE1 shortly before mating showed no negative effect on their pregnancy nor on their offspring. As a matter of fact, the offspring were protected against *B. pertussis* challenge.

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

IV. Study objectives

IV.1. PRIMARY OBJECTIVE: SAFETY

To assess the general safety and tolerability of the respiratory tract including specific adverse events such as cough/spasmodic cough during the 4-28 days after vaccination by a single ascending dose of the genetically modified *B. pertussis* strain BPZE1 in healthy adult volunteers.

Safety assessments recorded during the trial include:

- general safety, i. e. general well-being of the volunteers and any symptoms reported (or experienced) by the volunteers with onset within one month after vaccine administration.
  - vital signs: blood pressure, heart rate, respiratory rate, oral temperature.
  - abnormalities in the following laboratory data: haemoglobin (i.e. b-haemoglobulin), total and differential white blood cell count (i.e. granulocytes: neutrophils, eosinophils, and basophils, non-granulocytes: lymphocytes and monocytes) platelets (thrombocytes) and RBC).
- specific side effects:
  - cough during the 28 days period (colonization period) after vaccination.
  - spasmodic cough, defined as rapid repetitive cough without intervening inspiration usually occurring as sudden intensified attacks of involuntary coughing (paroxysms) that may occur at different times in a way that is not regular several times per 24 hours during the 28 days period (colonization period) after vaccination.
  - other symptoms from the respiratory tract: sneezing, swollen nose, cough, bleeding from the nose, pain or other symptoms from the ear, symptoms from the eyes (redness, secretion).

IV.2. SECONDARY OBJECTIVES

To assess after vaccination by a single ascending dose of the genetically modified *B. pertussis* strain in healthy adult volunteers:

1) the safety and tolerability of the vaccine over 6 months post-vaccination
2) the frequency of colonization of the human respiratory tract by live attenuated B. pertussis strain and its magnitude (in terms of colony forming units) over 28-days post-vaccination.
3) the levels of serum IgG and IgA antibodies to 4 different *B. pertussis* antigens (pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3) over 6 months after vaccination.

IV.3. EXPLORATORY OBJECTIVES

To assess the B and T cell immune responses to 4 different *B. pertussis* antigens (pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3) over 6 months after vaccination by:

1) the levels of IgG and IgA in nasopharyngeal aspirate
2) the levels of cytokine after stimulation of PBMC with *B. pertussis* antigens and unrelated antigens.
3) the cytokine levels in nasopharyngeal aspirate.

To explore the frequency and magnitude of colonization and as well as antibody, B and T cell responses in a small group of healthy adult volunteers with high PRN antibody levels (only highest vaccine dose).

V. Selection of volunteers

V.1. INCLUSION CRITERIA

Subject will be included in the study if he/she meets all the following criteria:

1. Healthy male or female between 18 and 32 years of age, vaccinated or unvaccinated with acellular pertussis vaccine.
2. Female subject of child bearing potential must be willing to ensure to use one of the following methods of contraception from 2 weeks before vaccination till one month after vaccination to avoid pregnancy during the study:
   - Complete abstinence from penile-vaginal intercourse
   - Double barrier method (male condom/spermicide, male condom/diaphragm, diaphragm/spermicide)
   - Any intrauterine device (IUD) with published data showing that the expected failure rate is <1% per year
   - Approved hormonal contraception (except low-dose gestagen), which is not contraindicated to study vaccine administration
3. Informed consent form (ICF) signed by the subject.
4. Subject shall be able to attend all scheduled visits and to understand and comply with the study procedures.

V.2. EXCLUSION CRITERIA

If any of the following criteria are met, the subject must not be included in the study:

1. Individual with PT and /or PRN serum IgG antibodies ≥ 20 IU/ml. NOTE! One control group with PRN serum IgG antibodies ≥ 20 IU/ml and PT serum IgG antibodies <20 IU/ml will be included.
2. Vaccinated with the study vaccine in the Child Innovac study (EudraCT number 2010-019936-11).
3. Pregnant or lactating women. Pregnancy not planned and to be avoided during the study by use of effective contraceptive methods.
4. Blood pressure after resting ≥ 150/90 mm Hg at screening.
5. Heart rate after resting ≥ 80 bpm at screening.
6. Respiratory rate after resting ≥ 20/minute at screening.
7. Unwillingness to refrain from the use of nicotine products from screening through day 28.
8. Use of narcotic drugs and/or a history of drug/alcohol abuse whitin the past 2 years prior to screening.
9. The subject has donated blood or suffered from blood loss of at least 450 ml (1 unit of blood) within 60 days prior to screening or donated plasma within 14 days prior to screening.
10. Receipt of immunoglobulin, blood derived products, systemic corticosteroids or other immunosuppressant drugs within 90 days prior to day 0.
11. Asthma or other chronic respiratory problems.
12. Use of corticosteroids in the respiratory tract (e.g. nasal steroids, inhaled steroids) 30 days prior to day 0.
13. Receipt of a vaccine within the last 30 days prior to day 0 or planned vaccination within the next 30 days after day 0.
14. Known hypersensitivity to any component of the study vaccine.

15. Current participation in any other clinical trial or participation (and during the whole study) in any clinical trial in the previous 3 months prior to day 0.
16. Inability to adhere to the protocol, including plans to move from the area.
17. Family history (first degree) of congenital or hereditary immunodeficiency.
18. Past or present infection with HIV, hepatitis B or C.
19. Chronic conditions requiring ongoing active medical interventions, such as diabetes mellitus or cardiovascular disease.
20. Any autoimmune or immunodeficiency disease/condition (inherited or iatrogenic).
21. Any medical condition which, in the opinion of the investigator, might interfere with the evaluation of the study objective or might affect the safety of the individual, e.g. evolving encephalopathy not attributable to another identifiable cause within 7 days of administration of a previous dose of any vaccine, hospitalization due to major depression or history of suicidal attempt. .
22. Abnormal laboratory values outside the limit of normal values for the screening laboratory with clinical significance at the discretion of the investigator.
23. Person in frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc) or residence in the same household as persons with known immunodeficiency including persons on immunosuppressive treatment.

V.3. TEMPORARY CONTRAINDICATIONS

Reschedule vaccination and visit 2 if

- Febrile illness and/or oral temperature ≥ 38.0°C at the time of vaccination.
- Upper respiratory infection (URI), rhinitis and/or rhinoconjunctivitis.

VI. Study plan and design

VI.1. PLANNED STUDY CALENDAR

FVFS	*August 2015*
FVLS	*February 2016*
LVLS	*September 2016*
Antibody assays	*August 2015 – October 2016*
Exploratory laboratory assays	*April 2016 – December 2016*
Statistical analysis	*October 2016 – December 2016*
Clinical study report	*February 2017*

The end of the trial will be the last visit of the last subject.

VI.2. GENERAL STUDY PLAN AND DESIGN

Year	2015					2016							
Month	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
		Vaccination											
Group 1		↓											**Low dose 10^7 cfu**
		x	xxxxx					x					BPZE1 Nasal aspirate
	x	x	xxxx					x					Blood collection for serology, clinical chemistry and /or hematology
				Vaccination									
Group 2				↓									**Medium dose 10^8 cfu**
				xxxx	xx					x			BPZE1 Nasal aspirate
			x	xxxx	x					x			Blood collection for serology, clinical chemistry and /or hematology
						Vaccination							
Group 3						↓							**High dose 10^9 cfu**
						xxxx	xx				x		BPZE1 Nasal aspirate
					x	xxxx	xx				x		Blood collection for serology, clinical chemistry and /or hematology
								Vaccination[a]					
Group 4								↓					**High dose 10^9 cfu**
								xxxxxx				x	BPZE1 Nasal aspirate
							xx	xxx				x	Blood collection for serology, clinical chemistry and /or hematology

[a] Group 4 will either be run in parallel with group 3 or as indicated in the schedule.

This is a phase Ib dose-escalating study with consecutive dose groups, and a randomized placebo control group in each dose group (Groups 1 – 3). Participants will be randomized between active vaccine and placebo in a 3:1 ratio.

The volunteers will be recruited in a step-wise fashion with 16 individuals in each of three groups.

- Group 1: 12 individuals will be vaccinated once intranasally with 10^7 cfu of the organism. 4 individuals will be randomized to placebo (the diluent alone).

- Group 2: 12 individuals will be vaccinated once intranasally with 10^8 cfu of the organism. 4 individuals will be randomized to placebo (the diluent alone).
- Group 3: 12 individuals will be vaccinated once intranasally with 10^9 cfu of the organism. 4 individuals will be randomized to placebo (the diluent alone).

The administration of vaccine or diluent alone will be performed in a double-blind fashion with administration of coded vials (see below).

The volunteers in each group will be included sequentially in the study and vaccinated according to the following schedule.

The 16 individuals will be randomized as described in VI.4 *"Screening number and randomization procedure"*.

- Day 1: 2 individuals with at least 4 hours interval
- Day 2: 2 individuals with at least 4 hours interval
- Day 3: 3 individuals with at least 2 hours interval
- Day 4: 4 individuals with at least 1 hour interval
- Day 5: 5 individuals with at least 1 hour interval

Interim safety meetings with the DSMB for recommendation to the sponsor will be held before administering the next dose. At the interim safety meetings, safety data covering 28-days of post-vaccination follow-up of group 1 and group 2, respectively, will be available. Taking into account the time for the DSMB to meet and to give its recommendations for dose escalation, group 1 volunteers will have been followed up during at least 6 weeks by the time the group 2 volunteers become vaccinated. Likewise, group 2 volunteers will have been followed up during at least 6 weeks by the time the group 3 volunteers become vaccinated.

A non-controlled group (group 4) consisting of 6 individuals will be vaccinated with 10^9 cfu of the organism (open-label) to investigate the frequency of colonization in subjects with high anti-PRN antibody levels (≥20 IU/ml) and PT serum IgG antibodies <20 IU/ml.

VI.3. STUDY VISITS

This study will include 9, or if required, 10 study visits. An overview of all study visits with interviews, clinical examinations and samples to be taken are presented in I.4 "*Procedural flow chart*".

- Visit 1, recruitment visit 1-6 weeks before vaccination: medical history, physical examination and collection of blood and urine sample and ECG.
- Visit 2, inclusion visit, 1-6 weeks after visit 1: physical examination, collection of blood and urine (females only) sample and vaccination with vaccine or placebo.
- Visit 3, 4±1 days after visit 2: physical examination, safety follow up of vaccination and collection of blood (in the high dose group 3 only) and nasopharyngeal sample.
- Visit 4, 7±1 days after visit 2: physical examination, safety follow up of vaccination and collection of blood and nasopharyngeal sample.
- Visit 5, 11±1 days after visit 2: physical examination, safety follow up of vaccination and nasopharyngeal sample.
- Visit 6, 14±1 days after visit 2: physical examination, safety follow up of vaccination and collection of blood and nasopharyngeal sample.
- Visit 7, 21±1 days after visit 2: physical examination, safety follow up of vaccination and collection of blood and nasopharyngeal sample.
- Visit 8, 28 (- 1; +7) days after visit 2: physical examination, safety follow up of vaccination and collection of blood, urine (females only) and nasopharyngeal sample.

- Visit 8', 45 (-5; +10) days after visit 2: physical examination, safety follow up of vaccination and collection of nasopharyngeal sample in volunteers in which BPZE1 bacteria were still detectable on day 28 (- 1; +7).
- Visit 9, 6±1 months after visit 2: physical examination, safety follow up of vaccination and collection of blood and nasopharyngeal sample.

VI.3.1. First contact

The clinical trial site will either find the healthy volunteers by the use of Karolinska Trial Alliance database or by advertising in the local periodicals or on Facebook.

All subjects will have a personal contact by phone and a screening visit can be booked.

Written information about the study will be sent to the potentially healthy volunteers before they come to their first visit at the clinic.

VI.3.2. Visit 1, screening 1-6 weeks before vaccination

During this visit the investigator will:

1. Inform the subject about the study and obtain a signed ICF. ICF should be dated and signed by the volunteer and the investigator before any study-related activities take place.
2. Verify the subject's eligibility by reviewing inclusion and exclusion criteria.
3. Assign a unique screening number to the subject (refer to section VI.4. "*Screening number and randomization procedure*").
4. Collect information regarding the subject's demography. Record the information in the CRF.
5. Collect information regarding the subject's personal and medical history and concomitant treatment as defined in section VII.1. "*Prior medications and vaccination*". Record the information in the CRF.
6. Perform a full physical examination including ECG and measure
   a) oral temperature
   b) blood pressure
   c) pulse
   d) respiratory rate
   e) height
   f) weight

   and record the information in the subject's eCRF
7. Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate document provided in the investigator or delegate Site file.
8. Collect urine samples as described in section XII.5. *"Urine samples"*. Complete the appropriate document provided in the investigator or delegate Site file.

VI.3.3. Visit 2, day 0, vaccination

During this visit the investigator or delegate will:

1. Verify the subject's eligibility by reviewing inclusion and exclusion criteria.
2. Confirm that a signed ICF is on file for the subject. Perform a limited physical examination and measure vital signs (oral temperature, blood pressure, heart rate and respiratory rate) and record the information in the subject's CRF.
3. Check of temporary contraindications to vaccination.
4. Collect urine sample from females and do a dip stick pregnancy testing.
5. Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate

document provided in the investigator or delegate Site file.

6. Assign the next consecutive vial number of the given dose group to the subject (refer to section VI.4. "*Screening number and randomization procedure*").
7. Record the allocated vial number in the participant medical record (source document).
8. Administer the study vaccine assigned to the subject by the investigator or delegate according to the randomization described in section VI6.4 *'Screening number and randomization procedure'* and according to section X.7 *'Handling and administration of study vaccine to participants'*.
9. Vaccination should be administered intranasally by two tuberculin syringes, one to each nostril. The fluid and the air cushion are pushed slowly out of the tuberculin syringe to ensure administration of the total volume (400 µl) into each nostril of the volunteer. In case of volunteer sneezing or blowing nose immediately after vaccination this should be recorded in the subject's CRF, but a new vaccine dose should not be given.

   The staff and the volunteer should adhere to the safety instructions described in the Standard operating procedure.
10. Observe the subject for 2 hours after vaccination for immediate adverse events and record any immediate adverse event following immunization (AEFI).
11. Give the subject the Diary and an oral thermometer.
12. Inform the subject to record in the Diary.
    a. Any respiratory tract adverse reaction (AR) occurring until Visit 8 and to specify the intensity of the reaction using definitions given in the Diary.
    b. Any other medical event occurring until Visit 8 and to specify the intensity of the reaction using definitions given in the Diary.
    c. The temperature as measured orally with the provided thermometer daily in the evening from day 0 until day 28. If the temperature is taken more than once during a day the highest temperature of the day should be recorded in the Diary.
    d. Any medication taken from day 0 until Visit 8.
13. Inform the subject to
    a. Immediately contact the investigator or delegate in case of any serious event and/or any visit to another physician that may occur at any time between visits.
    b. Complete the whole Diary and to bring the Diary back at the next visit.
14. Arrange for an appointment for visit 3 (4(±1) days after visit 2.
15. The subject should stay at the clinic for 6 hours after administration of the vaccine

VI.3.4. Visit 3, 4±1 days after visit 2

During this visit the investigator or delegate will:

1. Collect and review the Diary. Check for any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 2, or any adverse event corresponding to the definition of a SAE (refer to Section XIII. "*Recording and reporting of adverse events*), and check for any concomitant medication.
2. Perform a limited physical examination and measure vital signs (blood pressure, heart rate and respiratory rate) and record the information in the subject's CRF.
3. For the high dose group 3 only: Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate document provided in the investigator or delegate Site file.
4. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.
5. Arrange for an appointment for visit 4 (7±1 days after visit 2).

