A Phase II/III randomized, double-blind controlled study to compare the safety and immunogenicity of 1 or 2 doses of acellular pertussis vaccines containing genetically-detoxified pertussis toxin in young adults previously primed with acellular pertussis vaccines.

Short title: Pertagen2x

Sponsor	Geneva University Hospitals 4 Rue Gabrielle Perret-Gentil 1205 Geneva, Switzerland
Principal Investigator	Dr. Géraldine Blanchard-Rohner, MD, PhD Immunology and Vaccinology Division Department of Pediatrics University Hospitals of Geneva (HUG) Rue Willy-Donzé 6 1211 Genève 14 Phone +41 79 553 4482 Email: Geraldine.blanchardrohner@hcuge.ch

Funding source	Request for funding has been submitted. Center for Vaccinology Medical Faculty (UNIGE) and University Hospitals of Geneva (HUG) CMU, 1 Michel-Servet 1211 Geneva 4

CONFIDENTIALITY STATEMENT

GENERAL INFORMATION/ STUDY ADMINISTRATIVE STRUCTURE

Study type	Phase II/III clinical trial
Study categorization	C
Study product	Pertagen® (BioNet-Asia acellular pertussis vaccine)
Sponsor	Geneva University Hospitals Center for Vaccinology 4 Rue Gabrielle Perret-Gentil 1205 Geneva, Switzerland
Vaccine manufacturers	**BioNet-Asia Co., Ltd. (BNA)** 19 Soi Udom Suk 37, Sukhumvit 103 Rd., Prakanong, Bangkok, 10260 Thailand **Sanofi Aventis (Suisse) SA** Route de Montfleury 3 1214 Vernier/Geneva Switzerland
Qualified Person	Dario Franscesco Creschi
Principal Investigator	Dr. Géraldine Blanchard-Rohner, MD, PhD Vaccinology and Immunology Division Department of Pediatrics University Hospitals of Geneva (HUG) Rue Willy-Donzé 6 1211 Genève 14 Phone +41 79 553 4482 Email: Geraldine.blanchardrohner@hcuge.ch
Trial site	Plateforme de Recherche Clinique de Pédiatrie, Gynécologie et Obstétrique (HUG)
Local laboratories	University Hospitals of Geneva Rue Gabrielle-Perret-Gentil 4 Geneva, Switzerland 1205
Immunology laboratories	Vaccinology Laboratory, HUG Center for Vaccinology, University of Geneva Biology Laboratory of BioNet-Asia Co., Ltd.
Clinical investigators	Dr. Géraldine Blanchard-Rohner, MD, PhD Dr. Natasha Loevy
Immunologic evaluator	Prof. Claire-Anne Siegrist, MD
External collaborators	Collaborating immunology research laboratories
External monitor	Center for Clinical Research (HUG)
Current protocol version	1.2

Version history

Date	Version	Changes	By
24.09.2020	1.0	Initial draft	G. Blanchard Rohner
11.05.2021	1.0	Final version v1.0	G.Blanchard Rohner
01.07.2021	1.1	Revised version following CCER review	G.Blanchard Rohner
23.07.2021	1.2	Revised version following CCER review	G. Blanchard Rohner

SIGNATURE PAGE

Declaration of Sponsor

Title: **A Phase II/III randomized, double-blind controlled study to compare the safety and immunogenicity of 1 or 2 doses of acellular pertussis vaccines containing genetically-detoxified pertussis toxin in adults previously primed with acellular pertussis vaccines.**

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, [2013] (APPENDIX 1) and the guidelines on Good Clinical Practice.

Dr. Géraldine Blanchard Rohner		
Sponsor representative	**Signature**	**Date**

Declaration of Principal Investigator

I, the undersigned, have reviewed the protocol entitled **"A Phase II/III randomized, double-blind controlled study to compare the safety and immunogenicity of 1 or 2 doses of acellular pertussis vaccines containing genetically-detoxified pertussis toxin in adults previously primed with acellular pertussis vaccines,"** version 1.2, dated 23.07.2021, including appendices. I will conduct the clinical study as described and I will adhere to Good Clinical Practices/International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (GCP/ICH)and all ethical and regulatory considerations stated under the Federal Law on Medicinal Products and Medical Devices (TPA/LPTh, 15 December 2000), Federal Law on Human Subjects Research (HRA/LRH, 30 September 2011) and the Ordinance on Clinical Trials in Human Research (ClinO/OClin, 20 September 2013).

Dr. Géraldine Blanchard Rohner		
Principal Investigator	**Signature**	**Date**

TABLE OF CONTENTS

CONFIDENTIALITY STATEMENT 1

GENERAL INFORMATION/ STUDY ADMINISTRATIVE STRUCTURE 2

SIGNATURE PAGE 4

PROTOCOL SYNOPSIS 8

STUDY FLOW CHART 15

ABBREVIATIONS AND DEFINITIONS 16

1 BACKGROUND AND RATIONALE 17

1.1 The recurrence of pertussis and need for optimized pertussis vaccines 17

1.2 Clinical evidence to date: development of aP vaccines including genetically-detoxified PT 17

1.3 Rationale for the current study and proposed vaccine schedule 20

2 OBJECTIVES AND ENDPOINTS 21

2.1 Primary objective 21

2.1.1 Primary outcome measures 21

2.2 Secondary objectives 22

2.2.1 Safety outcome 22

2.2.2 Main secondary immunogenicity outcomes 22

2.3 Exploratory Outcomes Measures 22

3 CLINICAL STUDY MATERIALS 23

3.1 Study vaccines 23

3.2 Packaging, Labeling, and Storage of Vaccines 23

3.3 Preparation and Administration 24

3.4 Clinical Study Supply, Dispensing, and Accountability 24

4 STUDY DESIGN 24

4.1 Overall Study Design 24

4.2 Study population and entry criteria 25

4.2.1 Informed consent 25

4.2.2 Inclusion and exclusion criteria 25

4.2.3 Compliance with dosing regimen 27

4.3 Randomization 27

4.4 Formal safety analyses 27

5 CONDUCT OF STUDY 29

5.1 Study visits 29

5.1.1Visit 1: Randomization, assessment and immunization, Day 0 29

5.1.2Phone contact (Day 7 +/-1) 30

5.1.3Visit 2 (Day 28 +7) 30

5.1.4Visit 3 (Day 180 +/- 30) 30

5.1.5Phone contact (Day 187 +/-1) 31

5.1.6Visit 4 (Day 210 +7) 31

5.1.7Visit 5 (Day 365 +/- 30) 31

5.2 Unscheduled visits 31

5.3 Study assessments 32

5.3.1 Safety tests 32

5.3.2 Immunogenicity tests 32

6 STUDY DISCONTINUATION 32

6.1 Study termination 32
6.2 Study withdrawal 32
6.3 Procedures for handling subjects with premature study discontinuation 33
6.4 Breaking the randomization code 34

7 SAFETY ASSESSMENT 34
7.1 Adverse events 34
7.2 Adverse reactions 34
7.3 Serious adverse events 35
7.4 Serious adverse drug reaction (SADR) 35
7.5 Suspected unexpected serious adverse reaction (SUSAR) 35
7.6 Causality assessment 35
7.7 Reporting procedures for all adverse events 36
7.7.1 Reporting procedures for SAE 36
7.7.2 Reporting procedures for SUSAR 36
7.8 Assessment of severity 37
7.9 Summary of risk mitigation strategies 40

8 STATISTICAL ANALYSIS 40
8.1 Sample size calculation 40
8.2 Safety analysis 40
8.3 Immunogenicity analyses 40

9 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES 41
9.1 Investigator procedures 41
9.2 Monitoring 41
9.3 Modification to protocol 41
9.4 Protocol and GCP deviation 41
9.5 Trial progress 41
9.6 Publication policy 41

10 ETHICAL AND REGULATORY ASPECTS 42
10.1 Declaration of Helsinki 42
10.2 ICH Guidelines for Good Clinical Practice (GCP) 42
10.3 Study registration 42
10.4 Categorization of study 42
10.5 Ethics committee and regulatory agency review 42
10.6 Declaration of interest 42
10.7 Informed consent 42
10.8 Benefits and risks to the participant 42
10.9 Subject confidentiality 42

11 DATA HANDLING AND RECORD KEEPING 44
11.1 Data handling and management 44
11.2 Record keeping 44
11.3 Source data and case report forms (CRFs) 44
11.4 Data protection and ownership 45
11.5 Retention and destruction of study data and biological material 45

12 FINANCING AND INSURANCE 46
12.1 Financing 46
12.2 Insurance 46
12.3 Compensation of subjects 46

REFERENCES 47

List of Tables

Table 1. Study groups 11
Table 2. Composition of each dose of 0.5mL presented in pre-filled syringe 11
Table 3. Schedule of Assessment 15
Table 4. Anti-PT and anti-FHA antibodies by study groups 20
Table 5. Toxicity grading scale for local adverse events 37
Table 6. Toxicity grading scale for Vital signs 38
Table 7. Toxicity grading scale for systemic AEs excluding the vital signs listed above 39
Table 8. Risk mitigation strategies 40

List of Figures

Figure 1. Study Flow Chart 15
Figure 2. Binding (left) or neutralizing (right) anti-PT IgG antibodies before and after immunization with BNA aPor Tdap vaccines or Adacel® 19
Figure 3. Antibody responses to recombinant acellular pertussis vaccine (r-aP Pertagen®) compared to the standard vaccine containing chemically detoxified pertussis toxin (cd/Tdap Boostrix®) 21
Figure 4. Memory B-cell response to recombinant acellullar pertussis vaccine (r-aP Pertagen®) compared to the standard vaccine containing chemically detoxified pertussis toxin (cd/Tdap Boostrix®) 21
Figure 5. Workflow for reporting of serious adverse events and SUSAR 37

PROTOCOL SYNOPSIS

PROTOCOL SYNOPSIS – PHASE II/III 2 doses rPT-VACCINE TRIAL		
PROTOCOL TITLE:	**A Phase II/III randomized, double-blind controlled study to compare the safety and immunogenicity of 1 or 2 doses of acellular pertussis vaccines containing genetically-detoxified pertussis toxin in adults previously primed with acellular pertussis vaccines.**	
PRINCIPAL INVESTIGATOR:	Dr Géraldine Blanchard Rohner, MD, PhD	
STUDY CENTER:	University Hospitals of Geneva, Clinical Trials Unit, Geneva, Switzerland 1205	
SPONSORING INSTITUTION:	Geneva University Hospitals 4 Rue Gabrielle Perret-Gentil 1205 Geneva, Switzerland	
MANUFACTURERS:	BioNet-Asia Co., Ltd. (BNA) 19 Soi Udom Suk 37, Sukhumvit 103 Rd., Prakanong, Bangkok, 10260 Thailand	Sanofi Aventis (Suisse) SA 1214 Vernier/Geneva Switzerland
Qualified person:	Dario Franscesco Cresci	
CLINICAL INVESTIGATORS:	Dr. Géraldine Blanchard-Rohner, MD, PhD Dr. Natasha Loevy	
IMMUNOLOGICAL EVALUATOR:	Prof. Claire-Anne Siegrist, MD	
EXTERNAL COLLABORATORS:	Collaborating immunology research laboratories	
EXTERNAL MONITOR:	Center for Clinical Research (HUG)	
NAME OF INVESTIGATIONAL PRODUCT:	Pertagen® (BioNet-Asia acellular pertussis vaccine)	
NAME OF ACTIVE INGREDIENTS:	Genetically detoxified pertussis toxin (rPT) and filamentous hemagglutinin (FHA), alum adsorbed	
INVESTIGATIONAL PRODUCT DOSAGE, SCHEDULE, AND MODE OF ADMINISTRATION:	**Product dosage:** Group 1: Pertagen® (BioNet-Asia): recombinant Pertussis Toxin (rPT) 5□g; filamentous hemagglutinin (FHA) 5 □g Revaxis® (Sanofi Aventis): tetanus toxoid, 20 UI; diphtheria toxoid 2 UI; inactivated polio viruses. Group 2: Pertagen® **Schedule:** One vaccination on Day 0 and 6 months later for each volunteer. Group 1 will receive one dose of Revaxis® followed by 1 dose of Pertagen®. Group 2 will receive two doses of Pertagen®. A dose of Revaxis® will be offered at the last visit if useful for protection. **Mode of Administration:** Intramuscular injection into the deltoid region of the upper extremity, using a syringe with a 1 to 1.5 inch #25-gauge sterile needle.	

PROTOCOL SYNOPSIS – PHASE II/III 2 doses rPT-VACCINE TRIAL	
TRIAL DESIGN:	Phase II/III, randomized, double-blind controlled trial
STUDY POPULATION:	Adults aged 18-30 years previously primed with acellular pertussis vaccines (see entry criteria below).
SAMPLE SIZE:	100 volunteers
FOLLOW-UP PERIOD:	6 months following the second injection
STUDY PERIOD:	July 2021 – September 2023
RATIONALE:	A significant increase of pertussis incidence is reported in a growing number of countries. This resurgence is considered as resulting from the limited durability of aP-vaccine-induced immunity and is associated with increased mortality in young infants and morbidity at all age groups. As the pertussis immunity acquired through immunization or infection is short-lived, its maintenance or reactivation requires repeat boosting at regular time points. Thus, novel strategies capable of reactivating pertussis immunity are needed. The efficacy of current acellular pertussis vaccines (which contain chemically-detoxified pertussis toxoid (PT)) rapidly wanes, in part because priming and repeat immunization with acellular vaccines induce antibodies specific for the chemically-detoxified PT but unable to efficiently recognize the native PT expressed by *B. pertussis.* Clinical studies have shown the superior immunogenicity profile of acellular pertussis vaccines including genetically-detoxified PT (rPT) in adults and adolescents previously primed with aP. In particular, we showed in a past Geneva study in teenagers previously primed with aP that rPT/FHA induced a stronger recall response than the current aP-vaccine at one month post-vaccination. However, the difference was less clear one year after vaccination, suggesting that 2 doses may be needed for more sustained immunity. In the present study, we would like to assess whether giving two doses of rPT/FHA at 6 months interval induces stronger immune responses than a single dose.
PRIMARY OBJECTIVE:	To assess B cell responses to two doses compared to one dose of an acellular pertussis vaccine (Pertagen®) including genetically detoxified pertussis toxin (rPT) when delivered by the intramuscular route to adult volunteers previously primed and boosted with chemically-detoxified PT. • The primary endpoint will be the geometric mean concentration (GMC) of anti-PT neutralizing antibodies assessed 4 weeks (early immunity) and 6 months (sustained immunity) following one or two injections of Pertagen® given at 6 months interval.
SECONDARY OBJECTIVES:	• To assess the safety of two doses at 6 months interval of intramuscular immunization with Pertagen® administered to adults as compared to one dose of Pertagen®. • To assess the humoral responses elicited by two doses of Pertagen® as compared to a single dose. The following parameters will be assessed at Day 0, Day 28 and Month 6 post first and second vaccination: o Geometric mean concentrations (GMCs) of PT, FHA, tetanus and diphtheria-toxoid specific IgG antibodies measured by ELISA o Seroresponse rates of PT, FHA, tetanus and diphtheria-toxoid specific IgG antibodies measured by ELISA o Seroresponse rates of PT, FHA, tetanus and diphtheria-toxoid specific neutralizing antibodies.