VI.3.5. Visit 4, 7±1 days after visit 2

During this visit the investigator or delegate will:

1. Collect and review the Diary. Check for any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 3, or any adverse event corresponding to the definition of a SAE (refer to Section XIII *"Recording and reporting of adverse events*), and check for any concomitant medication.
2. Perform a limited physical examination and measure blood pressure, heart rate, respiratory rate and record the information in the subject's CRF.
3. Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate document provided in the investigator or delegate Site file.
4. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.
5. Arrange for an appointment for visit 5 (11±1 days after visit 2).

VI.3.6. Visit 5, 11±1 days after visit 2

During this visit the investigator or delegate will:

1. Collect and review the Diary. Check for any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 4, or any adverse event corresponding to the definition of a SAE (refer to Section XIII. "*Recording and reporting of adverse events*"), and check for any concomitant medication.
2. Perform a limited physical examination and measure blood pressure, heart rate, respiratory rate and record the information in the subject's CRF.
3. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.
4. Arrange for an appointment for visit 6 (14±1 days after visit 2).

VI.3.7. Visit 6, 14±1 days after visit 2

During this visit the investigator or delegate will:

1. Collect and review the Diary. Check for any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 5, or any adverse event corresponding to the definition of a SAE (refer to Section XIII. "*Recording and reporting of adverse events*"), and check for any concomitant medication.
2. Perform a limited physical examination and measure blood pressure, heart rate, respiratory rate and record the information in the subject's CRF.
3. Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate document provided in the investigator or delegate Site file.
4. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.
5. Arrange for an appointment for visit 7 (21±1 days after visit 2)

VI.3.8. Visit 7, 21±1 days after visit 2

During this visit the investigator or delegate will:

1. Collect and review the Diary. Check for any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 6, or any adverse event corresponding to the definition of a SAE (refer to Section XIII. "*Recording and reporting of adverse events*"), and check for any concomitant medication.
2. Perform a limited physical examination and measure blood pressure, heart rate, respiratory rate and record the information in the subject's CRF.

3. Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate document provided in the investigator or delegate Site file.
4. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.
5. Arrange for an appointment for visit 8 (28 (-1: +7) days after visit 2)

VI.3.9. Visit 8, 28 (-1; +7) days after visit 2

During this visit the investigator or delegate will:

1. Collect and review the Diary. Check for any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 7, or any adverse event corresponding to the definition of a SAE (refer to Section XIII. "*Recording and reporting of adverse events*"), and check for any concomitant medication.
2. Perform a limited physical examination and measure blood pressure, heart rate, respiratory rate and record the information in the subject's CRF.
3. Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate document provided in the investigator or delegate Site file.
4. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.
5. Collect urine sample from females and do a dip stick pregnancy testing.
6. Arrange for an appointment for visit 9 (6±1 months after visit 2).

VI.3.10. Visit 8', 45(-5; +10) days after visit 2

In case the nasopharyngeal aspirate contains detectable vaccine strain bacteria on day 28 the laboratory contacts the clinical investigator or delegate, who contacts the subject for a new visit.

During this visit the investigator or delegate will:

1. Collect information on any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 8, or any adverse event corresponding to the definition of a SAE (refer to Section XIII. "*Recording and reporting of adverse events*"), and check for any concomitant medication.
2. Perform a limited physical examination and measure blood pressure, heart rate, respiratory rate and record the information in the subject's CRF.
3. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.

VI.3.11. Visit 9, 6±1months after visit 2

During this visit the investigator or delegate will:

1. Collect information on any AEFI, interview on AR from the respiratory tract and systemic adverse events that might have occurred since visit 8 (or visit 8'), or any adverse event corresponding to the definition of a SAE (refer to Section XIII. "*Recording and reporting of adverse events*"), and check for any concomitant medication.
2. Perform a limited physical examination and measure blood pressure, heart rate, respiratory rate and record the information in the subject's CRF.
3. Collect blood samples as described in section XII.3. "*Blood samples*". Complete the appropriate document provided in the investigator or delegate Site file.
4. Collect nasopharyngeal aspirate as described in section XII.4. "*Nasopharyngeal samples*". Complete the appropriate document provided in the investigator or delegate Site file.
5. Complete the End of study section in the CRF.

VI.4. SCREENING NUMBER AND RANDOMIZATION PROCEDURE

Subject

At visit 1 after a signed and dated ICF has been obtained from the subject, each subject will be assigned a **unique screening number** consisting of 3 digits (for example, 001) and the initials of the subject consisting of 3 letters (for example ATA for Anders Tomas Andersson). In case of no middle name there should be a hyphen in the middle (for example S-S for Sven Svensson).

If the consent to participate in the study is withdrawn before randomization at Visit 2, Day 0, this screening number will be kept and not reassigned to another subject, for any reason.

Randomization procedure and blinding

Vaccine and placebo will come in identical, coded vials.

The vials will be coded with group number and vial number: group1: 101 – 116, group 2: 217 – 232, group 3: 333 – 348 and group 4: 449 -454, for the respective groups described above under point VI.2 "*General study plan and design*".

To ensure randomized allocation, the vials must be used by the site in their consecutive order of vial numbers within a given dose group. The vial number **given to the volunteer (and the time of administration)** will be recorded both in the CRF and the medical record (source data).

Vaccine and placebo are not distinguishable when frozen, allowing for allocation concealment, and are not distinguishable at the 10^7 dose level when thawed. For higher dose levels (10^8, 10^9), where vaccine and placebo may be distinguishable when thawed, observer-blinding will be established, by the fact that the vaccine will be prepared and administered by a study nurse not involved in any other trial procedures.

The randomization list, establishing the order of vials and the correspondence between vial number and active vaccine or placebo group will be generated centrally by the statistician at the EUCLID clinical trials platform prior to the start of the study and kept confidentially in a secure environment.

The list with the codes/information if a vial contains vaccine or placebo will only be available at the statistics center (EUCLID), Lund University Hospital pharmacy (for emergency unblinding), at the manufacturer's site (Q Biological; for labelling the vials) and at the sponsor pharmacovigilance site. The decision whether the code shall be broken for an individual volunteer or all participants will be made by the investigator.

For purposes of interim analyses, the statistician responsible for the analysis will maintain the codes in strict confidence with no communication of any individual codes until final data lock.

An identical series of back up vials will be generated for each group. These vials will be kept and stored at the manufacturer's site.

VII. Prior and concomitant medications and non-study vaccines

VII.1 Prior medications and vaccination

All prior medications and vaccinations will be asked for and documented (in subjects' medical record) at visit 1 and updated at visit 2 as indicated below:

The following therapies must be excluded prior to the time of the vaccination (Visit 2):

- Receipt of immunoglobulin, blood derived products, systemic corticosteroids or other immunosuppressant drugs within the previous 90 days and receipt of corticosteroids for use in the respiratory tract (e.g. nasal steroids, inhaled steroids) within 30 days prior to Visit 2.
- Non-study vaccines 30 days prior to Visit 2.

All medications received by the subject within 7 days prior to Visit 2 (Day 0) should be recorded in the CRF on page "*Concomitant medications*". The trade name, route, dose, start date, stop date and indication will be documented.

VII.2. Concomitant medications and non-study vaccines

Any administration of concomitant medications or non-study vaccines after the Visit 2 (Day 0) will be recorded in the Diary by the subject and will be reported in the CRF by the investigator or delegate according to the following rules:

For **medications**, the trade name, route, dose, start date, stop date and indication should be recorded in the CRF.

For **non-study vaccines**, the trade name, route, injection side and injection site (if applicable) and date of vaccination should be recorded in the CRF.

The principal investigator will decide whether the volunteer can continue in the study or not.

VII.3. Not allowed medications & vaccines

Not allowed medications and vaccines during the first month after vaccination

The following therapies should be avoided during the first month after vaccination.

- Antibacterial agents with an effect on *B. pertussis*, notably macrolides, azitromycin, tetracyklines, trimetoprim-sulphamethoxazole, quinolones.
- Immunoglobulin, corticosteroids for systemic use or for use in the respiratory tract (e.g. nasal steroids, inhaled steroids), other immunosuppressive or immunostimulating agents.
- Topical nasal therapies.
- Pertussis vaccines. No other vaccine is known to cross-react with pertussis vaccines. Therefore the volunteer can continue in the study if other vaccines are given. Vaccines which can be anticipated, though only rarely, are tetanus vaccine in case of a wound, influenza vaccines in case of an outbreak of influenza, vaccines intended for travellers in case the volunteer will perform a visit in other countries with short notice. If a journey is known and vaccines are planned already at the screening visit, the volunteer will not be included in the study.

If it is necessary to give a medication or a vaccine, this shall for obvious reasons be permitted. All medications and vaccines will be documented in the CRF. The volunteer will be followed as pre-planned for adverse events. The immunological part of the study can also continue, but the results from the volunteer should be reported separately in the statistical analyses.

If the volunteer needs a medication or a vaccine more than one month after inclusion in the study this will be registered and the principal investigator will decide whether the volunteer can continue in the study or not. But as far as possible the subject must be followed as scheduled.

VIII. Withdrawals/study termination

Subjects may be withdrawn from the study and/or the study vaccination schedule for the following reasons:

- At their own request
- On PI decision
- The DSMB, PI and the sponsor have the right to terminate the study at any time if adverse events which may be related to the study vaccine occur.

In all cases, the reason for withdrawal can if available be recorded in the CRF. The subject must be followed up to establish whether the reason was an adverse event, and, if so, this must be reported in accordance with the procedures in Section XIII. *"Recording and reporting of Adverse Events following immunization*". The adverse event should be followed-up by the investigator until its complete resolution or stabilization.

As far as possible, as many examinations as possible scheduled during follow-up and in particular for the final study day (at 6±1months) must be performed on all subjects who received the investigational product (including withdrawn subjects) but did not complete the study according to protocol.

The investigator must make every effort to contact subjects lost to follow-up. This information will be documented in the CRF. Withdrawn subjects will not be replaced.

IX. Follow up of volunteers

After successful completion and evaluation of the present study the volunteers may be asked if they would like to participate in a follow up study after due approval from the Medical Product Agency and the Local Ethics Committee.

X. Investigational Medicinal Products i.e. Study vaccine

X.1. Identity of study vaccine

Live attenuated *Bordetella pertussis* BPZE1 bacteria or placebo.

X.2. Supply of study vaccine

Formulated attenuated *Bordetella pertussis* BPZE1 at 3 different doses (Colony Forming Unit) (see Investigational Medicinal Product (IMP) Dossier).

Liquid formulation of live attenuated *Bordetella pertussis* BPZE1 bacteria in phosphate buffered saline (containing sodium chloride and potassium chloride) and 5% saccharose at three different colony-forming units (see IMP Dossier).

X.3. Doses and treatment regimens

A dose escalation placebo controlled study will be performed, starting with a low dose (10^7 cfu), followed by a medium dose (10^8 cfu), followed by a high dose (10^9 cfu) + placebo consisting of the formulation buffer (phosphate buffered saline (containing sodium chloride and potassium chloride) and 5% saccharose).

Vaccine or placebo will be given as single administrations by nasal application in 0.4 mL in each nostril (0.4 mL per nostril containing half the dose $5x10^6$; $5x10^7$; $5x10^8$ bacteria to give a total dose of 10^7; 10^8; 10^9, respectively). The dose of BPZE1 active ingredient or placebo will be administered to the volunteer after thawing within 2 hours after leaving the freezer.

X.4. Labelling and packaging of study vaccine and placebo

Vials of vaccine and placebo are provided in 1.8 ml sterile polypropylene cryotubes (CE marked medical device). The vial closure system is a polypropylene screw cap (see Information Manufacturing Drug Product).

Vaccine and placebo will come in identical vials. Vaccine and placebo are not distinguishable when frozen, allowing for allocation concealment, and are not distinguishable at the 10^7 dose level when thawed. For higher dose levels (10^8, 10^9), where vaccine and placebo may be distinguishable when thawed, observer-blinding will be established, by the fact that the vaccine will be prepared and administered by a study nurse not involved in any other trial procedures.

Per dose/group, the vials with the vaccine or placebo will be identically coded. Only the group/subject number on the vials (i.e. vial number) will be different.

Per subject, the vials (2 vials for administration and one spare via) will be packed in a small plastic bag (outer package).

The bags with the vaccine vials or placebo vials will receive an identical label. Only the group/subject number on the bag (i.e. vial number) will be different.

The bags containing either the vaccine or the placebo will be packed in 2 boxes of 8 bags per group/dose.

Primary labels for the cryovials and secondary labels for the plastic bags are designed.

Primary labels for the 4 different groups in the BPZE1 Drug Product Phase 1 study

Group 1: Low dose

Trial code: C14-80
BPZE1 $5x10^6$ CFU/ Placebo-400µl
Nasal administration
Batch: XXXXXX
Retest date: DDMMYYYY
Store below -60°C
For clinical use only
Sponsor INSERM
Tel: +33 (0)1 44 23 60 41
Patient No: 1XY*

Group 2: Middle dose

Trial code: C14-80
BPZE1 $5x10^7$ CFU/ Placebo -400µl
Nasal administration
Batch: XXXXXX
Retest date: DDMMYYYY
Store below -60°C
For clinical use only
Sponsor: INSERM
Tel: +33 (0)1 44 23 60 41
Patient No: 2XY*

Group 3: High dose

Trial code: C14-80
BPZE1 $5x10^8$ CFU/ Placebo -400µl
Nasal administration
Batch: XXXXXX
Retest date: DDMMYYYY
Store below -60°C
For clinical use only
Sponsor: INSERM
Tel: +33 (0)1 44 23 60 41
Patient No: 3XY*

Group 4: High dose

Trial code: C14-80
BPZE1 $5x10^8$ CFU-400µl
Nasal administration
Batch: XXXXXX
Retest date: DDMMYYYY
Store below -60°C
For clinical use only
Sponsor: INSERM
Tel: +33 (0)1 44 23 60 41
Patient No: 4XY*

*: XY varies from 01 to 16 for group 1, from 17 to 32 for group 2, from 33 to 48 for group 3 and from 49 to 54 for group 4. This corresponds to the vial number. To ensure randomized allocation, vials must be used by the site in their consecutive order of vial number within a given group.

Label for the outer package for the BPZE1 Phase 1 study

INSERM — ISP- 75013 Paris — FRANCE	Telephone: +33 1 44 23 60 41
Clinical trial: C14-80 BPZE1 Phase Ib	EudraCT Number: 2015-001287-20
Nasal drops	Administration: nasal only
Produced by: Q Biologicals - Belgium	
Investigator: Karolinska University Hospital- Nabil Al-Tawil	
BPZE1 xx CFU-400 µL Batch n°: xxxxxx	
Retest date xx/xx/xxxx	**Storage below - 60°C**
Genetically Modified Organism	
Use under medical surveillance	
For clinical trial use only	
Group/Subject N°: XXY	

BPZE1 xx CFU is 5 x 10^6 CFU for group 1, 5 x 10^7 CFU for group 2 and 5 x 10^8 CFU for group 3 and 4.

Group/ Patient N° is 101 to 116 for group 1, 217 to 232 for group 2, 333 to 348 for group 3 and 449 to 454 for group 4. This corresponds to the vial number. To ensure randomized allocation; vials must be used by the site in their consecutive order of vial number within a given group.

X.5. SHIPMENT OF STUDY VACCINE AND PLACEBO

The 2 boxes containing the vaccine and placebo vials will be shipped on dry ice in boxes with temperature charts directly to the clinical trial site. The reason for not shipping the study vaccine to the local pharmacy is to minimize the risk of breaking the cold chain (see below). At the clinical center the vaccine will be stored in a freezer below -60°C.

The boxes will be shipped by a certified door-to-door service company.

- At the moment of receipt, check the temperature charts and the presence of dry ice. No temperature recording should be above -60°C
- Care must be taken to minimize the exposure to room temperature (RT) remove the material out of the dry ice box in front of the freezer with set point below -60°C

X.6. STORAGE OF STUDY VACCINE AND PLACEBO

Frozen storage, below -60°C at the clinical trial site.

It is critical that the vaccine is kept frozen at -60°C until the thawing just prior to vaccination to maintain the viability of the pertussis BPZE1 bacteria. The storage of the BPZE1 at RT should not be longer than 2 hours. It is recommended that the thawed BPZE1 solution is administered within one hour after leaving the freezer. Once the product has been thawed, it should be used. It is not allowed to freeze the product after it was thawed and to use it afterwards.

The vaccine will be kept in a locked freezer with alarm and continuous temperature monitoring (temperature charts).

- *Remember that samples which were thawed should never be refrozen.*

X.7. HANDLING AND ADMINISTRATION OF STUDY VACCINE AND PLACEBO TO PARTICIPANTS

It is critical that the vaccine is kept frozen below -60°C until the thawing just prior to vaccination to maintain the viability of the *B. pertussis* BPZE1 bacteria. Initial stability studies have shown that the BPZE1 Drug Product is very sensitive to freeze-thaw cycles and therefore the following actions must be taken to eliminate the risk of to obtain delivery of a uniform dose of BPZE1 colony forming units:

- Take the 3 cryovials out of the plastic bags immediately after removal out of the freezer
- Put the cryovials immediately in a "cryovial rack", make sure that the cryovial is in vertical position
- Check that the cryovials are not damaged.
- Leave cryovials for about 15 min at RT before administration
- ***Do not invert or shake the cryovials before use***
- Attention: 1 cryovial was added as a spare vial. Do not use this spare vial without approval of the investigator or delegate.
- Extract slowly the total volume of one (1) cryovial by placing a 1 mL- tuberculine syringe on the bottom of the cryovial.
- Expel the liquid from the syringe back into the vial slowly to mix the liquid (only once!)
- ***NB! Do not remove the air present in the syringe***
- The volunteer should lay down during and 5 - 7 minutes after administration.
- Administer to one nostril the whole content of the syringe by slowly pushing the fluid and the air cushion out of the tuberculine syringe.
- Take a new 1-mL tuberculine syringe and repeat the extraction and administration procedure for the second cryovial
- Administer the whole content of the syringe to the other nostril.

Thawed samples, which were not administered (delivered) within the maximum time of exposure to RT (max. 2 hours), may "NOT" be used and may "NOT" be refrozen below -60°C. These cryovials must be destroyed according to the applicable procedure.

X.8. ACCOUNTABILITY OF STUDY VACCINE AND PLACEBO

The investigator or delegate is responsible for the management of study vaccine available at the investigational site and will have to maintain accountability records of the study vaccine. The accountability will include quantity of study vaccine delivered to the site, study vaccine inventory at the site (stock), study vaccine administered to subjects, study vaccine wasted/lost and unused product destroyed (see X.9. below).

The **"*IMP Accountability Form*"** must be completed. The monitoring staff will verify the investigational site's product accountability records against the record of administered study vaccine in the CRF.

X.9. DESTRUCTION OF STUDY VACCINE AND PLACEBO

All vials (empty, unused, unusable) must be kept at the investigator site until the reconciliation has been performed by the clinical Monitor.

After authorization by the sponsor the empty vials will be destroyed at the investigational site.

Unusable study vaccine, i.e. expired vaccine and vaccine having experienced a cold chain break **must not** be administered.

Following verification of vaccine accountability, all unused study vaccine will be destroyed at the clinical trial site.

XI. Collection of study variables

XI.1. Recording of data

All results of physical examinations, medical history, vital signs and hematology will be completed on every visit in an electronic CRF.

XI.2. Screening and demographic procedures

At the screening visit, the subject will be informed of the study objectives, obligations, benefits and risks and will be given time to ask questions. The subject must sign an informed consent document before any study-related activities take place. The subject will be allocated a screening number. The subjects' eligibility for the study will be examined with regard to the inclusion/exclusion criteria and will include medical history, a full physical examination, assessment of vital signs and ECG, clinical laboratory evaluation, screening for hepatitis B and C and HIV, drug screening, measurement of PT and PRN IgG antibody levels and human chorionic gonadotropin (hCG) (females only). Collection of demographic information will also be done at the screening visit.

XI. 3. Safety

- Recording of AEFI. All adverse events, including findings at physical examination and vital signs, will be collected, documented and reported by the principal investigator/delegates as described in *XIII.2 Recording of safety parameters.*
- Reporting of AEFI. The principal investigator or delegate will report all adverse events in the eCRF.

XI.4. Laboratory safety assessment

All laboratory abnormalities which occur during the study will be evaluated by the principal investigator or delegate and those deemed clinically significant will be reported in the eCRF.

XII. Collection, transport and storage of biological samples

XII.1. RESPONSIBLE LABORATORIES

	Responsible laboratory	Time for delivery of samples
Safety and other Labs:		
Hematology, haemoglobin, WBC with total and differential cell count, (i.e. granulocytes: neutrophils, eosinophils, and basophils, non-granulocytes: lymphocytes and monocytes) platelets (thrombocytes) and RBC.	Labmedicine Karolinska University hospital	
Blood chemistry include. pregnancy testing	Labmedicine Karolinska University hospital	
Screening HIV, hepatitis B and C	Labmedicine Karolinska University hospital	
Urine	Labmedicine Karolinska University hospital	
Urine drug and pregnancy analysis dipstick	Karolinska Trial Alliance	
Serum PT and PRN IgG antibodies	Public Health Agency of Sweden	<24 h RT
Nasopharyngeal aspirate for BPZE1 culture	Public Health Agency of Sweden	<4 h RT
Immunogenicity Labs and Sample Archive:		
Binding pertussis serum and nasopharyngeal antibodies	Public Health Agency of Sweden	<6 h RT
Cytokine determination after lymphocyte proliferation	Public Health Agency of Sweden	<6 h RT
Cytokine determination in nasopharyngeal aspirate	Public Health Agency of Sweden	<4 h RT
Cryo-preserved cells, optional assays*	Public Health Agency of Sweden	<6 h RT

* A vial of the sample will be shipped in batch to Prof. Camille Locht, Inserm, Lille, France from the Public Health Agency for analysis of cellular immune responses
RT = room temperature

XII.2. LABELLING OF SAMPLES

The Laboratory Analytical Plan will contain detailed information about the labelling of the samples. All samples will have information about the study (BPZE1 Phase 1b), sponsor name (INSERM), the unique screening number (USN) or vial number, initials of the subject and date of collection.

XII.3. BLOOD SAMPLES

Blood Collection

After the subject's eligibility has been verified and after the ICF has been signed by the subject and by the investigator all subjects will have blood samples collected according to the following schedule for

- Blood cell counts i.e. hematology (haemoglobin, total and differential WBC (i.e. granulocytes: neutrophils, eosinophils, and basophils, non-granulocytes: lymphocytes and monocytes) platelets (thrombocytes) and RBC.
- Blood chemistry
- Pregnancy testing (females only)
- HIV and hepatitis screening
- Pertussis specific humoral and cellular immunity

Blood samples

Study Visit	1	2	3	4	5	6	7	8	9
	1-6 weeks prior to visit 2	Day 0	Day 4±1	Day 7±1	Day 11±1	Day 14±1	Day 21±1	Day 28 -1;+7	Month 6±1
Safety labs:									
Hematology[a]	4 ml	4 ml		4 ml		4 ml		4 ml	4 ml
Blood chemistry[b]	11 ml								
Screening for HIV, hepatitis B and C (no anticoagulant)	3.5 ml								
Serum PT and PRN IgG antibodies (no anticoagulant)	4.5 ml								
Immunogenicity Labs and Sample Archive:									
Binding pertussis IgG/IgA antibodies (no anticoagulant)		9 ml		4.5ml		9 ml	4.5 ml	9 ml	9 ml
Lymphoproliferative response		x	x[c]			x		x	x
Cryo-preserved cells, optional assays (Na-heparin)		x				x		x	x
Volume for cellular immunogenicity (Na-heparin)(ml)		12x8 ml	2x8 ml			12x8 ml		12x8 ml	12x8 ml
TOTAL BLOOD VOLUME APPROXIMATELY (ML)	23	109	16[c]	8.5		109	4.5	109	109

[(a)] Hematology (haemoglobin, total and differential WBC (i.e. granulocytes: neutrophils, eosinophils, and basophils, non-granulocytes: lymphocytes and monocytes) platelets (thrombocytes) and RBC.

[(b)] Blood chemistry (potassium, calcium, sodium, creatinin, albumin, serum bilirubin, alkaline phosphatases alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), glutamyltransferase (GT), high sensitive C-reactive protein, blood glucose, thyroid stimulating hormone (TSH), human chorionic gonadotropin (hCG) (females only).

[(c)] Blood will only be collected from the high dose group 3 subjects (n=16).

The blood sample should be drawn using aseptic techniques (see detailed procedure in the investigator's file). Blood must be collected in tubes with or without anticoagulant as indicated.

Blood samples transport

The blood samples for safety analysis should be transported to the department of Lab Medicine at the Karolinska University Hospital, Solna.

The blood samples for immunology analysis should be transferred to the laboratory at the Public Health Agency of Sweden within 6 hours. At the Public Health Agency the samples are handled according to the Laboratory analytical plan. Blood samples for screening of serum PT and PRN IgG antibodies should be transferred to the Public Health Agency of Sweden within 24 hours.

Samples for analysis of cellular immune responses will be shipped from the Public Health Agency to Prof. Camille Locht, Inserm, Lille, France after completion of the sample collection in accordance with Laboratory analytical plan.

Serum samples management and storage

The samples without anticoagulant should be left at RT for 1-2 hours for clotting before centrifugation. After aliquoting, serum samples should be kept frozen at -18ºC or lower in two different freezers. The temperature of the freezers will be monitored weekly and recorded during the entire study period.

Peripheral blood mononuclear cells management and storage

The samples with sodium heparin as anticoagulant should be handled at the Public Health Agency within 6 hours after collection. After purification of PBMC according to the Laboratory analytical plan they will be aliquoted and stored frozen in two different liquid nitrogen containers.

XII.4. NASOPHARYNGEAL SAMPLES

Nasopharyngeal aspirate collection

After the subject's eligibility has been verified and after the ICF has been signed by the subject and by the investigator all subjects will have nasopharyngeal aspirates collected according to the following schedule for determination of

- Colonization of *B. pertussis* strain BPZE1 in the nasopharyngeal mucosa
- Analysis of pertussis specific IgG/IgA antibodies
- Cytokines

Nasopharynx samples

Study Visit	1	2	3	4	5	6	7	8	8'	9
	1-6 weeks prior to visit 2	Day 0	Day 4±1	Day 7±1	Day 11±1	Day 14±1	Day 21±1	Day 28 -1;+7	Day 45-5; +10	6±1months
Safety and Other Labs:										
Nasopharyngeal aspirate for BPZE1			x	x	x	x	x	x[a]	x[a]	
Immunogenicity Labs and Sample Archive:										
Binding pertussis IgG/IgA nasopharyngeal antibodies				x		x	x	x		x
Nasopharyngeal cytokines			x	x	x	x	x	x		x

[(a)] If positive nasopharyngeal BPZE1 culture after 28 days (±1) a new nasopharyngeal sample should be collected 2-3 weeks later for culture.

The nasopharyngeal aspirate will be collected from the posterior pharynx along the floor of the nasopharynx using a syringe aspiration kit. Further details will be described in the Laboratory analytical plan.

Nasopharyngeal samples transport, management and storage

Detailed information will be available in the Laboratory analytical plan.

The tip of the nasal catheter will immediately (within 1 hour) be transferred to enrichment medium at the clinical trial site. The enrichment tube and the nasopharyngeal aspirate will thereafter be transported to the Public Health Agency to be seeded on Bordet-Gengou blood agar plates.

The remaining aspirate will be aliquoted into at least 2 vials. For long term storage (after the study analysis are completed) the nasopharyngeal aspirate aliquots will be kept at -50ºC or lower.

XII.5. URINE SAMPLES

Urine collection

After the subject's eligibility has been verified and after the ICF has been signed by the subject and by the investigator all subjects will have urine collected for safety analysis of pH, erythrocytes, leucocytes, protein, glucose, ketones and bacteria (nitrite) and the drugs cocaine, amphetamine, cannabis, morphine, benzodiazepines, and methylenedioxymethamphetamine.

Samples for pregnancy testing will be collected from females before vaccination (day 0) and on day 28 (-1; +7) after vaccination.

Urine samples and transport

The urine samples for safety analysis should be transported to the department of Lab Medicine at the Karolinska University Hospital, Solna.

Drug analysis and pregnancy test will be performed with dipstick test at Karolinska Trial Alliance.

The samples will not be stored after completed analysis.

XII.6. WITHDRAWAL OF INFORMED CONSENT FOR DONATED BIOLOGICAL SAMPLES

All volunteers have the right to withdraw their consent for storage of biological samples at any time of the study without giving any reason. As a consequence the sample would be destroyed if asked by the subject.

XII.7. BIOBANK STORAGE OF BIOLOGICAL SAMPLES

After completion of the study all biological samples (i.e. from blood, and nasopharynx samples) will be stored in the biobank at the Public Health Agency of Sweden according to Swedish law with possibility for future analysis. If such analysis will be planned outside the scope of this study or the next study the project should be approved by the Local Ethics Committee who is the one to decide if the volunteers should give their consent. The samples are coded and identification of the donor of the samples will be available in a locked safe. Responsible for the samples at the Public Health Agency of Sweden is the Director General.

XIII. Recording and reporting of Adverse Events Following Immunization

XIII.1. DEFINITIONS

Adverse Event Following Immunization

In this study AEFI are defined according to the current definition generally used in vaccine pharmacovigilance (Report of CIOMS/WHO Working Group on vaccine pharmacovigilance 2012) as: "*any untoward medical occurrence which follows immunization and which does not necessarily have a causal relationship with the usage of the vaccine. The adverse event may be any unfavourable or unintended sign, abnormal laboratory finding, symptom or disease.*"

Serious Adverse Event

An AEFI is considered as serious adverse event (SAE) or a serious adverse reaction (SAR)
• results in death;
• is life-threatening (means that the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
• requires hospitalization or prolongation of existing hospitalization;
• results in persistent or significant disability or incapacity;
• is a congenital anomaly or birth defect;
• is an "important medical event" (medical events, based upon appropriate medical judgment, which may jeopardize the subject or may require medical or surgical intervention to prevent one of the above characteristics/consequences);

Examples: allergic bronchospasm requiring intensive treatment at an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization, suspected transmission of an infectious agent via a medicinal product…

Is a special interest Adverse Event:
- clinical pertussis in a colonized individual if wild type *B. pertussis* is not detected by culture or PCR.