PROTOCOL SYNOPSIS – PHASE II/III 2 doses rPT-VACCINE TRIAL	
EXPLORATORY OBJECTIVES:	• To assess antibody titers to specific neutralizing and non-neutralizing epitopes of PT, by competitive ELISA, following one or two doses of Pertagen® • To compare antibody avidity following one or two doses of Pertagen® • To assess the frequency and phenotype of PT-specific memory B cell responses following one or two doses of Pertagen®
STUDY POPULATION:	Volunteers, 18-30 years of age
NUMBER OF SUBJECTS:	100 (50 subjects per each vaccine group)

PROCEDURE AND FOLLOW-UP:

This is a phase II/III, single center, randomized, double-blind controlled vaccine trial in 18 to 30 years old volunteers. It will be performed at the Plateforme de Recherche Clinique de Pédiatrie, Gynécologie et Obstétrique (PGO) of the HUG.

Volunteers who have signed the informed consent and who gave their written assent will be screened clinically for general health status and those who fulfill all exclusion and inclusion criteria will be enrolled into the study.

At Day 0, eligible volunteers will undergo a venous bleed for the determination of baseline values and enter the randomization scheme, being allocated to one of two groups: A (Revaxis® + Pertagen®), B (Pertagen® + Pertagen®) (Tables 1 and 2).

Group A	Revaxis® + Pertagen®	50 subjects
Group B	Pertagen® + Pertagen®	50 subjects

Table 1. Study groups

	Pertagen®	**Revaxis®**
Active substance		
Tetanus Toxoid (TT)	-	NLT 20IU
Diphtheria Toxoid (DT)	-	NLT 2.0 IU
Pertussis Toxoid (PT)	5 µg[a]	-
Filamentous Hemagglutinin (FHA)	5 µg	-
Pertactin (PRN)	-	-
Fimbriae type 2/3	-	-
Inactivated polio viruses type 1-2-3	-	40U (type 1), 8U (type 2), 32U (type 3)
Adjuvants		
Aluminum Hydroxide	0.3 mg/dose	0.35mg/dose
Volume for Injection	q.s. to 0.5 mL	q.s. to 0.5 mL
a: Recombinant Pertussis Toxin (rPT)		

Table 2. Composition of each dose of 0.5mL presented in pre-filled syringe

Visit 1: Randomized participants will receive one dose of Revaxis® (Group A) or one dose of Pertagen® (Group B) by intramuscular injection in the deltoid. All volunteers will be observed at the Plateforme de Recherche Clinique PGO for 30 minutes after immunization.

Diary cards will be distributed to study volunteers to record post-immunization local and systemic reactions avec adverse events. Volunteers will complete daily diaries for 7 days after immunization.

All volunteers will be followed for 28 days after immunization and all adverse events will be collected and monitored for 28 days after vaccination.

Visit 2: At Day 28, a second visit will take place for safety evaluation, blood draw and revision of diary cards. Investigators will reconcile and transcribe the Diary Card information in the CRF.

Visit 3: At 6 months, a third visit will take place for the second vaccination. Randomized participants will all receive one dose of Pertagen® (Groups A and B) by intramuscular injection in the deltoid. All volunteers will be observed at the Plateforme de Recherche Clinique PGO for 30 minutes after immunization.

Diary Cards will be distributed to study volunteers to record post-immunization local and systemic reactions and adverse events. Volunteers will complete daily diaries for 7 days after immunization.

All volunteers will be followed for 28 days after immunization and all adverse events will be collected and monitored for 28 days after vaccination.

Visit 4: At Day 28 post second immunization, a 4th visit will take place for safety evaluation, blood draw, and revision of diary cards. Investigators will reconcile and transcribe the Diary Card information in the CRF.

Visit 5: A final visit will take place at 6 months post-second immunization (blood draw).
At this visit, Group 2 participants will be offered one dose of Revaxis® if needed for their protection (last dose of tetanus-containing vaccine >= 10 years).

Blood draws performed on Day 0 (Baseline), Day 28, and Month 6 following each vaccination will be used to evaluate early and sustained vaccine responses.

The primary statistical analysis will be based upon visit 4 and visit 5 data to compare the early and sustained immunogenicity of one or two doses of Pertagen®.

PROTOCOL SYNOPSIS – PHASE II/III 2 doses rPT-VACCINE TRIAL	
SUBJECT INCLUSION CRITERIA:	• Has provided written informed consent; • Male or female, ages 18 to 30 years (inclusive) at the time of enrollment; • With documented history of acellular pertussis immunization (5 doses); • Free of clinically significant health problems, as determined by pertinent medical history and clinical examination at study screening; • Non-pregnant, non-lactating females : o A negative urinary pregnancy test at enrollment; o If sexually active, female subjects must be willing to use reliable birth control measures for 1 month after each vaccination; • Able to attend all scheduled visits during one year and to understand and comply with the study procedures;
SUBJECT EXCLUSION CRITERIA:	• Prior dTpa immunization within the last 5 years or prior dT immunization within the last 2 years, or any other investigational vaccine likely to impact on interpretation of the trial data; • Suspected or confirmed pertussis infection within the last 10 years or documented pertussis infection in a household member within the last 10 years; • History of severe local or systemic reactions to any vaccination; • Known hypersensitivity or allergy to diphtheria, tetanus, or pertussis-containing vaccines (including excipients); • Receipt of investigational product up to 30 days prior to enrollment or ongoing participation in another interventional clinical trial; • Receipt of licensed vaccines within 30 days of planned study immunization or ongoing participation in another clinical interventional trial likely to interfere with study results; • Acute or chronic, clinically significant psychiatric, hematologic, pulmonary, cardiovascular, or hepatic or renal functional abnormality as determined by the Investigator based on medical history and physical exam; • Any confirmed or suspected immunosuppressive or immunodeficient condition, including human immunodeficiency virus (HIV) infection, asplenia, cytotoxic therapy in the previous 5 years, and/or diabetes; • Has a known history of vaccine-induced Guillain-Barré Syndrome; • Has an active malignancy or recent (<10 years) history of metastatic or hematologic malignancy; • Suspected or known alcohol and/or illicit drug abuse within the past 5 years; • Pregnant or lactating female, or female intending to becoming pregnant during the study period; • Administration of immunoglobulins within the 120 days preceding study entry or planned administration during the study period; • History of blood donation (at least 450 ml) within 30 days of enrollment or plans to donate within the 30 days following and preceding each blood draw;

PROTOCOL SYNOPSIS – PHASE II/III 2 doses rPT-VACCINE TRIAL	
SUBJECT EXCLUSION CRITERIA (cont):	• Receipt of chronic (>14 days) immunosuppressants or other immune-modifying drugs within 6 months of study entry: o For corticosteroids, this will mean prednisone or equivalent ≥0.5 mg/kg/day, o Intranasal and topical steroids are allowed; • Any other significant finding that, in the opinion of the investigator, would increase the risk of the individual's having an adverse outcome by participating in this study.
SUBJECT TEMPORARY EXCLUSION CRITERIA:	• Acute disease at the time of randomization. (Acute disease in the context of this trial is defined as the presence of a moderate or severe illness with or without fever.) The vaccine can be administered to persons with a minor illness such as mild upper respiratory tract infection with or without low-grade febrile illness, i.e. temperature of ≤37.5°C.; • Body temperature documented ≥38°C within 3 days of the intended vaccination; • Any other significant finding that, in the opinion of the investigator, would temporarily increase the risk of the individual's having an adverse outcome by participating in this study.
ENDPOINTS • SAFETY:	The nature, frequency, and severity of AEs and/or SAEs associated with immunization: • Solicited AEs occurring from the time of immunization through 7 days following the procedure, facilitated with the use of a diary card. Solicited local reactogenicity adverse events include pain, erythema, swelling and pruritus at the treatment site. Solicited systemic reactogenicity AE include objective fever, subjective fever, chills, myalgia, arthralgia, sudation, fatigue, headache, nausea, vomiting and loss of appetite; • Unsolicited AEs from the time of immunization through 28 days following immunization; • SAEs from the time of the first treatment through the final study visit.
• IMMUNOGENICITY:	Development and characteristics of PT-and FHA specific antibody and cellular responses on Day 0 and Day 28 and Month 6 following first and second immunization.
STATISTICAL ANALYSIS:	The sample size calculation is based on the previous study using the rPT vaccin in teenagers in Geneva (Blanchard Rohner et al. CID 2019). In order to have a power of 90%, to expect to see a double increase in the neutralising PT antibodies following two doses of rPT in comparison to a single dose, we would need 2x35 volunteers. Safety analysis will be descriptive. Recorded solicited and unsolicited adverse events will be described by treatment group. Immunogenicity endpoints and confidence bounds will be calculated for each group and compared (using Chi-square or Fisher's exact test for binary endpoints, and Wilcoxon-Mann-Whitney's test or Kruskal-Wallis test for quantitative endpoints). All tests will be two-sided.
STUDY PERIOD:	One year for each volunteer

STUDY FLOW CHART

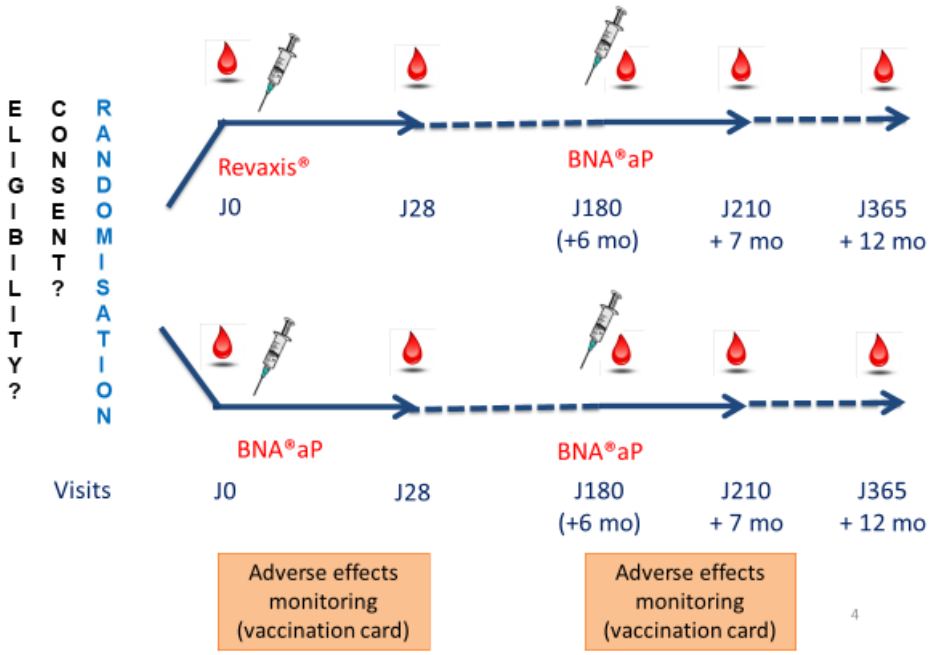

Figure 1. Study Flow Chart

Visits	V1		V2	V3		V4	V5
	Enrollment	Phone call			Phone call		
Timeline (days)	0	**7**	**28**	**180**	**187**	**210**	**365**
Window period (days/months)		**±1d**	**+7d**	**±30d**	**±1d**	**+7d**	**±30d**
Informed consent	X						
Demographics, Medical history, Immunization history	X						
Exclusion/Inclusion	X						
Physical examination	X		(X)	X		(X)	(X)
Enrollment	X						
Randomization	X						
Blood sampling	X		X	X		X	X
Immunization (Pertagen®/Revaxis®)	**X**			**X**			(X)
Diary card provided				X			
Diary card collection / review						X	
Post vaccination assessment*	X			X			
AE evaluation / diary use		X			X		
AEs & SAEs reviewed			X			X	
End-of-study visit							X
Complete blood count (mL)	3		3	3		3	3
Antibodies IgG (ELISA) (mL)	3		3	3		3	3
Anti-PT (neutralization) (mL)	3		3	3		3	3
Ficoll - PBMC freezing and plasma aliquots (mL)	20		20	20		20	20
Total blood draw (mL)	29		29	29		29	29

* After vaccination subjects will be observed for 30 minutes;

Table 3. Schedule of Assessment

ABBREVIATIONS AND DEFINITIONS

AE	Adverse event
aP-vaccine	Acellular pertussis vaccine
AR	Adverse reaction
BNA	BioNet-Asia Co., Ltd.
CHO	Chinese hamster ovary cell (CHO cell) assay
ClinO	Clinical trials ordinance (Ordonnance sur les essais cliniques)
CRC	Centre de Recherche Clinique
CRF	Case report form
CTU	Clinical trial unit
dT	Diphtheria – Tetanus
dTpa	Diphtheria – Tetanus – acellular pertussis
Tdap	Tetanus – diphtheria – acellular pertussis
EC	Ethics committee
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
GCP	Good clinical practice
GMO	Genetically modified organism
GMT	Geometric mean titer
GMC	Geometric mean concentration
GP	General Practitioner
HUG	Hôpitaux Universitaires de Genève
ICF	Informed consent form
IMP	Investigational medicinal product
ITT	Intention to treat
NSAID	Non-steroidal anti-inflammatory drug
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
PII	Personally identifiable information
PIS	Participant information sheet
PT	Pertussis toxin
rANOVA	Repeated measure analysis of variance
rPT	Genetically detoxified pertussis toxin
SADR	Serious adverse drug reaction (also SAR)
SAE	Serious adverse event
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TGFb	Transforming growth factor beta
TNF	Tumor necrosis factor
WNL	Within normal limits

1 BACKGROUND AND RATIONALE

1.1 The recurrence of pertussis and need for optimized pertussis vaccines

Pertussis is an important cause of infant death worldwide and continues to be a public health concern even in countries with high vaccination coverage. Estimates from WHO suggest that, in 2008, about 16 million cases of pertussis occurred worldwide, 95% of which were in developing countries, and that about 195,000 children died from the disease (World Health Organization, 2010). Immunization programs designed to protect young children against *Bordetella pertussis* started in 1944 (Cherry JD, 1999). The incidence of pertussis has been greatly reduced by mass vaccination, but even in countries with high vaccination coverage, the disease is still present (Centers for Disease Control and Prevention, 1993; Christie CD *et al*, 1994). In a cross-sectional study published in 2000 the prevalence of antibodies to pertussis toxin reached 52% in the 30 to 39 year age group, without a further increase thereafter (Garcia-Corbeira P *et al*, 2000).

These data suggest that there is a widespread circulation of the organism in the population and that pertussis infection is increasingly frequent in adolescents and young adults – which now represent the majority of cases. Although hospitalization, complications and mortality in adolescents and adults are rare, these populations serve as a reservoir for *B. pertussis* and play an important role in the transmission to the very young infants (Black S, 1997; Guris D *et al*, 1999; Althouse BM & Scarpino SV, 2015).