EXCEPTIONS: SAE not requiring immediate reporting to the sponsor
• Medical or surgical procedures (e.g.: surgery, endoscopy, tooth extraction, transfusion); only the condition that leads to the procedure should be notified if the condition is referring to a seriousness criteria described above.
• Pre-existing diseases or present conditions or detected prior to start of study drug administration that do not worsen.
• Situations where an untoward medical occurrence has not occurred (e.g.: hospitalization for elective surgery if known prior to start study, social and/or convenience admissions, pre-specified study hospitalizations for observation).
• Outpatient care; the volunteer has been formally admitted to a hospital for medical reasons with no seriousness criteria (described above) and does not require overnight hospitalization.

Adverse Reaction

AR are all untoward and unintended responses to an IMP related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as AR. This definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

An adverse event is considered an AR if the investigator or sponsor indicates that the adverse event is possibly, probably or definitely related to the study vaccine or placebo (relationship to an excipient).

In addition, any SAE assessed by the investigator as possibly, probably or definitely related to a protocol-specified intervention is considered a serious adverse reaction (SAR).

Suspected Unexpected Serious Adverse Reaction (SUSAR)

An unexpected serious adverse reaction is a serious adverse reaction of a nature, outcome or severity of which is not consistent with the investigational study vaccines Investigator's Brochure.

New fact

A new fact is defined as any safety data that could modify significantly the evaluation of the benefit/risk ratio of the clinical trial.
A new fact is defined as any new events related to the conduct of a trial or the investigational vaccine likely to affect the safety of subjects.
Examples: a serious adverse event which could be associated with the trial procedures and which could modify the conduct of the trial, a significant hazard to the subject population such as lack of efficacy of an investigational vaccine used for the treatment of a life-threatening disease, recommendations of the DSMB where relevant for the safety of subjects.

Relationship of an Adverse Event to the Study Vaccine

The relationship of an adverse event to the study vaccine has to be determined by the PI or delegate as follows:

- **1-NONE** [no relation with the study vaccine]: subject has not received the study vaccine or inconsistent temporal relationship (excessively long interval between administration of IMP and the onset of the symptom or the symptom appeared before administration of IMP) or there is *another obvious or more likely cause of the adverse event.*
- **2-POSSIBLE** [possible relation with the study vaccine]: has a temporal relationship with the study vaccine; however, a potential alternative aetiology that may be responsible for the symptom has not been investigated. *The adverse event could have been due to another equally likely cause.*
- **3-PROBABLE** [probable relation with the study vaccine]: has a relevant temporal relationship with the study vaccine, suggestive symptoms and a potential alternative aetiology is not apparent. *The adverse event is more likely explained by vaccine than by another cause.*
- **4-DEFINITE** [definite relation with the study vaccine]: has a relevant temporal relationship with the study vaccine and no alternative aetiology is apparent (after investigation other aetiologies have been ruled out, *the adverse event is most likely explained by vaccine than by another cause)*, or positive rechallenge with suggestive symptoms or reaction at the site of injection.

At the time of a SUSAR will be reported to the European Medicines Agency (EMA) through Eudravigilance, it is Inserm Clinical Research Safety Office's policy to map causality assessments 2-4 to "reasonable possibility" and the point 1 as "no reasonable possibility" in the binary scale in order to satisfy Eudravigilance requirement of a binary assessment.

Intensity of an AEFI / AR

The adverse events will be graded according to the Food and Drug Administration guidelines Appendix 4 *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials.*
http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf
For systemic adverse events, the intensity recorded in the CRF will be the maximum intensity observed.

- mild: awareness of sign or symptom but easily tolerated
- moderate: discomfort enough to cause interference with usual activity
- severe: incapacitating with inability to do usual activity

For fever, the intensity recorded in the CRF will be the highest observed temperature of the day measured oral in degrees Celsius if temperature is taken more than once a day otherwise the evening temperature.

XIII.2. RECORDING OF SAFETY PARAMETERS

Immediate AEFI

The subjects will be under the surveillance of the investigational staff for at least 2 hours following vaccination for evaluation of immediate adverse events. The subjects have to stay at the clinic in total 6 hours after vaccination.

Adverse events (respiratory tract adverse reactions and systemic adverse events, serious or not) occurring during this time period (2 hours) will have to be reported by the investigator or delegate in accordance with applicable laws (LVFS 2011:19, Patientdatalagen 2008:355).

AEFI from the respiratory tract

AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia) will be considered as serious and reported to the DSMB and sponsor. Other AEFI from the respiratory tract will be recorded in the volunteer´s CRF.

After the immediate medical surveillance at the investigator site, respiratory tract adverse events occurring from the day of vaccination (Day 0) to Day 28 following vaccination will be recorded in the Diary by the subject.

- Cough will be daily documented from Day 0 to Day 28
- Spasmodic cough, defined as rapid repetitive cough without intervening inspiration usually occurring as sudden intensified attacks of involuntary coughing (paroxysms) that may occur at different times in a way that is not regular several times per 24 hours, will be daily documented from Day 0 to Day 28
- Solicited AE from the respiratory tract that are pre-listed in the Diary will be daily documented from Day 0 to Day 28.
- Unsolicited AE from the respiratory tract will be spontaneously recorded in the Diary from Day 0 to Day 28

At Visit 8, 28 days (-1: +7) after vaccination, the Diary will have to be returned to the investigational site by the subject. The investigator or delegate will check the Diary, interview the subject and record AE from the respiratory tract in the CRF.

Systemic AEFI

After the immediate medical surveillance at the investigator site, systemic adverse events occurring from the day of vaccination (Day 0) to Day 28 following vaccination will be recorded in the Diary by the subject:

- Solicited systemic adverse events (fever, headache and tiredness) that are pre-listed in the Diary will be daily documented from Day 0 to Day 28
- Unsolicited systemic adverse events will be spontaneously recorded in the Diary from Day 0 to Day 28

At Visit 8 the Diary will have to be returned to the investigational site by the subject. The investigator or delegate should check the Diary, interview the subject and record systemic adverse events in the CRF.

Temperature

After the immediate medical surveillance at the investigator site, numeric values of temperature taken from the day of vaccination (Day 0) will be recorded in the Diary by the subject.

- Daily from Day 0 to Day 28

Tympanic measurement of temperature is not allowed. If temperature is measured more than once during a day, the highest temperature measured that day should be recorded in the Diary.

At Visit 8 the Diary will have to be returned to the investigational site by the subject. The investigator or delegate should check the Diary, interview the subject and record numeric values of temperature in the CRF.

Serious Adverse Event

All SAE should be reported from Visit 1 (screening visit) to Visit 9.

After the immediate medical surveillance at the investigator site, any hospitalisations and/or visits to a physician (within the definitions of SAE) from the day of vaccination at Visit 2 (Day 0) to Visit 8 will also be recorded on the Diary by the subject.

The subject should be instructed to immediately contact the investigator in case of any serious event.

At Visit 8, the Diary will have to be returned to the investigator site by the subject. The investigator or delegate should check the Diary, interview the subject and record the SAE in the CRF.

Any SAE should be recorded by the investigator in a "*Serious adverse event form*" to be sent to DSMB and to INSERM immediately, and no later than 24 hours of the investigator becoming aware of the event (see section 0 "*Reporting of Adverse Events*").

General requirements

All efforts should be made by the investigator to retrieve follow-up information for all SAE.

In case of premature discontinuation of a subject, particular efforts should be made to collect any adverse event, serious and not, that occurred at least within 28 days following the study vaccine administration.

The investigator should follow until their complete disappearance (resolution) or stabilisation:

- All SAE, which persist at the time of the last visit of the concerned subject (Visit 9). The investigator will inform the Data Safety Monitoring Board and INSERM of the date of resolution or stabilisation of the adverse event and will document it.
- Non-SAE assessed as possibly, probably or definitely related to the study vaccine by the investigator which persist 28 days after vaccination of the concerned subject (Visit 8)

Any SAE likely to be related to the study vaccine or to the protocol specified-intervention and occurring after subject's study termination will not be recorded into the CRF but should be reported by the investigator to the sponsor INSERM (see section 0 *"XIII.3. Reporting* of Adverse Events*"*).

Pregnancy notification

The investigator has to notify to the sponsor, immediately and no later than 48 hours after being made aware of it, any pregnancy and its outcome, concerning the enrolled woman.

The initial report should be notified as a detailed, written report, using the CRF "Initial pregnancy notification form" and specifying estimated date of delivery, obstetrician contact and name of maternity hospital.

The investigator has to follow the subject until the end of the pregnancy or its interruption and to notify the outcome to the sponsor using the "Final pregnancy notification form".

Follow-up of pregnant participant continues until the end of the trial. Any exceptions, related to the indication of maternal treatment, should be discussed between the investigator, the coordinating investigator, the sponsor and if necessary a specialist in terato-vigilance.

Warning:

- The medical surveillance of the women and their children should be reinforced: a particular attention must be given on serious pathology occurring during pregnancy abnormalities. A SAE initial report form should be filled if any **anomaly or birth defect** is detected.

- **All voluntary interruption of pregnancy, therapeutic interruption of pregnancy or miscarriage needed a hospitalization** is considered as a SAE/SAR and should be notify as mentioned in section XIII.3. SAE reporting.

Pregnancy management:

If the investigator has been informed that a participant woman is pregnant during the study period:

- The participant must not be treated with investigational study vaccines if the pregnancy is determined before the administration.
- The investigator must stop treatment administration and perform the planned safety assessments including a follow up in collaboration with a specialist in terato-vigilance if possible.

XIII.3. REPORTING OF ADVERSE EVENTS BY THE INVESTIGATOR

Contact persons at INSERM for the reporting of adverse events

INSERM - Pôle de Recherche Clinique (PRC)
8 rue de la croix Jarry
F-75013 Paris, France
Fax: + 33 1 44 23 67 10

Email: rqrc.siege@inserm.fr

The anonymity of subjects shall be respected when the investigator reports adverse events to the sponsor INSERM.

Reporting of AE

All biological and clinical adverse events have to be reported in the proper CRF AE form.

Reporting of SAE

SAE must be reported by the investigator or designee to INSERM immediately, and no later than 24 hours after being made aware of it. This notification must be made regardless of the relationship to vaccination.

The initial notification can be made via the e-CRF, or exceptionally (in case of unavailability of the e-CRF) by faxing the completed and signed "*Serious adverse event form **(initial)***" to:

Inserm – Pôle de Recherche Clinique (PRC)

Réglementation et Qualité et recherche clinique (RQRC)

Fax: +33 1 44 23 67 10

Email: rqrc.siege@inserm.fr

Any relevant information concerning the SAE that becomes available after the "*Serious adverse event form **(initial)***" has been sent (outcome, precise description of medical history, results of the investigation, copy of hospitalisation report, etc.) should be forwarded as soon as possible of knowledge by the investigator, by completing a "*Serious adverse event form **(follow-up)***".

This form should be signed by the investigator and sent by fax to the person listed above.

Reporting of other safety information

At any time throughout the study period the investigator could contact INSERM to report any suspected vaccine-related non-SAE occurring outside the period of collection defined in the CRF or any information

of safety interest (e.g. overdose, vaccine failure, misuse or medication error) - refer to section XIII.3 "*Reporting of adverse events*" for Pharmacovigilance contact details.

XIII.4. ASSESSMENT AND REPORTING OF ADVERSE EVENTS

In order to comply with European and local regulations on SAE reporting to Health Authorities and Ethics Committees and to allow INSERM to carry out a precise analysis of the safety of its vaccine, the investigator is committed to document accurately the event, to respect notification deadlines described in section XIII.3 *Reporting of adverse events* "to provide INSERM with all necessary information and, if requested by INSERM, to give access to de-identified source documents.

According to available information and current medical knowledge, INSERM will assess the causal relationship with the study vaccine. A SAE will be considered a vaccine-related SAE (i.e. SAR) if either the investigator or INSERM indicates that the SAE is possibly, probably or definitely related to the study vaccine. The causality assessment given by the investigator will not be downgraded by INSERM. The opinion of both the investigator and INSERM will be provided with the report.

The expectedness:

Assessment on expectedness is usually done by the sponsor drug safety managers.

If information on expectedness has been made available by the reporting investigator, this should be taken into consideration by the sponsor.

The expectedness of an adverse reaction is determined by the sponsor in the reference safety information ('RSI') (Investigator's Brochure of study vaccine), which contained the information on the frequency and nature of adverse reactions.

XIII.5. REPORTING OF SUSAR

All Suspected Unexpected Serious Adverse Reactions (SUSARs) have to be reported to EMA and to the national competent authority of the Member State concerned, within the legal timeframe, by the sponsor, directly in the EudraVigilance Clinical Trial Module (EVCTM). After SUSAR being reported to EudraVigilance no additional reporting to Swedish Medical Product Agency is needed. At the same time, the SUSARs will be sent to the Concerned Ethic Committee by the sponsor.

A contemporaneous copy of all reports sent to Swedish Medical Product Agency, EMA Eudravigilance database will be sent by e-mail to the Public Health Agency of Sweden:

rigmor.thorstensson@folkhalsomyndigheten.se

Then the Public Health Agency of Sweden on behalf of the sponsor will send them to the involved investigators.

The timelines for expedited initial reporting (day 0) starts as soon as the information containing the minimum reporting criteria has been received by the sponsor.

For fatal and life-threatening SUSAR, the sponsor should report at least the minimum information as soon as possible and in any case no later than 7 calendar days after being made aware of the case. Relevant complementary information should be collected and notified within 8 extra-days.

SUSAR which are not fatal and not life-threatening are to be reported within 15 calendar days.

If significant new information on an already reported case is received by the sponsor, this information should be reported as a follow-up report within 15 days.

XIII.6. ANNUAL SAFETY REPORT

Once a year throughout the clinical trial, the sponsor should submit to the national competent authority and the Ethics Committee of the Member States, an annual safety report, the Development Safety Update Report (DSUR - guideline ICH Topic E2F).

The DSUR should be submitted no later than 60 calendar days from the date of the sponsor's first authorization to conduct the clinical trial in any country. This date is termed the "Development International Birth Date" (DIBD).

Where clinical trials are ongoing in one country and are later initiated in another country (ies), one DSUR based on the same DIBD should be used for all countries.

The Reference Safety Information (RSI) in effect at the start of the reporting period serves as RSI during the reporting period. The DSUR should clearly indicate the version number and date of the Investigator's Brochure or SmPC used for this purpose. If there are significant changes to the RSI during the reporting period, they should be listed in the DSUR. Despite the change to the RSI, the RSI in effect at the start of the reporting period serves as RSI during the reporting period.

The DSUR is prepared in collaboration between the Inserm Clinical Research Safety Office and the Clinical Project Manager, and includes:

- a line-listing of all suspected serious adverse reactions which have occurred over this period (expected and unexpected SAR);
- a cumulative summary tabulation of the all expected and unexpected SAR by System Organ Class (SOC) name;
- a cumulative summary tabulation of all SAE by SOC name;
- a line-listing of deaths;
- DSMB opinion and Steering committee report;
- a concise, critical analysis of the subjects' safety.

The DSUR may be submitted to the principal clinical investigator for approval.

This report will be reported to the Medical Product Agency and the concerned Ethic Committee.

XIII.7. DATA SAFETY MONITORING BOARD

The Data Safety Monitoring Board will consist of 4 members.

The DSMB is tasked with 1) ensuring the safety of the participants in the Trial throughout and 2) maintaining the scientific and ethical integrity of the Trial. The DSMB is an advisory committee in charge of giving the Sponsor and the Executive Committee formed for this biomedical research an opinion and recommendations on the implementation of the Trial.

The DSMB may help the Trial sponsor and investigators to make decisions during the Trial for which independent judgment is desirable. In this regard, it may particularly recommended:

- additional amendments enabling the findings of an intermediate data examination to be interpreted
- minor or major amendments to the protocol that have become necessary because of recruitment or follow-up of the trial or to take new scientific data into consideration
- the amendment of statistical analysis data
- the premature stoppage of the trial if it believes that:
    - elements (publications) or new facts (SAE) show that the safety of participants is no longer ensured, that the study is dangerous, that the study is pointless, etc.

The DSMB shall meet whenever necessary during the Trial, at least four times in total for regular meetings (before the Trial gets under way, before each dose escalation i.e. before the start of groups 2 and 3), for the progress analysis or review and for the final analysis), and at least once a year while the trial is ongoing (whichever is more frequent).

Interim safety meetings with the DSMB for recommendation to the sponsor will be held before administering the next dose. At the interim safety meetings, safety data covering 28-days of post-vaccination follow-up of group 1 and group 2, respectively, will be available. Taking into account the time for the DSMB to meet and to give its recommendations for dose escalation, group 1 volunteers will

have been followed up during at least 6 weeks by the time the group 2 volunteers become vaccinated. Likewise, group 2 volunteers will have been followed up during at least 6 weeks by the time the group 3 volunteers become vaccinated.

Additional ad-hoc DSMB meetings will be convened in case of occurrence of an SAE. This also includes AEFIs which lead to difficulties in breathing (asthma, spasmodic cough, pneumonia).

In case of an SAE occurring during the trial (triggering an ad-hoc DSMB meeting), the following non-binding guidance should be considered by the DSMB:

- The study may be stopped if a volunteer within the period 0-28 days after vaccination suffers from clinical whooping cough without laboratory demonstration of wild-type B. pertussis. In such a case it must be suspected that the symptoms are caused by BPZE1.
- The study may be stopped if a volunteer has a SAE which is considered by the DSMB to be most probably caused by BPZE1.

At the regular DSMB meetings before the start of dose group 2 and 3, respectively, the DSMB will review all accumulated safety data in the trial (AEs and SAEs per dose and randomized allocation) and make a recommendation whether the next higher dose can be administered.

A DSMB charter will describe the functioning of the DSMB.

XIV. Endpoints

XIV.1. PRIMARY SAFETY ENDPOINT

The primary safety endpoint will be the number and proportion of participants, per dose group and randomized allocation, with at least one of the following adverse events between Day 0 (Visit 2) and Day 28 (Visit 8):

- Cough and spasmodic cough of grade 2 or higher.
- Other respiratory tract AE related or possibly related to vaccination of grade 3 or higher.
- Any other AE related or possibly related to vaccination of grade 3 or higher.

XIV.2. SECONDARY SAFETY ENDPOINT

The second safety endpoint will be the number and proportion of participants per dose group and randomized allocation, with:

- at least one episode of cough or spasmodic cough, described by grade, between Day 4 and Day 28
- at least one solicited or unsolicited AEFI from respiratory tract between Day 0 and Day 28
- at least one AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia) between Day 0 and Day 28
- at least one other AEFI from the respiratory tract between Day 0 and Day 28
- at least one episode of cough, described by grade, between Day 0 and Day 28
- at least one episode of spasmodic cough, described by grade, between Day 0 and Day 28
- at least one solicited systemic AEFI between Day 0 and Day 28 (fever, headache, tiredness)
- at least one unsolicited AEFI between Day 0 and Day 28

Adverse events following immunization (AEFI) should be reported at study visits during the first 28 days of the concerned subject.
Serious adverse events (SAE) should be reported immediately but no later than 24 hours after detection. AEFI which lead to difficulties in breathing (asthma, spasmodic cough, pneumonia) will be considered as serious and reported to the Data Safety Monitoring Board (DSMB) and the sponsor.

XIV.3. OTHER SECONDARY ENDPOINTS

1) Colonization of the BPZE1 strain in the nasopharyngeal mucosa:

Per dose, group and randomized allocation:

- Number and proportion of participants who have been colonized by the modified B. pertussis strain BPZE1 at each post-vaccination visit
- Number of visits during which bacteria are detected
- Magnitude of bacterial colonization (number of cfu) per post-vaccination visit

2) Immunogenicity:

Per dose, group and randomized allocation:

- IgG and IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in serum at visits 2, 4, 6, 7, 8 and 9
    - Geometric mean levels
    - Number and proportion of participants with positive responses, defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD (minimum level of detection) after vaccination

Sera will be tested blindly for pertussis specific antibodies in batch for each group. Antibody levels to PT, FHA, PRN, and fimbriae 2/3 will be expressed in IU/mL calibrated against reference antisera from the National Institute for Biological Standards and Controls.

XIV.4. EXPLORATORY ENDPOINTS

- IgG and IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in nasopharyngeal aspirate at visits 2, 4, 6, 7, 8 and 9
    - Geometric mean levels
    - Number and proportion of participants with positive responses, defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD after vaccination
- Median levels of cytokines in nasopharyngeal aspirate
- Median levels of cytokines in cell culture supernatants after stimulation of PBMC with PT, FHA, PRN and unrelated antigen

XV. Statistical considerations

XV.1. Sample Size Considerations

Acknowledging that due to the small sample size, the power of Phase I trials is in general very low, and that only frequent events can be detected with sufficient power, 12 volunteers receiving active vaccination per dose level in groups 1, 2 and 3 constitute a trade-off between detectable event rate and power in the context of a phase I trial (Buoen, J Clin Pharmacol 2003). A sample size of 12 volunteers allows for observing at least one primary safety endpoint event with 80% power if the underlying event rate is at least 12.6%.
If no primary safety endpoint event is observed among 12 volunteers, the upper bound of a two-sided 95% confidence interval for the event rate would be 26%.

If no primary safety endpoint event is observed among 36 volunteers (pooled across groups 1,2,3), then the upper bound of the 95% confidence interval for the event rate would be 10%.

XV.2. Statistical methods

XV.2.1. Analysis strategy

Statistical analyses will be performed by the EUCLID Clinical Trials Platform (except for the interim analysis of the colonization and safety data when culture results are available from twenty-eight days after vaccination of the high dose group 3, mentioned below).

All participants having received at least on injection will be included in the analysis. All primary analyses will be performed per dose group and randomized allocation (for arms receiving active vaccination) and for the pooled placebo arms (unless there is evidence for a time trend in placebo observations between group).

The main analysis of the primary endpoint is conducted:

- By modified intention to treat: This means that all randomized participants who received a vaccine dose are included in the analysis in the group and intervention arm to which they were initially randomized and all their data is used regardless of protocol deviations during the trial/study.
- In this analysis, participants with missing follow-up data will be assumed to be event-free, unless the site has any knowledge of a safety event having occurred and has notified this in the e-CRF.

No statistical comparisons between dose groups or randomized allocations will be performed but two-sided 95% confidence intervals will be displayed..

Intra-group statistical comparisons will be performed for immunogenicity markers.

In ancillary analyses, appropriate statistical models for the analysis of longitudinal data may be used to estimate the slopes of changes in immunogenicity markers per group. Methods for high-dimensional data may be used to assess the interrelationships between different immunogenicity markers.

Quantitative variables

Quantitative variables will be described in terms of absolute frequency, median, interquartile range, minimum, and maximum.

Intra-group comparisons will be made using paired Student t-test or Wilcoxon paired signed-ranks test according to the distribution of variable of interest for analyzing change from baseline to post-vaccination within a given dose group or randomized allocation. Transformations to normalize the variables can be made if necessary.

Qualitative variables

Qualitative variables will be described in terms of number, proportion and exact binomial confidence interval of proportion.

Intra-group comparisons will be made using McNemar test or other, depending on the number of modalities of the variables of interest.

Titers

Geometric means and their 95% confidence interval will be calculated for titers.

XV.2.2 Analysis software

Analyses will be performed using SAS® software (version 9.3 or higher) and R software.

XV.2.3 Analysis plan

A detailed statistical analysis plan will be established before the first statistical analyses of the trial.

Descriptive statistics will be provided for demographic and other baseline characteristics.

Hematology

Pre- and post-vaccination values of cell blood counts (haemoglobin, total and differential WBC, RBC and platelets) will be described for each group separately. Within group comparisons may be done using appropriate tests for paired measurements.

Primary Safety endpoint

The primary endpoint wil be described per group and randomized allocation, in modified ITT population, with participants with missing follow-up data considered event-free in primary analysis. Numbers, percentages and 95% confidence intervals will be shown. Each individual component of the primary endpoint will also be described.

Secondary endpoints

Secondary endpoints will be described per group and randomized allocation, displaying numbers, percentages and 95% confidence intervals (qualitative variables); medians and interquartile intervals (quantitative variables) or geometric mean and 95% confidence interval (antibody titers).

Number, time of occurrence and duration of secondary safety endpoints will be described.

Colonization endpoints will be described (number and proportion of participants with colonization at each post-vaccination visit; number of visits during which bacteria are detected; magnitude of bacterial colonization (number of cfu) per post-vaccination visit)

Pre- and post- vaccination serum IgG and IgA antibody endpoints will be described per group and randomized allocation. Within group comparisons may be done using appropriate tests for paired measurements.

Cough, spasmodic cough and any symptom from the respiratory tract, as well as antibody titers, will be described for each of the vaccine groups and the pooled placebo group, and for the groups of colonized and non-colonized individuals.

Exploratory endpoints

Pre- and post- vaccination nasopharyngeal IgG and IgA antibodies endpoints will be described per group and randomized allocation. The median of cytokines levels in nasopharyngeal aspirate will be displayed for pre- and post-vaccination samples in each group. The median levels of T cell responses by measuring cytokines after stimulation with PT, FHA, PRN and unrelated antigen will be displayed for pre- and post-vaccination samples in each group. Intra-group comparisons by statistical tests for paired samples will be done for each dose group and randomized allocation. Multiplicity adjustments methods may be considered for the analysis of high dimensional immunogenicity assays.

In addition, a description of exploratory immunogenicity endpoints per groups of colonized versus non-colonized individuals will be performed.

Interim analyses

Unblinded interim safety analyses using descriptive statistical methods for the DSMB will be performed when all volunteers of a given dose group will have reached day 28, before vaccination of the next dose group (next dose). These interim reports will be prepared by a statistician at EUCLID.

In addition, an interim analysis of the final colonization and safety data in groups 1, 2 and 3 until D28 will be performed by a statistician not involved in any other trial procedures (outside of EUCLID), when culture results are available from twenty-eight days after vaccination of the high dose group 3. This interim analysis will provide unblinded summary data only (i.e. no information about individual volunteers) for the sponsor/ILiAD to present to the FDA and potentially other regulatory agencies to aid in designing a Phase II study.

XVI. Administrative and regulatory requirements

XVI.1. Study documents and retention of records

Study documents include CRF, "*Serious Adverse Event*" Forms, data correction/ request forms, source documents, study books and appointment schedules, investigator correspondence and regulatory documents (*e.g. confidentiality agreement, signed protocol and amendments, Ethics Committee correspondence and approval, approved and signed consent forms, receipts for clinical supplies and records for their dispensing*).

Source documents include all recorded observations or notes of clinical activities and all reports and records necessary for the evaluation of the clinical study. They include, but are not limited to, laboratory reports, progress notes, and any other reports or records of procedures performed in accordance with the protocol. The Diary is a source document.

Whenever possible, the original recording of an observation should be retained as a source document. However, a photocopy is acceptable if certified by the investigator, provided it is clear, legible and an exact duplication of the original document.

The investigators and/or institutions will have to permit study-related monitoring, audits, Independent Review Board and/or Independent Ethic Committee review and regulatory inspections, providing direct access to source data and source documents.

The investigator will have to retain the study documents for 15 years after completion or discontinuation of the trial unless specified otherwise by the sponsor for a longer retention period.

XVI.2. Recording of data

The subject medical journal and the Diary are source documents.

However, study-related data obtained during the study visit and which, from a medical perspective, are not essential for subject follow up in routine medical practice, may be recorded directly in the CRF (source data). All study data location will be described in the "source data location form". All data in the protocol will be documented in the CRF.

XVI.3. Data quality assessment and informed consent

Electronic CRF will be provided by EUCLID on behalf of the sponsor to record all participant data.

Periodically, a CRA working on behalf of INSERM will review study documents to verify compliance with the protocol, GCP and the accuracy of the data referring to the source documents.

Periodicity of the monitoring visits will be defined in the monitoring plan.

All information requested in the CRF will have to be completed. Whenever information is not available, this should be noted.

To avoid possible inconsistencies, logical controls and tests of validation will be carried out on this database which will be subject to both human visual and computer driven procedures in order to maximize logical consistencies in the collected CRF data.

Inconsistencies will generate queries, which will be sent to the investigator. The investigator or delegate will have to answer queries and sign them to authorize any database modification.

The data manager will apply self-evident corrections (SEC) in the database following rules defined in the SEC Plan validated and signed by the sponsor and the site investigator prior to implementation.

Written informed consent must be obtained from the subject before participation in the study and before any study related procedure commences (e.g. collection of blood sample). The signature on the ICF must be dated by the subject. Consent form must be dated and signed by the principal investigator or sub-investigator.

One signed consent must be kept by the investigator or delegate and a copy of the signed consent must be given to the subject.

XVI.4. DATA MANAGEMENT

The study will be reported to the person responsible for handling with personal identifiers under the Swedish law ("personuppgiftslagen") at the Karolinska University Hospital.

All data management activities and responsibilities' are summarized in the data management plan. The Data management procedure will be performed in accordance with ICH guidelines and Standard Operating Procedures and conducted in accordance with good clinical, scientific and data management principles.

Case report form – data management software

The data management software used is Clinsight® (CS) version 7 developed by the company Ennov-Clinsight. The software Clinsight® has been developed to meet the regulatory requirements. The software complies with the FDA requirements regarding IT systems used in clinical trial ("Guidance for Computerized systems Used in Clinical Trials") as well as electronic signature ("21CFR part 11") and various international norms (CDISC/CDASH, ICH, GCP 2001/20/CE).

Data entry

The study will be conducted by the Karolinska Trial Alliance, where investigators and study nurses will enter data on vaccinations and adverse events following immunization in the CRF. If the investigator authorizes other persons in their staff to make entries on the eCRF, the names, positions, signatures and initials must be documented in writing (e.g., site delegation tasks form). eCRF must be completed during/after each study visit. All persons entering data in the eCRF must be trained beforehand and appointed to do this task.

The eCRF is developed via Clinsight® software by the Data Manager (DM) of the study at EUCLID. Access to the eCRF for data entry or review will require distinct individual access code assigned uniquely to the site staff members who will be entering study data and those involved in study oversight who may review study data. The sponsor and site monitors have "read only" access to data collected in the eCRF exclusively.

Volunteers diaries and immune response data

The data in diaries will be reviewed for legibility before entered into the eCRF.

Approved data of the immune response determinations will be entered into the eCRF.

Database closure

When all data are received, all data problems are solved and all data checks and quality controls have been performed, a data review meeting has been held, the study database is considered to be clean and can be locked via Clinsight software.