These observations have important implications for *B. pertussis* vaccination policy. In November 2012, the WHO Strategic Advisory Group of Experts (SAGE) on immunization expressed concern about resurgence of pertussis despite high vaccine coverage with aP vaccines. As pertussis immunity acquired through immunization or even infection is short-lived, its maintenance or reactivation requires repeat boosting. In 2014, WHO specified that the countries currently using an aP vaccine may continue using this vaccine but should consider the need for additional booster doses and strategies to prevent early childhood mortality in case of resurgence of pertussis (World Health Organization, 2014a). Countries with demonstrable nosocomial transmission are encouraged to vaccinate health-care workers, particularly maternity and pediatric staff, if economically and logistically feasible (World Health Organization, 2014b).

A puzzling observation is that the efficacy of current acellular pertussis vaccines (which contain chemically-detoxified pertussis toxoid (PT) is higher in infants and children than in adolescents, in whom vaccine efficacy is limited and rapidly wanes. A recent publication about the 2012 US Washington state pertussis epidemic showed that protection wanes within 2 to 4 years after adolescent boosting, where the vaccine effectiveness declined to 34% (Acosta AM *et al*, 2015).

The resurgence of pertussis is considered as resulting largely from the limited durability of aP-vaccine-induced immunity in adolescents. This may derive – at least in part – from priming and repeat immunizations with acellular vaccines containing chemically-detoxified PT and thus the induction of antibodies specific of the chemically-detoxified PT but unable to efficiently recognize the native PT expressed by *B. pertussis*.

The development of optimized acellular pertussis vaccines should thus include antigens with a similar immunogenicity profile as native PT but deprived of its toxic properties. This is best achieved through the genetic rather than chemical detoxification of PT, which results in nontoxic and immunogenic PT.

1.2 Clinical evidence to date: development of aP vaccines including genetically-detoxified PT

The development of optimized acellular pertussis vaccines should include the use of antigens with a similar immunogenicity profile as native Pertussis Toxin (PT) but deprived of its toxic properties. This is best achieved through genetic rather than chemical detoxification, which results in both nontoxic and optimally immunogenic PT.

In adults, children and toddlers below one year, the safety and immunogenicity of a 9K/129G genetically-detoxified rPT was first demonstrated with either a monovalent PT vaccine (15 µg/intramuscular) or in DTaP combined vaccine trials (Rappuoli R, 1999; Podda A *et al*, 1993). A comparative study with either monovalent pertussis vaccine, combined acellular vaccine or combined genetically detoxified PT vaccine showed a similar safety profile between all vaccine products. In this study, the genetically detoxified vaccine was the most immunogenic when similar PT dose levels were compared (Keitel WA *et al*, 1999). In infants, aP vaccines containing genetically-detoxified recombinant PT (rPT) were shown to be safe, highly immunogenic, efficacious and able to elicit antibodies and protection which persisted up to 6 years (Greco D 1996; Salmaso S 2001). The superior immune response of rPT-containing vaccines was associated with the conservation of 75-80% of native PT, allowing efficient binding of immune cells to B and T cell epitopes (Ibsen PH 1996; Di Tommaso A 1994). The development of this first generation vaccine was unfortunately interrupted by patent issues.
Thus, genetically detoxified rPT was proven safe, immunogenic and effective in several clinical trials.

1.2.1 Phase I/II study of BNA rPT-containing vaccines in healthy adults

Following the expiration of the patents, BioNet-Asia developed a new *B. pertussis* strain expressing a recombinant PT (rPT) (Buasri W *et al*, 2012) which retains the functional antigenic properties of native PT but with loss of its toxicity.

This genetically-detoxified recombinant PT (rPT) (Buasri W *et al*, 2012)was used in a Phase I/II randomized, observed-blind, controlled study conducted by BioNet-Asia in 2014 to assess the safety and immunogenicity of BNA aP vaccine containing rPT, FHA and PRN given alone or in combination with tetanus-diphtheria vaccine in adults (Protocol No. TDA101). A total of 60 healthy volunteers aged 18-35 years were enrolled and randomized in a 1:1:1 ratio to receive BNA aP vaccine, BNA Tdap vaccine (both with genetically inactivated PT) or a marketed Tdap vaccine (Adacel®, Sanofi Pasteur), which is a chemically inactivated vaccine – see Investigator's Brochure.

The evaluated safety endpoints included local and systemic post-immunization reactions, adverse events, and serious adverse events within 28 days after vaccination. None of the subjects showed any clinical relevant modification of the complete blood count, erythrocyte sedimentation rate, renal and liver function tests and ECG, as reported by the investigator. None of the subjects had any immediate reaction within the 4-hour observation period after vaccination. Similar incidence of local and systemic post-immunization reactions was observed among the three vaccine groups, with one subject in the BNA Tdap group reporting local induration of Grade 3 severity which resolved without sequelae before study end.

The incidence of Adverse Events (AEs) for 28 days after vaccination was similar in the three vaccine groups. Those related to vaccine administration were mostly local post-immunization reactions. All AEs were resolved without sequelae. One Serious Adverse Event (SAE) ('dysfunctional uterine bleeding') reported as unrelated to the study vaccine administration occurred in a female subject in the BNA aP vaccine group approximately two weeks after vaccination.

Thus, BNA aP and Tdap vaccines showed a similar tolerability and safety profiles to the marketed vaccine in healthy adults.

The immunogenicity endpoints were the antibody response on Days 7 and 28 post vaccination, including seroresponse rates and antibody titers to pertussis antigens, diphtheria and tetanus, as well as functional antibody titers - determining PT neutralizing antibody titer in CHO cell assay. Briefly, for PT: on Day 28 after vaccination, ELISA anti-PT geometric mean antibody titers (GMTs) as well as PT neutralizing antibody GMTs were statistically significantly higher in the BNA aP and Tdap groups than those in the marketed vaccine group (Figure 2).

GMT ELISA Anti-PT Antibody GMT Anti-PT Neutralizing Antibody

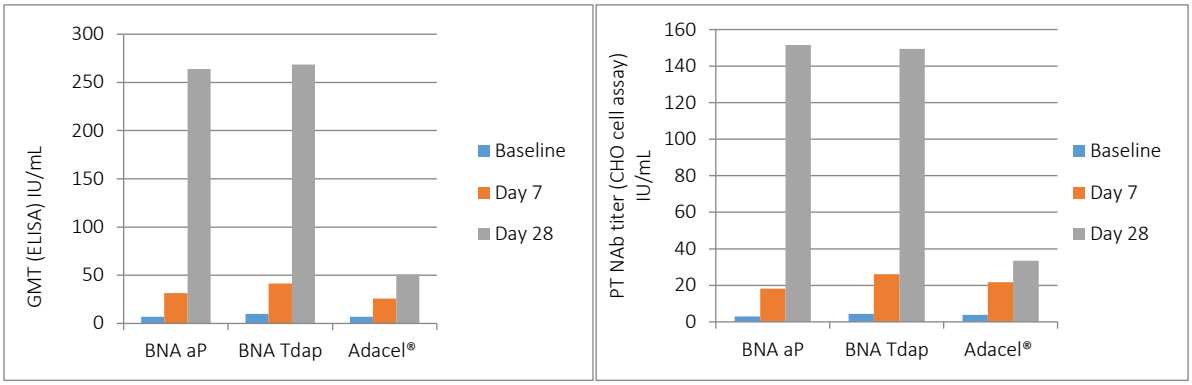

Figure 2. Binding (left) or neutralizing (right) anti-PT IgG antibodies before and after immunization with BNA aPor Tdap vaccines or Adacel®

Thus, BNA aP (Pertagen®) and Tdap vaccines elicited statistically significant higher antibody titers to PT, as measured by ELISA or by the CHO cell assay for the quantification of neutralizing antibodies.

1.2.1 Phase II/III study in Thai adolescents

BNA's TdaP and aP (Pertagen®) vaccines were subsequently tested and compared to the licensed Sanofi Pasteur Adacel® vaccine in 450 (150 per each vaccine group) healthy 12-17 years old subjects in Bangkok, Thailand (BioNet TDA202). In this country, whole-cell vaccines are used for priming and acellular vaccines have not yet been introduced as childhood boosters.

Preliminary results indicate similar frequencies and intensities of local and systemic solicited adverse reactions during 7 days after immunization and of adverse events during 28 days (see Investigator's Brochure). A single SAE was reported, unrelated to study vaccines.

Seroresponse rates (percentages of subjects with ≥ 4-fold increase of anti-PT titers) were 96%, 97% and 55% following immunization with BNA aP (Pertagen®), BNA TdaP (Boostagen®) and Adacel®, respectively. Superior seroconversion rates were also observed for FHA.

This reflected the induction of significantly higher anti-PT and anti-FHA antibodies (Table 4).

A. ELISA anti-PT GMCs by study groups

Vaccine	Baseline	Day 28 post-vaccination	Geometric Mean Change	P-value
	GMT (IU/mL) (95% CI)	GMT (IU/mL) (95% CI)	GMT (IU/mL) (95% CI)	
BNA aP	13.64 (11.55-16.11)	561.87 (467.79-674.86)	527.51 (435.57-638.87)	<0.0001*
BNA TdaP	12.91 (11.24-14.83)	365.23 (315.07-423.38)	343.08 (294.46-399.73)	<0.0001*
Adacel®	15.57 (13.21-18.36)	63.26 (51.05-78.37)	48.09 (36.99-62.50)	<0.0001*
P-value	0.3631	<0.0001*	<0.0001*	

B. ELISA anti-FHA GMCs by study groups

Vaccine	Baseline	Day 28 post-vaccination	Geometric Mean Change	P-value
	GMT (IU/mL) (95% CI)	GMT (IU/mL) (95% CI)	GMT (IU/mL) (95% CI)	
BNA aP	38.99 (32.54-46.72)	923.80 (809.39-1054.4)	836.13 (725.13-964.12)	<0.0001*
BNA TdaP	41.42 (34.60-49.58)	632.11 (549.85-726.67)	549.67 (471.95-640.18)	<0.0001*
Adacel®	45.71 (37.64-55.50)	241.85 (208.86-280.05)	178.19 (148.94-213.19)	<0.0001*
P-value	0.5369	<0.0001*	<0.0001*	

C. Neutralizing anti-PT ELISA GMCs by study groups in a subset of 50 subjects

Vaccine	Baseline	Day 28 post-vaccination	Geometric Mean Change	P-value
	GMT (IU/mL) (95% CI)	GMT (IU/mL) (95% CI)	GMT (IU/mL) (95% CI)	
BNA aP	9.75 (7.23-13.14)	275.74 (181.63-418.59)	309.08 (214.09-446.23)	<0.0001*
BNA TdaP	7.93 (6.13-10.27)	215.92 (164.16-284.02)	201.27 (151.11-268.09)	<0.0001*
Adacel®	8.83 (6.50-12.01)	36.26 (25.74-51.08)	33.66 (24.36-46.52)	<0.0001*
P-value	0.7993	<0.0001*	<0.0001*	

Table 4. Anti-PT and anti-FHA antibodies by study groups

Thus, BNA aP (Pertagen®) and Tdap vaccines elicited similar reactogenicity and significantly higher antibody responses to PT and FHA in 12-17 year-old Thai adolescents who had been primed in childhood with whole-cell vaccines.

Additional information is available in the Investigator's Brochure.

1.3 Rationale for the current study and proposed vaccine schedule

The superior responses to rPT-containing BNA vaccines observed in Thai adults and adolescents strongly suggest a higher immunogenicity of the BNA genetically-detoxified rPT antigen. Importantly, however, this superior immunogenicity was observed in adults (Phase I/II) and adolescents (Phase II/III) who had been primed as infants with whole-cell pertussis vaccines – i.e. with inactivated vaccines containing native PT. In Switzerland, Europe and the US, the use of whole-cell vaccines for pertussis priming was replaced in 2000 by the use of the better tolerated acellular vaccines – which contain chemically-detoxified PT (aP).

A previous Geneva study (Blanchard Rohner et al. CID 2019) reported that boosting aP-primed adolescents with one dose of the rPT-containing vaccine (Pertagen®) was well tolerated and elicited 2-fold stronger anti-PT neutralizing and binding antibody responses than a sixth dose of cd/Tdap. However, this superior immunogenicity was found to be transient; a potential solution being repeat doses of the rPT-containing BNA vaccine.

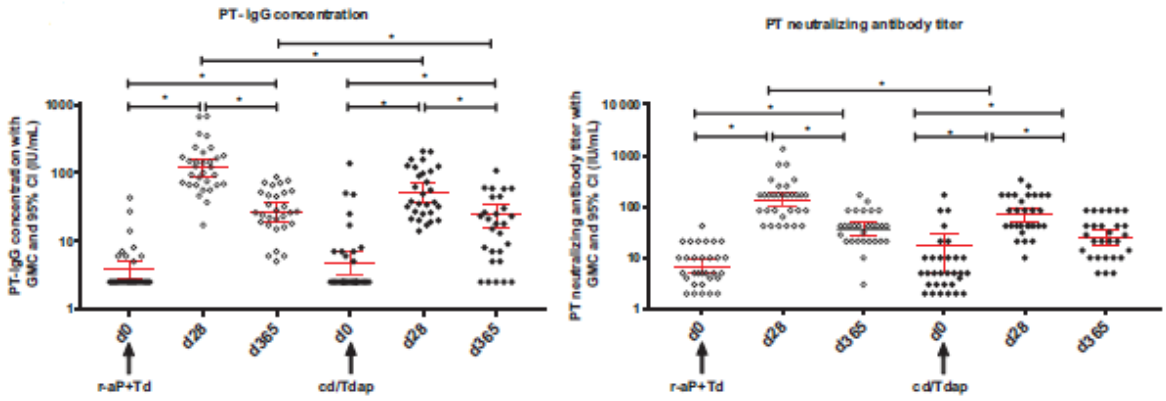

Figure 3. Antibody responses to recombinant acellular pertussis vaccine (r-aP Pertagen®) compared to the standard vaccine containing chemically detoxified pertussis toxin (cd/Tdap Boostrix®)

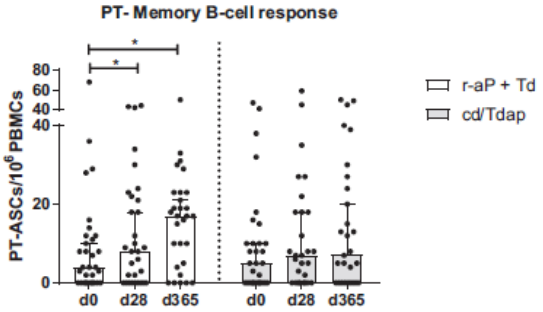

Figure 4. Memory B-cell response to recombinant acellullar pertussis vaccine (r-aP Pertagen®) compared to the standard vaccine containing chemically detoxified pertussis toxin (cd/Tdap Boostrix®)

The critical question to be addressed here is whether two doses of rPT/FHA will prove to be more effective than one dose in adults previously exposed to at least 5 doses (2 months, 4 months, 12 months, 4-7 years and 11-14/15 years) of chemically-detoxified PT-containing vaccines. This question is fundamental to define the potential of repeat doses of rPT/FHA to overcome the limited and rapidly waning efficacy of PT-containing boosters in adults.

We therefore propose to assess the safety and immunogenicity of two doses of BNA aP (Pertagen®) administered at a 6 month interval to adults 18-30 years old previously primed with 5 doses of chemically-inactivated acellular pertussis vaccines and to compare the results with responses elicited by a single dose of BNA aP (Pertagen®) in the same population.