XVI.5. ETHICS COMMITTEE

Karolinska Trial Alliance, Karolinska University Hospital will submit an application to the relevant Swedish Ethics Committee for approval of the clinical study. A copy of the Ethics Committee's approval must be received by INSERM and the Public Health Agency of Sweden before study starts.

Any substantial amendment that becomes necessary during the course of the study and approved by the sponsor must be submitted to the same Ethics Committee and needs to receive a positive approval before entering into force (*in case of refusal, the subjects will still have to be followed*).

Any minor change or modification (*e.g., modification of clinical supply procedure with no change in dose and/or route and/or schedule of administration*) will be notified to the Ethics Committee for information only.

INSERM (or delegate) will notify the end of the clinical study to the Ethics Committee within 90 days following the end of the clinical study.

XVI.6. HEALTH AUTHORITIES

INSERM (or delegate) will submit an application to the relevant Swedish Health Authorities for approval of the clinical study. A copy of the Health Authorities approval must be received by INSERM (or delegate) before study starts.

Any substantial amendment that becomes necessary during the course of the study must be submitted to the same Health Authorities and needs to receive a positive approval before entering into force (*in case of refusal, the subjects will still have to be followed*).

Any minor change or modification (*e.g., modification of clinical supply procedure with no change in dose and/or route and/or schedule of administration*) will be notified to the Health Authorities for information only.

INSERM (or delegate) will notify the end of the clinical study to the Health Authorities within 90 days following the end of the clinical study.

XVI.7. CONFIDENTIALITY

By signing this protocol, the principal investigator undertakes that the protocol and all attached information are and will be kept confidential. The Principal investigator agrees that after providing the protocol and all information attached to the Ethics Committee, affiliated institution and/or specified employees, he/she remains responsible for their overall confidentiality. Such obligation is detailed in the confidentiality agreement signed by the investigator prior to the initiation of the study.

The Principal investigator agrees that, subject to local regulations and ethical considerations, an INSERM representative or any regulatory agency may consult directly and/or copy study documents in order to verify a case report, provided that the subject's identity remains anonymous.

The investigator undertakes to treat all subjects' data used or disclosed in connection with the conduct of study in compliance with European and Swedish applicable laws relating to data protection.

XVI.8. COMPLIANCE WITH LAW AND AUDIT

The investigator and INSERM agree to conduct the study in an efficient and diligent manner in accordance with this protocol, ICH Good Clinical Practices standards, Declaration of Helsinki and applicable regulatory requirements [see GCP 6.2.5] as well as any European and Swedish applicable laws and regulations relating to the conduct of the study.

The principal investigator will prepare and maintain complete and accurate study documents for each subject participating in the study. The investigator will promptly submit to INSERM (or delegate) reports as required by this protocol following completion or termination of the clinical study or as otherwise required by INSERM.

Study documents and source documents will be promptly and fully disclosed to INSERM by the investigator upon request and will be made available at the investigational site upon request for inspection, copying, review and audit by INSERM representatives or any regulatory agency representatives, provided that the subject´s identity remains anonymous.

Persons prohibited from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on studies sponsored by INSERM. The investigator will immediately inform INSERM in writing if any person involved in conducting the study is prohibited from conducting or working on clinical studies by any court or regulatory agency or if any proceeding against this person is pending.

XVI.9. INSURANCE

The Swedish Patient Insurance Scheme, i.e. Patientskadelagen (SFS 1996:799) covers all subjects in the clinical trial in a similar way as during other medical care. INSERM as sponsor takes out an insurance (Responsabilité Civile) for the duration of study.

XVI.10. PUBLICATIONS AND DATA PROPERTY

Intellectual property rights and dissemination activities including but not restricted to publications and presentations shall be governed by the rules defined in the Agreement between INSERM and the Public Health Agency of Sweden.

XVII. Study management

XVII.1. Training of study site personnel

The Public Health Agency of Sweden will provide SOPs describing the procedures for handling, and storage of the study vaccine, vaccination, sample collection, transport and storage.

The procedure for vaccination and collection of nasopharyngeal specimens will be demonstrated by staff from the Public Health Agency of Sweden.

XVII.2. Monitoring of the study

The study will be performed in compliance with the Protocol, the Declaration of Helsinki and the GCP guidelines.

All documents related to quality procedures will be monitored according to the procedures of the sponsor.The clinical research associate (CRA) in charge of the quality control is mandated by the sponsor. He has access to individuals data strictly required for this control. The CRA is obliged to respect professional secret. Each visit will be followed by a complete written report.

At least an initiation visit, one follow-up visit after each escalating doses and a close-out visit will be performed.

Initiation visit:

An initiation visit will be performed before the inclusion of the first subject.

The CRA shall ensure that:

- The material and the documents to be used during the clinical trial have been received
- The investigational team has been properly informed about the study, regulatory requirements, and all applicable SOPs.

Follow-up visits:

The CRA will carry out regular follow up visits according to the monitoring plan.

The investigator commits himself to be available for these visits and to allow the monitoring staff direct access to subject medical files and eCRFs.

During the visits, the CRA will:

- Carry out a quality control of the clinical trial progress,
- Review compliance of the clinical trial with the protocol and SOPs, data collection, signature of consent forms, reporting of adverse events, sample and product management.
- Review the completed eCRFs.

The CRA will discuss any problem with the investigator and define with him the actions to be taken.

Close-out visit:

A close-out visit will be performed at the end of the clinical trial to make sure that:

- The centre has all the trial documents necessary for archiving
- All data have been collected and all data queries are resolved
- All samples have been shipped and adequately stored

Quality control modalities will be detailed in the Monitoring Plan.

XVIII. List of appendices

Appendix 1. A Phase I Clinical Study of a Live Attenauted *Bordetella pertussis* Vaccine - BPZE1; A Single Centre, Double-Blind, Placebo-Controlled, Dose-Escalating Study of BPZE1 Given Intranasally to Healthy Adult Male Volunteers (2014) PLoS One 9(1); E83449

Appendix 2. Environment risk assessment for *B. pertussis* strain BPZE1

Appendix 3 Copy of official French agreement on the downgrading of BPZE1 as a class I organism

Appendix 4. FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

C14-80 – BPZE1 PHASE IB TRIAL

PHASE IB (HIGH DOSE), DOUBLE-BLIND, SINGLE CENTRE, DOSE-ESCALATING, PLACEBO-CONTROLLED, RANDOMIZED STUDY OF A LIVE ATTENUATED B. PERTUSSIS STRAIN GIVEN AS A SINGLE INTRANASAL DOSE TO HEALTHY ADULT VOLUNTEERS

TABLE OF APPROVALS

	Developed by	Validated by				Approved by
Name Position	Céline Colin EUCLID Statistician Dr. Laura Richert EUCLID Methodologist	Dr. Céline Roy EUCLID Project leader	Gabriella DEROCLE Sponsor	Funder	Scientific Responsible (Camille Locht)	Pr. Geneviève Chêne EUCLID Methodologist
Date Signature	05/09/2016	05/09/2016 p.o.	13/09/2016	14/09/2016	21/10/2016	24/10/2016

REVISIONS		
Version		**Justification**
1.0	03/11/2015	Creation
2.0	14/03/2016	Section 3.11 filled by Dr Gary Stevens
3.0	05/09/2016	Amendment to the protocol with a 12-months visit added

DISSEMINATION	EUCLID, Sponsor and the principal investigator
DISSEMINATION METHODS	▪ Electronic: in the "Statistics" folder of the trial ▪ Original signed paper version stored by the trial statistician
IMPLEMENTATION DATE	31/10/2016

CONTENTS

ACRONYMS

AEFI	Adverse Event Following Immunization
AR	Adverse Reaction
BMI	Body Mass Index
CI	Confidence Interval
CTU	Clinical Trials Unit
e-CRF	Cahier d'observation électronique/Case Report Form
EUCLID	EUropean CLInical trials & Development
FDR	False Discovery Rate
ICH	International Conference on Harmonisation
INSERM	Institut National de la Santé Et de la Recherche Médicale
IQR	Interquartile Range
MCAR	Missing Completely At Random
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified Intent To Treat
MLD	Minimum Level of Detection
N	Frequencies
PT	Preferred Terms
RBC	Red Blood Cells
SAE	Serious Adverse Event
SOC	System Organ Class
WBC	White Blood Cells

DEFINITIONS

The analysis plan is a document that contains a more detailed and technical description of the main aspects of the analysis outlined in the protocol, and defines the terms of the statistical analysis conducted for primary and secondary endpoints as well as for other data (1).

SCOPE

This document applies to the statistical analysis of the trial. It includes main final analysis, as well as statistical reports to DSMB and interim analysis.

1. TRIAL SUMMARY

1.1. TRIAL OBJECTIVES

1.1.1. MAIN OBJECTIVE

To assess the general safety and tolerability in the respiratory tract including specific adverse events such as cough/spasmodic cough, during the 4- 28 days after vaccination by a single ascending dose of the genetically modified *B. pertussis* strain BPZE1 in healthy adult volunteers.

1.1.2. SECONDARY OBJECTIVES

To assess after vaccination by a single ascending dose of the genetically modified *B. pertussis* strain in healthy adult volunteers:

1. the safety and tolerability of the vaccine over 6 months (group 1-4) and then 12 months (group 1-3) post-vaccination
2. the frequency of colonization of the human respiratory tract by live attenuated *B. pertussis* strain and its magnitude (in terms of colony forming units) over 28-days post-vaccination.
3. the levels of serum IgG and IgA antibodies to 4 different *B. pertussis* antigens (*pertussis* toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3) over 6 months (group 1-4) and then 12 months (group 1-3) after vaccination.

Exploratory objectives

To assess the B and T cell immune responses to 4 different *B. pertussis* antigens (*pertussis* toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae 2/3) over -12 months after vaccination by :

- the levels of IgA in nasopharyngeal aspirate.
- the cytokine levels in nasopharyngeal aspirate.
- the levels of secreted cytokines as well as the number of pertussis-specific T- and memory B-cells after stimulation of PBMC with *B. pertussis* antigens and unrelated antigens.
-

To explore the frequency and magnitude of colonization and as well as antibody, B and T cell responses in a small group of healthy adult volunteers with high PRN antibody levels (only highest vaccine dose).

1.2. TRIAL DESIGN

1.2.1. DESIGN/METHODS

This is a phase Ib, double-blinded, single center, dose-escalating study with consecutive dose groups, and a randomized placebo control group in each dose group (Groups 1 – 3). Participants will be randomized between active vaccine and placebo in a 3:1 ratio.

The volunteers will be recruited in a step-wise fashion with 16 individuals in each of three groups.

- Group 1: 12 individuals will be vaccinated once intranasally with 10^7 cfu of the organism. 4 individuals will be randomized to placebo (the diluent alone).
- Group 2: 12 individuals will be vaccinated once intranasally with 10^8 cfu of the organism. 4 individuals will be randomized to placebo (the diluent alone).
- Group 3: 12 individuals will be vaccinated once intranasally with 10^9 cfu of the organism. 4 individuals will be randomized to placebo (the diluent alone).

The administration of vaccine or diluent alone will be performed in a double-blind fashion with administration of coded vials (see below).

The volunteers in each group will be included sequentially in the study and vaccinated according to the following schedule.

The 16 individuals will be randomized as described in section VI.4 "Screening number and randomization procedure" of the protocol.

- Day 1: 2 individuals with at least 4 hours interval
- Day 2: 2 individuals with at least 4 hours interval
- Day 3: 3 individuals with at least 2 hours interval
- Day 4: 4 individuals with at least 1 hour interval
- Day 5: 5 individuals with at least 1 hour interval

Interim safety meetings with the DSMB for recommendation to the sponsor will be held before administering the next dose. At the interim safety meetings, safety data covering 28-days of post-vaccination follow-up of group 1 and group 2, respectively, will be available. Taking into account the time for the DSMB to meet and to give its recommendations for dose escalation, group 1 volunteers will have been followed up during at least 6 weeks by the time the group 2 volunteers become vaccinated. Likewise, group 2 volunteers will have been followed up during at least 6 weeks by the time the group 3 volunteers become vaccinated.

A non-controlled group (group 4) consisting of 6 individuals will be vaccinated with 10^9 cfu of the organism (open-label) to investigate the frequency of colonization in subjects with high anti-PRN antibody levels (≥20 IU/ml).

1.2.2. TRIAL TIMELINE

Site opening	: August 2015
First Visit First Subject (group 1)	: September 2015
First randomization (group 1)	: September 2015
First Visit First Subject (group 2)	: October 2015
First randomization (group 2)	: November 2015
First Visit First Subject (group 3)	: January 2016
First randomization (group 3)	: January 2016
First Visit First Subject (group 4)	: March 2016
First vaccination (group 4)	: March 2016
First Visit Last Subject	: February 2016
Last Visit Last Subject	: February 2017
Antibody assays	: August 2015 – April 2017
Exploratory laboratory assays	: April 2016 – December 2017
Final statistical analysis	: October 2016 – June 2017
Clinical study report	: February 2017 and then August 2017

1.3. SAMPLE SIZE

Acknowledging that due to the small sample size, the power of Phase I trials is in general very low, and that only frequent events can be detected with sufficient power, 12 volunteers receiving active vaccination per dose level in groups 1, 2 and 3 constitute a trade-off between detectable event rate and power in the context of a phase I trial (Buoen, J Clin Pharmacol 2003). A sample size of 12 volunteers allows for observing at least one primary safety endpoint event with 80% power if the underlying event rate is at least 12.6%.

If no primary safety endpoint event is observed among 12 volunteers, the upper bound of a two-sided 95% confidence interval for the event rate would be 26%.

If no primary safety endpoint event is observed among 36 volunteers (pooled across groups 1, 2, 3), then the upper bound of the 95% confidence interval for the event rate would be 10%.

1.4. RANDOMIZATION AND BLINDING

Vaccine and placebo will come in identical, coded vials.

The vials will be coded with group number and vial number: group1: 101 – 116, group 2: 217 – 232, group 3: 333 – 348 and group 4: 449 -454, for the respective groups described above under point VI.2 "General study plan and design" of the protocol.

To ensure randomized allocation, the vials must be used by the site in their consecutive order of vial numbers within a given dose group. The vial number given to the volunteer (and the time of administration) will be recorded both in the CRF and the medical record (source data).

Vaccine and placebo are not distinguishable when frozen, allowing for allocation concealment, and are not distinguishable at the 10^7 dose level when thawed. For higher dose levels (10^8, 10^9), where vaccine and placebo may be distinguishable when thawed, observer-blinding will be established, by the fact that the vaccine will be prepared and administered by a study nurse not involved in any other trial procedures.

The randomization list, establishing the order of vials and the correspondence between vial number and active vaccine or placebo group will be generated centrally by the statistician at the EUCLID clinical trials platform prior to the start of the study and kept confidentially in a secure environment.

The list with the codes/information if a vial contains vaccine or placebo will only be available at the statistics center (EUCLID), Lund University Hospital pharmacy (for emergency unblinding), at the manufacturer's site (Q Biological; for labelling the vials) and at the sponsor pharmacovigilance site. The decision whether the code shall be broken for an individual volunteer or all participants will be made by the investigator.

For purposes of interim analyses, the statistician responsible for the analysis will maintain the codes in strict confidence with no communication of any individual codes until final data lock.

An identical series of back up vials will be generated for each group. These vials will be kept and stored at the manufacturer's site.