2 OBJECTIVES AND ENDPOINTS

2.1 Primary objective

The primary objective is to assess the immunogenicity of two doses compared to a single dose of an acellular pertussis vaccine (Pertagen®) including genetically-detoxified pertussis toxin (rPT) administered at 6 months interval and delivered by the intramuscular route to adults aged 18-30 years previously primed and boosted with chemically-detoxified PT.

2.1.1 Primary outcome measures

The main immunogenicity endpoints will be the geometric mean concentration (GMC) of neutralizing antibodies to PT.

2.2 Secondary objectives

- To assess the safety of two doses at 6 months interval of intramuscular immunization with Pertagen® administered to adults, compared to one dose of Pertagen®.
- To assess the humoral responses elicited by two doses of Pertagen® when administered to adults, compared to one single dose.

2.2.1 Safety outcome

Genetically detoxified rPT was proven safe in several clinical trials (see 1.2 Clinical development of aP vaccines including genetically-detoxified PT).
Solicited local and systemic reactions will be recorded on a diary card during 7 days and unsolicited AEs for 28 days after immunization.

2.2.2 Main secondary immunogenicity outcomes

The following parameters will be assessed at Day 0, Day 28 and Month 6 post first and second vaccination:

- Geometric mean concentrations (GMCs) of PT, FHA, tetanus and diphtheria-toxoid specific IgG antibodies measured by ELISA.
- Seroresponse rates of PT, FHA, tetanus and diphtheria-toxoid specific IgG antibodies measured by ELISA.
- Seroresponse rates of PT specific neutralizing antibodies.

2.3 Exploratory Outcomes Measures

Exploratory immunological measures will be performed at each visit pending sufficient or additional funding.
These are anticipated to include:

- The assessment of antibody titers to specific neutralizing and non-neutralizing epitopes of PT, by competitive ELISA, following one for two doses of Pertagen®
- To compare antibody avidity following one or two doses of Pertagen®
- To assess the frequency and phenotype of PT-specific memory B cell responses following one or two doses of Pertagen®

Exploratory immunological measures and the quantification of neutralizing anti-PT antibodies will involve collaboration with other specialized laboratories and research groups in Switzerland and/or abroad. This will involve transfer of coded serum/plasma and/or frozen peripheral blood mononuclear cells (PBMC) samples, traceable only by means of a subject identifier and as described in the signed informed consent form.

3 CLINICAL STUDY MATERIALS

3.1 Study vaccines

Two vaccines will be used in the study. The BNA study vaccine (Pertagen®) and the control vaccine (Revaxis®).

Group A	Revaxis® + Pertagen®	50 subjects
Group B	Pertagen® + Pertagen®	50 subjects

Table 1. Study groups

	Pertagen®	**Revaxis®**
Active substance		
Tetanus Toxoid (TT)	-	NLT 20IU
Diphtheria Toxoid (DT)	-	NLT 2.0 IU
Pertussis Toxoid (PT)	5 µg[a]	-
Filamentous Hemagglutinin (FHA)	5 µg	-
Pertactin (PRN)	-	-
Fimbriae type 2/3	-	-
Inactivated polio viruses type 1-2-3	-	40U (type 1), 8U (type 2), 32U (type 3)
Adjuvants		
Aluminum Hydroxide	0.3 mg/dose	0.35mg/dose
Volume for Injection	q.s. to 0.5 mL	q.s. to 0.5 mL
a: Recombinant Pertussis Toxin (rPT)		

Table 2. Vaccine composition of each dose of 0.5 mL presented in pre-filled syringe

The investigational product Pertagen® will be manufactured, labeled, packaged and released for clinical use by BNA, in accordance with the requirements of Good Manufacturing Practices. The quality control standards and requirements for the investigational vaccine will be described in separate release protocols and the required approvals will have been obtained. One batch will be used for the entire study.

The Revaxis® vaccine used in this study is commercially available in Switzerland and will be purchased for the study. One batch will be used for the entire study.

3.2 Packaging, Labeling, and Storage of Vaccines

Study vaccines will be provided in appropriate boxes.
The investigational vaccine container and the syringe labels will contain the following information: Sponsor name, protocol number, vaccine code, storage instructions, and **"For Clinical Study Use Only"**. The licensed vaccines will be re-labeled according to the applicable local requirement.

All study products will be stored in a safe, locked, and secure place with no access by unauthorized personnel. They will be kept in a refrigerator (+2°C to +8°C) and **not be frozen**. Storage temperature will be monitored every day.

3.3 Preparation and Administration

Both Pertagen® and Revaxis® vaccines are presented in 0.5 mL pre-filled syringes to be administered by intramuscular injection in the non-dominant deltoid.

The package containing the used syringe (empty syringe), and any vaccine dispensing form / document (if applicable) will be stored at study site until end of the study.

3.4 Clinical Study Supply, Dispensing, and Accountability

All study products will be provided by BNA and shipped to the study site. Shipment content and condition (including temperature control) will be checked at reception. All study products used in the study will be accounted for in a Study Vaccine Accountability Record indicating the date of administration to the subject.

An additional quantity of each vaccine will be supplied. In case a syringe of vaccine is broken or unusable, the unblinded site staff will replace it. Documentation of the use of the replacement syringe and reason for using it will be recorded by the site staff.

At the end of the study, all unused vaccines will be, at BNA's option, either returned to BNA or destroyed in accordance with written instructions provided by BNA. If to be returned, a shipment will be recorded, identifying each syringe being included in the shipment. The used study vaccines will be destroyed at study site according to the procedure of study site institution.

4 STUDY DESIGN

4.1 Overall Study Design

This is a phase II/III randomized, double-blind controlled study to compare the safety and immunogenicity of 1 or 2 doses of acellular pertussis vaccines containing genetically-detoxified pertussis toxin in adults aged 18-30 years, previously primed and boosted with acellular pertussis vaccines.

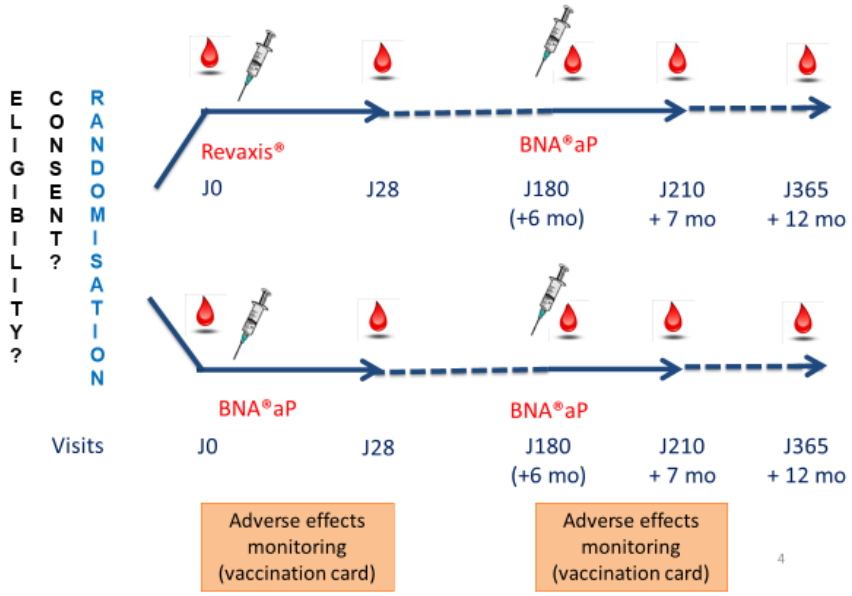

Figure 1. Study Flow Chart

4.2 Study population and entry criteria

The study population will include adults aged 18-30 years previously primed and boosted with acellular pertussis vaccines (see entry criteria below).