Since access to results of the cultivation of BPZE1 from the nasopharynx samples and the antibody determination may lead to treatment unblinding, these results will be strictly controlled within the respective laboratory. The access of these results outside the laboratory will be contingent on having a previous access to the randomization list and therefore will only be authorized after the completion of the study, with the exception a statistician in order to perform an interim analysis after 28-days follow-up. At the end of the trial, the study will be unblinded after all safety and laboratory results from 6 months follow up are monitored and the data bases are locked. The volunteers will be blinded until after the 12 month-follow up.

Unblinded interim reports for the DSMB will be prepared before escalation to the next dose by the trial statistician. An additional interim analysis will be performed when culture results are available from twenty-eight days after vaccination of the high dose group 3 and 4, respectively. For the latter, another statistician will get access to the randomization list for groups 1-3 to provide a summary of safety and colonization data to the Sponsor/ILiAD for use in discussion with regulatory agencies about a Phase II study. The interim analysis will provide no information about individual volunteers that could reveal the vial codes and the statistician performing the interim analysis will not be involved in any other trial procedure or in the final statistical analyses.

A clinical study report containing data of the primary and secondary end-points will be prepared when the safety and laboratory results from 6 months follow up are monitored and the data bases are locked. A separate report will be prepared containing the results of the 12 months follow up.

2. STATISTICAL ASPECTS

Final statistical analyses and interim reporting to the DSMB will be performed by the EUCLID Clinical Trials Platform. Confidential statistical analysis for the FDA during the ongoing trial, including safety and colonization data up to Day 28 in groups 1, 2 and 3, will be performed by a statistician outside of EUCLID, designated by Iliad.

2.1. ANALYTICAL STRATEGIES AND STUDY POPULATIONS

Exclusion from the analysis can be considered for those participants who meet, in particular, one of the following conditions:

- participants who never exposed to the trial intervention (provided they do not have knowledge of the group to which they were randomized);
- participants who withdrew their consent;
- participants who were wrongly enrolled due to major eligibility criterion(a) not being met

The decision to exclude a participant from the analysis is taken by the Executive Committee after documentation by the Clinical Trials Unit, blinded to study intervention and to the participants' evolution after enrolment.

2.1.1. PRIMARY SAFETY ENDPOINT

- **Definition**

The primary safety endpoint will be the number and proportion of participants, per dose group and randomized allocation, with at least one of the following adverse events between Day 0 (Visit 2) and Day 28 (Visit 8):

- Cough and spasmodic cough of grade 2 or higher.
- Other respiratory tract AE related or possibly related to vaccination of grade 3 or higher.
- Any other AE related or possibly related to vaccination of grade 3 or higher.

- **Strategy for the main analysis**

The main analysis is conducted:

- By modified intention to treat: This means that all randomized participants who received a vaccine dose are included in the analysis in the group to which they were initially randomized and all their data is used regardless of protocol deviations during the trial/study.
- In this analysis, participants with missing follow-up data will be assumed to be event-free, unless the site has any knowledge of a safety event having occurred and has notified this in the e-CRF.

- **Strategy for secondary analyses**

- With the strategy of *definitely missing follow-up data (i.e. premature termination of follow-up) = failure*.
  (For non-compliant participant, it will be assumed that no safety event occurred during the missing visits)

2.1.2. SECONDARY SAFETY ENDPOINT

The second safety endpoint will be the number and proportion of participants per dose group and randomized allocation, with:

- at least one episode of cough or spasmodic cough, described by grade, between Day 4 and Day 28
- at least one solicited or unsolicited AEFI from respiratory tract between Day 0 and Day 28
- at least one AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia) between Day 0 and Day 28
- at least one other AEFI from the respiratory tract between Day 0 and Day 28
- at least one episode of cough, described by grade, between Day 0 and Day 28
- at least one episode of spasmodic cough, described by grade, between Day 0 and Day 28
- at least one solicited systemic AEFI between Day 0 and Day 28 (fever, headache, tiredness)
- at least one unsolicited AEFI between Day 0 and Day 28

AEFI should be reported at study visits during the first 28 days of the concerned subject.

SAE should be reported immediately but no later than 24 hours after detection. AEFI which lead to difficulties in breathing (asthma, spasmodic cough, pneumonia) will be considered as serious and reported to the DSMB and the sponsor.

- **Analysis strategy**

Analytical strategies will be the same as the primary endpoint analytical strategies.

2.1.3. OTHER SECONDARY ENDPOINT

1) Colonization of the BPZE1 strain in the nasopharyngeal mucosa:

Per dose, group and randomized allocation:

- Number and proportion of participants who have been colonized by the modified *B. pertussis* strain BPZE1 at each post-vaccination visit
- Number of visits during which bacteria are detected
- Magnitude of bacterial colonization (number of cfu) per post-vaccination visit
- Cough, spasmodic cough and any symptom from the respiratory tract will be described for colonized and not colonized participants

2) Immunogenicity:

Per dose, group, colonized/not colonized and randomized allocation:

- IgG and IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in serum at visits 2, 4, 6, 7, 8, 9 and 10
    - Geometric mean levels
    - Number and proportion of participants with positive responses, defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD after vaccination

Samples will be tested blindly for *pertussis* specific antibodies in batch for each group. Antibody levels to PT, FHA, PRN, and fimbriae 2/3 will be expressed in IU/mL calibrated against reference antisera from the National Institute for Biological Standards and Controls.

- **Analysis strategy**

The immunogenicity criteria analysis will be performed on the mITT population and based on available data (without missing data handleling) with the hypothesis of MCAR.

A robustness analysis will be performed and missing values will be imputed by the median of immunogenicity values in the placebo group.

2.1.4. EXPLORATORY ENDPOINT

- IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in nasopharyngeal aspirate at visits 2, 4, 6, 7, 8, 9 and 10
  - Geometric mean levels
  - Number and proportion of participants with positive responses, defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD after vaccination
- Median levels of cytokines in nasopharyngeal aspirate
- Median levels of cytokines in cell culture supernatants after stimulation of PBMC with PT, FHA, PRN and unrelated antigen
- Median number of spot-forming units determined by ELISpot/FluoroSpot assay of pertussis-specific cytokine-positive T-cells after stimulation of PBMC with PT, FHA, PRN and unrelated antigens.
- Median number of spot-forming units determined by ELISpot assay of pertussis-specific IgG-producing memory B-cells after stimulation of PBMC with PT, FHA, PRN and unrelated antigens.

2.1.5. INTERIM REPORTING TO DSMB

Interim safety reports using descriptive statistical methods for the DSMB will be performed when all volunteers of a given group will have reached day 28, before opening enrolment to the next cohort (next higher dose).

2.1.6. CONFIDENTIAL INTERIM ANALYSIS FOR DECISION MAKING FOR PHASE II DESIGN

An analysis of the safety data and BPZE1 nasopharyngeal colonization data will be performed when culture results are available from twenty-eight days after vaccination of the last volunteer in the high dose group 3 and include analyses of the safety endpoints and the colonization endpoints up to D28 in groups 1, 2 and 3. The analysis report will provide summary data only (i.e. no information about individual volunteers) for ILiAD to present to regulatory agencies and expert consultants to aid in designing a Phase II study and its financial requirements. Since the long-term follow-up in the trial will still be ongoing at that time, this analysis will be performed by a statistician not involved in the rest of the trial procedures and analysis (i.e. a statistician designated by ILiAD outside of the EUCLID platform). The Interim Analysis statistician will provide the summary data to the VP of Development as a representative of ILiAD, who will only share this data on a need to know basis with regulatory agencies, as well as employees, consultants, or prospective partners of ILiAD who have signed a confidentiality agreement. In order not to bias the conduct of the long-term follow-up and other trial procedures of the ongoing trial, the Interim Analysis data will not be shared with any individuals directly involved with executing the Phase Ib study until after data lock.

2.2. STATISTICAL METHODS

2.2.1. DESCRIPTIVE ANALYSIS

The frequency and proportion of available data are described for each variable.

Quantitative variables will be described in terms of absolute frequency, mean, standard deviation, confidence interval of the mean, median, interquartile range, minimum, and maximum. Geometric means and their confidence interval will be performed for titers.

Qualitative variables will be described in terms of number, proportion and exact binomial confidence interval of proportion.

Descriptive analysis is performed overall and by intervention group.

2.2.2. COMPARATIVE ANALYSIS

No statistical comparisons between arms or cohorts will be performed, as the trial is not designed for such comparisons. Intra-group comparisons will be performed for immunogenicity endpoints.

Intra-group comparisons for quantitative variables will be made using paired Student t-test or Wilcoxon paired signed-ranks test according to the distribution of variable of interest for analyzing change from baseline to post-vaccination within a given cohort arm. Transformations to normalize the variables can be made if necessary.

Intra-group comparisons for qualitative variables will be made using McNemar test or other, depending on the number of modalities of the variables of interest.

2.3. CALCULATION CONVENTIONS FOR STATISTICAL ANALYSIS

2.3.1. CONVENTIONS FOR TIME CALCULATIONS

- Time between 2 dates **in months** $= \frac{(\text{Date 2} - \text{Date 1})}{30.4375}$
- Time between 2 dates **in years** $= \frac{(\text{Date 2} - \text{Date 1})}{365.25}$

2.3.2. CONVENTIONS FOR THE CALCULATION OF THE DIFFERENCE (DELTA) BETWEEN A MEASUREMENT AND THE ENROLMENT VISIT (D00)

The date of the enrolment visit (D00) is defined by the date of vaccination.

The difference to a D00 visit for a measurement taken at a visit posterior to D00 (follow-up visit DXX) will be calculated using the following formula:

- Delta = (measurement at DXX – measurement at D00)

2.3.3. CONVENTIONS FOR BMI CALCULATIONS

- **European** BMI $= \frac{Weight(kg)}{\text{Height}^2(\text{m})}$
- **US** BMI $= \frac{Weight(Pounds) x 703}{Height^2(inches)}$

2.3.4. MISSING DATA

- Conventions for dates

Except in special cases, if the day is missing, the convention is to use the value "15", i.e.mid-month. If the day and month are missing, the convention is to use the value "1" for days and the value "7" for month, i.e.

mid-year. This convention allows us to measure time estimates. The statistician makes these imputations at the time of analysis and consistency is checked at the time of data review.

- Conventions for handling missing data

The following values are used in the database to describe the missing data:

- « NK » or « K »: Unknown
- « ND » or « D »: Not done
- « NA » or « A »: Not applicable

There is no distinction in the statistical analyses according to the type of missing data.

2.4. SOFTWARE USED

The analyses are performed using SAS® software (version 9.3 or higher).

3. ANALYSIS PLAN

3.1. DESCRIPTION OF THE DEVIATIONS TO THE PROTOCOL IDENTIFIED BY THE CLINICAL MONITOR/PROJECT LEADER

3.1.1. ELIGIBILITY CRITERIA ASSESSMENT

- Eligibility criteria not met (n, %)

All eligibility criteria (inclusion and non inclusion criteria) are listed below, with a description of the verification performed by the statistician, where appropriate.

- **Inclusion criteria:**

For all inclusion criteria, the statistician checks that the response to the inclusion criteria is « yes » in the corresponding SAS table. Additional verifications are listed below:

Inclusion criteria	Description of the verification performed by the statistician
1. Healthy male or female between 18 and 32 years of age, vaccinated or unvaccinated with a cellular *pertussis* vaccine.	Age calculated by subtraction of date of inclusion and date of birth.
2. Female subject of child bearing potential must be willing to ensure to use one of the following methods of contraception from 2 weeks before vaccination till one month after vaccination to avoid pregnancy during the study (e.g. complete abstinence from penile-vaginale intercourse, double barrier method, intrauterine device, approved hormonal contraception).	
3. Informed consent form (ICF) signed by the subject.	
4. Subject shall be able to attend all scheduled visits and to understand and comply with the study procedures.	

- **Non inclusion criteria:**

For all non inclusion criteria, the statistician checks that the response to the inclusion criteria is « no » in the corresponding SAS table. Additional verifications are listed below:

Non inclusion criteria	Description of the verification performed by the statistician
1. Individual with PT and /or PRN serum IgG antibodies ≥ 20 IU/ml. NOTE! One control group with PRN serum IgG antibodies ≥ 20 IU/ml and PT serum IgG antibodies<20 IU/ml will be included.	
2. Vaccinated with the study vaccine in the Child Innovac study (EudraCT number 2010-019936-11).	
3. Pregnant or lactating women. Pregnancy not planned and to be avoided during the study by use of effective contraceptive methods.	
4. Blood pressure after resting ≥ 150/90 mm Hg at screening.	
5. Heart rate after resting ≥ 80 bpm at screening.	
6. Respiratory rate after resting ≥ 20/minute at screening.	
7. Unwillingness to refrain from the use of nicotine products from screening through day 28.	
8. Use of narcotic drugs and/or a history of drug/alcohol abuse whitin the past 2 years prior to screening.	
9. The subject has donated blood or suffered from blood loss of at least 450 ml (1 unit of blood) within 60 days prior to screening or donated plasma within 14 days prior to screening.	
10. Receipt of immunoglobulin, blood derived products, systemic corticosteroids or other immunosuppressant drugs within 90 days prior to day 0.	
11. Asthma or other chronic respiratory problems.	
12. Use of corticosteroids in the respiratory tract (e.g. nasal steroids, inhaled steroids) 30 days prior to day 0.	
13. Receipt of a vaccine within the last 30 days prior to day 0 or planned vaccination within the next 30 days after day 0.	
14. Known hypersensitivity to any component of the study vaccine.	
15. Current participation in any other clinical trial or participation (and during the whole study) in any clinical trial in the previous 3 months prior to day 0.	
16. Inability to adhere to the protocol, including plans to move from the area.	

Non inclusion criteria	Description of the verification performed by the statistician
17. Family history (first degree) of congenital or hereditary immunodeficiency.	
18. Past or present infection with HIV, hepatitis B or C.	Check if the laboratory result of "Screening of HIV, hepatitis B and C" is negative.
19. Chronic conditions requiring ongoing active medical interventions, such as diabetes mellitus or cardiovascular disease.	
20. Any autoimmune or immunodeficiency disease/condition (inherited or iatrogenic).	
21. Any medical condition which, in the opinion of the investigator, might interfere with the evaluation of the study objective or might affect the safety of the individual, e.g. evolving encephalopathy not attributable to another identifiable cause within 7 days of administration of a previous dose of any vaccine, hospitalization due to major depression or history of suicidal attempt.	
22. Abnormal laboratory values outside the limit of normal values for the screening laboratory with clinical significance at the discretion of the investigator.	
23. Person in frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc) or residence in the same household as persons with known immunodeficiency including persons on immunosuppressive treatment.	

3.1.2. MAJOR PROTOCOL DEVIATIONS

The other following deviations are described per category:

- Randomized participants who did not receive the trial intervention
- Participants who received the wrong intervention or incorrect dose
- Participants who received a forbidden concomitant intervention. The following therapies should be avoided during the first month after vaccination:
  - Antibacterial agents effective against *B pertussis*.
  - Immunoglobulin, corticosteroids for systemic use or for use in the respiratory tract (e.g. nasal steroids, inhaled steroids), other immunosuppressive agents or immunostimulating agents.
  - *Pertussis* vaccines.
  - Topical nasal therapies.

  If it is necessary to give a medication or a vaccine, this shall be permitted for obvious reasons be permitted. All medications and vaccines will be documented in the case report form (CRF). The volunteer will be followed as pre-planned for adverse events. The immunological part of the study can also continue, but the results from the volunteer should be reported separately in the statistical analyses.

Deviations per participant are described.

3.2. DESCRIPTION OF ENROLMENT AND FOLLOW-UP

Participant enrolment and follow-up are described based on the study profile as defined in the CONSORT recommendations (3).

3.2.1. FLOWCHART

According to CONSORT diagram,

- Participants pre-enrolled (n)
- Pre-enrolled participants not enrolled (n)
- Reasons for non-enrolment (n, %)
- Participants enrolled (n, %)
- Participants having received the trial intervention (n, %)
- Withdrawals (n, %)
- Participants to include in the analysis (n, %)
- Participants to exclude from the analysis (n, %)
- Participants to include in the mITT analysis (n, %)

3.2.2. DESCRIPTION OF SCREENING

- Participants screened (n, %)
- Participants screened/non-enrolled (n, %)
- Reasons for screen failure (n, %)

3.2.3. DESCRIPTION OF ENROLMENT

- Participants enrolled (n, %)
- Enrolment graph (trend in the number of participants enrolled from 1st enrolment to last)
- Enrolled participants per group and randomized allocation (n, %)

3.2.4. DESCRIPTION OF FOLLOW-UP

- Expected visits for to the number of participants enrolled (n, %)
- Visits actually conducted (n, %)
- Ratio of observed/expected visits
- Cumulative follow-up duration, i.e. amount of participation time for each participant enrolled, that is the difference, in days, between the date of enrolment and the date of last contact with the trial. The cumulative follow-up duration is also presented in person-days.

3.2.5. DESCRIPTION OF WITHDRAWALS

- Participant withdrawals (before/after enrolment) (n, %)
- Reasons for withdrawal (before/after enrolment) (n, %)
- Time between randomization date and date of withdrawal
- Participant lost to follow-up (before/after enrolment) (n, %)
- Time between randomization date and date of follow-up
- Deceased participants (n, %)

3.2.6. DESCRIPTION OF PARTICIPANTS WHO DIDN'T TAKE THE TRIAL INTERVENTION

- Enrolled participants who did not take the trial intervention (i.e. vaccination) (n, %)

- Reason for not taking the trial intervention (n, %)

3.3. PARTICIPANTS CHARACTERISTICS AT SCREENING (VISIT 1) AND ENROLMENT (D00)

The following characteristics are described (as numbers for qualitative variables) for all enrolled participants overall, per group (1, 2, 3, 4), and per group and randomized allocation (active vaccine or placebo) in groups 1, 2 and 3:

- Socio-demographic characteristics at visit 1 (screening);
- Clinical characteristics at visit 1 (screening) and visit 2 (D00);
- Biological characteristics at visit 1 (screening) and visit 2 (D00) (whenever planned in the protocol);
- Concomitant treatment characteristics visit 2 (D00).

3.3.1. SOCIO-DEMOGRAPHIC CHARACTERISTICS

- Age (in years; and in classes)
- Gender
- Ethnicity

3.3.2. CLINICAL CHARACTERISTICS

- Anthropometric characteristics
  - Height (in m)
  - Weight (in kg)
  - BMI (in kg/m²; in classes)
- Physical examination
  - Oral temperature (in °C)
  - General appearance (normal/abnormal)
  - Heart (normal/abnormal)
  - Respiratory (lung) (normal/abnormal)
  - Skin (normal/abnormal)
  - Nose/Throat (normal/abnormal)
  - Neurological (normal/abnormal)
  - Abdomen (normal/abnormal)
  - Lymph nodes (normal/abnormal)
  - Other (normal/abnormal)
- ECG examination
  - ECG result (in classes: normal/abnormal, not clinical significant/abnormal, clinical significant)
- Cardiovascular characteristics

- Systolic and diastolic blood pressure (in mmHg)
- Heart rate (in bpm)
- Respiratory rate (in breath per minute)

- Medical history

- Frequency and proportion of participants presenting at least one:
  - Past medical history regardless of its nature
  - Medical history with any relevant medical findings
  - Medical history at pre-enrolment, regardless of grade and grade 3 or 4
  - History of (past or current, and separately):
    - Gastrointestinal
    - Endocrine/Metabolic
    - Cardiovascular
    - Musculoskeletal
    - Nervous
    - Respiratory
    - Eyes, ears, nose and throat
    - Skin
    - Immune/Allergic
    - Blood/Lymphatic
    - Psychiatric
    - Other

3.3.3. BIOLOGICAL CHARACTERISTICS

- Laboratory sampling (taken or not)
  - Blood sample for *pertussis* antibodies
  - Blood sample for clinical chemistry and hematology
  - Blood sample for HIV, hepatis B and C
  - Urine safety analysis
  - Urine drug screen
  - Reason for not have been taken the sample

- Laboratory results
  - Screening of HIV (positive/negative)
  - Screening for hepatitis B (positive/negative)
  - Screening for hepatitis C (positive/negative)
  - Screening for anti-PT *pertussis* IgG antibodies in serum (in classes : < 20 IU/ml; ≥ 20 IU/ml)
  - Screening for anti-pertactin *pertussis* IgG antibodies in serum (in classes : < 20 IU/ml; ≥ 20 IU/ml)

- Urine analysis (in classes: normal/abnormal, not clinical significant/abnormal, clinical significant)

  - U-Erythrocytes
  - U-Leucocytes
  - U-Protein
  - U-Glucose
  - U-Ketones
  - U-Nitrites
  - U-pH

- Drug screen (negative or positive)
  - Cocaine
  - Amphetamine
  - Methylene dioxymeth-amphetamine
  - Morphine
  - Benzodiazepines
  - Cannabis

- Hematology and blood chemistry (in classes: normal/abnormal, not clinical significant/abnormal, clinical significant)
  - Hemoglobin
  - RBC
  - WBC
  - Neutrophil
  - Lymphocyte
  - Monocyte
  - Eosinophil
  - Basophil
  - Platlets
  - Potassium
  - Calcium
  - Sodium
  - Creatinin
  - Albumin
  - Bilirubin
  - Alkaline phosphatase
  - Alanine amino transferase
  - Glutamytransferase
  - C-reactive protein
  - Blood glucose
  - Thyreoidea stim. hormone

3.4. POOLING OF PARTICIPANTS ALLOCATED TO PLACEBO IN GROUPS 1, 2 AND 3

The description of the following characteristics will be used to assess whether participants allocated to placebo are described in groups 1, 2 and 3 can be pooled in the analysis:

- Age at screening (in years),
- Sex,
- BMI at D00 (in kg/m2),
- Occurrence of cough during follow-up in placebo recipients,
- IgG and IgA antibody titers in placebo recipients at 14 and 28 days to the following antigens:
    - PT
    - FHA
    - PRN
    - Fimbriae 2/3

Pooling of placebo recipients for all following analyses will be done if no clinically significant differences between groups (corresponding to different calendar enrolment periods) are observed.

3.5. DESCRIPTION OF SAFETY ASSESSMENTS DURING FOLLOW-UP

3.5.1. HEMATOLOGY

By group, randomized allocation and visit (pre/post vaccination – measured at visits 2, 4, 6, 8, 9 and 10):

- Hemoglobin
- RBC
- WBC
- Platelets

Intra-group comparisons may be made with appropriate tests for repeated quantitative measurements.

3.5.2. ADVERSE EVENTS

Adverse events during the whole follow-up, per group, randomized allocation and grade:

- Number and proportion of participants with at least :
    - one AEFI
    - one AR
    - one SAE
- Number of following AEs:
    - Solicited or unsolicited adverse reaction from respiratory tract
    - AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia)
    - Cough
    - spasmodic cough
    - solicited systemic adverse event
    - unsolicited adverse event

3.6. PRIMARY SAFETY ENDPOINT

The primary endpoints (see definition in chapter 2.1.1) will be described per group and randomized allocation (n, %, and 95% confidence interval).

Each individual component of the primary endpoint will also be described:

- Participants with at least one occurrence of cough or spasmodic cough of grade 2 or higher between Day 0 and Day 28 (n, %, 95% CI)
- Participants with at least one other respiratory tract AE related or possibly related to vaccination of grade 3 or higher between Day 0 and Day 28 (n, %, 95% CI)
- Participants with at least one other AE related or possibly related to vaccination of grade 3 or higher between Day 0 and Day 28 (n, %, 95% CI)

3.7. SECONDARY SAFETY ENDPOINT

Per group and randomized allocation:

- at least one episode of cough or spasmodic cough, described by grade, between Day 4 and Day 28
- at least one solicited or unsolicited adverse reaction from respiratory tract between Day 0 and Day 28
- at least one AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia) between Day 0 and Day 28
- at least one other AEFI from the respiratory tract between Day 0 and Day 28
- at least one episode of cough, described by grade, between Day 0 and Day 28
- at least one episode of spasmodic cough, described by grade, between Day 0 and Day 28
- at least one solicited systemic AEFI between Day 0 and Day 28 (fever, headache, tiredness)
- at least one unsolicited AEFI between Day 0 and Day 28

3.8. OTHER SECONDARY ENDPOINTS

1) Colonization of the BPZE1 strain in the nasopharyngeal mucosa:

Per dose, group and randomized allocation:

- Number and proportion of participants who have been colonized by the modified *B. pertussis* strain BPZE1 at each time point
- Number of days during which bacteria are detected
- Quantity of bacterial colonization (number of cfu) per post-vaccination visit
- Cough, spasmodic cough and any symptom from the respiratory tract will be described for colonized and not colonized participants

2) Immunogenicity:

Per dose, group, colonized/not colonized and randomized allocation:

- IgG and IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in serum at visits 2, 4, 6, 7, 8, 9 and 10
    - Geometric mean levels and 95%CI for pre and post vaccination (expressed in IU/mL calibrated against reference antisera from the National Institute for Biological Standards and Controls)

- Participants with positive responses (n, % and 95%CI), defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD after vaccination (n, % at each post-vaccination time point)

3.9. EXPLORATORY ENDPOINT

- IgA antibodies against PT, FHA, PRN, and fimbriae 2/3 in nasopharyngeal aspirate at visits 2, 4, 6, 7, 8, 9 and 10
  - Geometric mean levels
  - Number and proportion of participants with positive responses, defined by at least 100% increase from pre- to post-vaccination, to at least 4 times MLD after vaccination.
- Cytokine levels in nasopharyngeal aspirate (exploratory assay)
  - Median + IQR of concentration and fluorescence intensity per cytokine, assessed by Luminex pre and post vaccination
- Cytokine levels in cell culture supernatants after stimulation of PBMC with PT, FHA, PRN and unrelated antigen
  - Median + IQR of concentration and fluorescence intensity per cytokine, assessed by Luminex in PBMC supernatant after stimulation pre and post vaccination
- Median number of spot-forming units determined by ELISpot/FluoroSpot assay of pertussis-specific cytokine-positive T-cells after stimulation of PBMC with PT, FHA, PRN and unrelated antigens.
- Median number of spot-forming units determined by ELISpot assay of pertussis-specific IgG-producing memory B-cells after stimulation of PBMC with PT, FHA, PRN and unrelated antigens.

Intra-group comparisons compared to baseline will be done by statistical tests for paired samples. Multiplicity adjustments methods based on the FDR may be considered for the analysis of high dimensional immunogenicity assays (Luminex assays; assays on PBMC with various stimulation conditions).

In addition, in ancillary analyses, appropriate statistical models for the analysis of longitudinal data may be used to estimate the slopes of changes in immunogenicity markers per group. Methods for high-dimensional data may be used to assess the interrelationships between different immunogenicity markers.

3.10. INTERIM REPORTS FOR DSMB

Overall, per group and randomized allocation, and by period (pre/post vaccination):

- Number and proportion of participants with at least :
  - one AEFI during available follow-up
  - one AR during available follow-up
  - one SAE during available follow-up
  - one work accident
- Number of following AEs during available follow-up:
  - Solicited or unsolicited adverse reaction from respiratory tract
  - AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia)
  - Cough
  - spasmodic cough
  - solicited systemic adverse event
  - unsolicited adverse event

- Participants with at least one cough or spasmodic cough of grade 2 or higher between Day 0 and Day 28 (n, %)
- Participants with at least one other respiratory tract AE related or possibly related to vaccination of grade 3 or higher between Day 0 and Day 28 (n, %)
- Participants with any other AE related or possibly related to vaccination of grade 3 or higher between Day 0 and Day 28 (n, %)
- Participants with at least of the adverse events listed above (primary endpoint) (n, %, 95% CI)
- Participants with at least one solicited or unsolicited adverse reaction from respiratory tract between Day 0 and Day 28 (n, %, 95% CI)
- Participants with at least one AEFI with difficulties in breathing (asthma, spasmodic cough, pneumonia) between Day 0 and Day 28 (n, %, 95% CI)
- Participants with at least one other AEFI from the respiratory tract between Day 0 and Day 28 (n, %, 95% CI)
- Participants with at least one cough between Day 0 and Day 28 (n, %, 95% CI)
    - duration of symptoms (mean, median, SD, IQR, 95% CI)
- Participants with at least one spasmodic cough between Day 0 and Day 28 (n, %, 95% CI)
    - duration of symptoms (mean, median, SD, IQR, 95% CI)
- Participants with at least one solicited systemic adverse events between Day 0 and Day 28 (fever, headache, tiredness) (n, %, 95% CI)
- Participants with at least one unsolicited adverse event between Day 0 and Day 28 (n, %, 95% CI)

Detailed listings of individual events will be provided in an appendix of the reports.

3.11. CONFIDENTIAL INTERIM ANALYSIS FOR DECISION MAKING FOR PHASE II DESIGN

An interim analysis will be performed when culture results are available from twenty-eight days after vaccination of the high dose group 3. The interim analysis will provide summary data only (i.e. no information about individual volunteers) for the sponsor/ILiAD to present to regulatory agencies, partners, and funding sources to aide in designing and planning a Phase II study.

Descriptive statistics will be provided for demographic and other baseline characteristics.

COLONIZATION

The frequency of colonization of the human respiratory tract by live attenuated B. pertussis strain (BPZE1) and its magnitude (in terms of colony forming units, CFUs) will be summarized for each group. Descriptive statistics for the CFUs, including the mean, standard deviation, minimum, maximum, median, and 95% confidence intervals will be constructed and reported.

SYMPTOMS FROM THE RESPIRATORY TRACT

Cough, spasmodic cough and any symptom from the respiratory tract will be summarized using counts and percents for each of the vaccine groups and between the groups of colonized and non-colonized individuals.

HEMATOLOGY

Pre- and post-vaccination values of cell blood counts (haemoglobin, total and differential WBC, RBC and platelets) will be summarized for each group separately. Descriptive statistics, n, mean, standard deviation, minimum, maximum, median, and 95% confidence intervals will be constructed and reported.

REFERENCES

1. Guideline ICH - E9: Statistical Principles for Clinical Trials. 1998.

2. Guideline ICH - E3: Statistical Principles for Clinical Trials. 1995.

3. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet. 14 avr 2001;357(9263):1191‑1194.

4. Piaggio G, Elbourne DR, Pocock SJ, Evans SJW, Altman DG, CONSORT Group. Reporting of noninferiority and equivalence randomized trials: extension of the CONSORT 2010 statement. JAMA J Am Med Assoc. 26 déc 2012;308(24):2594‑2604.

5. Basic Statistical Reporting for Articles Published in Biomedical Journals: The « Statistical Analyses and Methods in the Published Literature » or The SAMPL Guidelines" | The EQUATOR Network [Internet]. [cité 9 mai 2014]. Disponible sur: http://www.equator-network.org/reporting-guidelines/sampl/