A leaflet describing the study objectives and modalities will be prepared to advertise the study to medical students of the University of Geneva, as well as other faculties if deemed necessary. An email containing the same information will also be sent out to the students' mailing list. Potentially interested volunteers will be invited to contact the study staff.

4.2.1 Informed consent

The participant information form will be made available to these potential volunteers in advance such that they have ample time and opportunity to inquire about details of the trial and to decide whether or not to participate. Consenting participants must sign and date the informed consent form before any study-specific procedures may be performed.

At the first visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasized:

- Participation in the study is entirely voluntary;
- Refusal to participate involves no penalty or loss of medical benefits;
- The volunteer may withdraw from the study at any time, without supplying a reason for withdrawal;
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved;
- The study involves research on an investigational vaccine;
- The volunteer's primary care practitioner may be contacted to corroborate their medical history or seek additional information;
- The volunteer's blood samples taken as part of the study will be anonymized and stored for a period of 15 years.

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If the volunteer does decide to participate, he or she will sign and date two copies of the consent form, one for his/her personal records, and one to be stored at site in the source documents. These forms will also be signed and dated by the Investigator.

4.2.2 Inclusion and exclusion criteria

This study will be conducted in adults aged 18-30 years, who meet the following inclusion and exclusion criteria:

Inclusion criteria

The volunteer must satisfy the following criteria to be eligible for the study:

- Has provided written informed consent before enrollment;
- Male or female, ages 18-30 years (inclusive) at the time of enrollment;
- With documented history of acellular pertussis immunization (5 doses);
- Free of clinically significant health problems, as determined by pertinent medical history and clinical examination at study screening;
- Non-pregnant, non-lactating female :

  - This implies a negative urinary pregnancy test at enrolment;
  - If sexually active, female subjects must be willing to use reliable birth control measures for 1 month after vaccination;
- Able to attend all scheduled visits and to understand and comply with the study procedures.

Exclusion criteria

The volunteer may not enter the study if any of the following apply:

- Prior dTpa immunization within the last 5 years or prior dT immunization within the last 2 years, or any other investigational vaccine likely to impact on interpretation of the trial data, as judged by the Principal Investigator;
- Suspected or confirmed pertussis infection within the last 10 years or documented pertussis infection in a household member within the last 10 years;
- History of severe local or systemic reactions to any vaccination or a history of severe allergic reactions;
- Known hypersensitivity or allergy to diphtheria, tetanus, or pertussis vaccine (including excipients);
- Receipt of investigational product up to 30 days prior to enrollment or ongoing participation in another interventional clinical trial;
- Receipt of licensed vaccines within 30 days of planned study immunization or ongoing participation in another clinical interventional trial;
- Acute or chronic, clinically significant psychiatric, hematologic, pulmonary, cardiovascular, or hepatic or renal functional abnormality as determined by the Investigator based on medical history, physical exam;
- Any confirmed or suspected immunosuppressive or immunodeficient condition, including human immunodeficiency virus (HIV) infection, asplenia, cytotoxic therapy in the previous 5 years, and/or diabetes;
- Any chronic or active neurologic disorder, including seizures, and epilepsy, excluding febrile seizures as a child;
- Has a known history of vaccine-induces Guillain-Barré Syndrome;
- Has an active malignancy or recent (<10 years) history of metastatic or hematologic malignancy;
- Suspected or known alcohol and/or illicit drug abuse within the past 5 years;
- Pregnant or lactating female, or female who intends to become pregnant during the study period;
- Administration of immunoglobulins and/or any blood products within the 120 days preceding study entry or planned administration during the study period;
- Receipt of chronic (>14 days) immunosuppressants or other immune-modifying drugs within 6 months of study entry:
  - For corticosteroids, this will mean prednisone or equivalent ≥0.5 mg/kg/day,
  - Intranasal and topical steroids are allowed;
- Any other significant finding that, in the opinion of the investigator, would increase the risk of the individual's having an adverse outcome by participating in this study.

Temporary exclusion criteria at the time of randomization

The following criteria constitute contraindications to administration of vaccine at that point in time; if any one of these occurs at the time scheduled for randomization, the subject may be randomized at a later date without the need for re-screening, at the discretion of the Investigator, or withdrawn at the discretion of the Investigator:

- Acute disease at the time of randomization. (Acute disease in the context of this trial is defined as the presence of a moderate or severe illness with or without fever.) The vaccine/placebo can be administered to persons with a minor illness such as mild upper respiratory tract infection with or without low-grade febrile illness, i.e. temperature of ≤37.5°C;
- Body temperature ≥38°C within 3 days of the intended vaccination;
- Any other significant finding that, in the opinion of the investigator, would temporarily increase the risk of the individual's having an adverse outcome by participating in this study.

4.2.3 Compliance with dosing regimen

The first intervention (study vaccine Pertagen® or Revaxis®) will be administered by the Clinical Investigator at Day 0, and the second (Pertagen®) at Day 180. At no time will the study medication be in the possession of the volunteer; compliance will not, therefore, be an issue.

4.3 Randomization

At Day 0, eligible subjects will be randomized in a 1:1 ratio into one of the following vaccine groups:

Group A	Revaxis® + Pertagen®	50 subjects
Group B	Pertagen® + Pertagen®	50 subjects

Table 1. Study groups

Randomization procedure

Randomization allocation will be performed on a 1:1 basis generated by computer randomization, with a 10-block size, concealed in sequentially labeled opaque envelopes. Each participant will be assigned a unique treatment number that corresponds to his or her treatment allocation. Only the unblended immunizing nurses will have access to the treatment allocation.

Randomization of subjects to treatment will occur at Visit 1 after all entry procedures have been performed and eligibility for the study confirmed. 100 volunteers will be allocated to either Group A or Group B at the ratio of 1:1 and will receive a 3-digit subject number preceded by the acronym of the study site (GE-_ _ _). The 3-digit subject number will be assigned according to the participant's chronological order of enrollment at the site. The subject number will be used as the subject identifier throughout the study.

Each participant will be randomized following the randomization list by chronological order and will be immunized according to the vaccine group assignment.

Participants will be blinded in order to keep the double-blind design of the study.

The personal responsible for study vaccine preparation, handling, storage and accountability, as well as immunization will be unblinded.

The team responsible for assessing adverse events will be blinded.

The laboratory staff performing the immunological assays will also be blinded.

4.4 Formal safety analyses

After vaccination, volunteers will be observed for 30 minutes at study site for any immediate post-immunization reactions. Reactogenicity and safety will be assessed during study visits at the clinical trial unit (CTU) on Day 0 and Day 28 after the first immunization, on Day 180 and Day 210 after the second, and finally on Day 365. At Day 0 and Day 180 (Visit 1 and 3, vaccination days), a Diary Card will be distributed to study volunteer to record post-immunization local and systemic reactions and adverse events. At Day 28 and Day 210 (Visit 2 and 4), the investigator will reconcile and transcribe the

Diary Card information in their corresponding CRF. Post-immunization local and systemic reactions will be collected and monitored for 7 days after each vaccination. All adverse events, including SAEs, will be collected and monitored for 28 days after each vaccination (until Visit 2 and until Visit 4).

5 CONDUCT OF STUDY

5.1 Study visits

Participants will be asked to attend 5 scheduled visits at the Plateforme de Recherche Clinique de Pédiatrie, Gynécologie et Obstétrique (PGO) of the HUG. All subjects will be asked to remain in the study throughout its entire duration (one year). If a subject does not appear at a scheduled visit, every effort will be made to contact him or her to confirm that she/he is well and to reschedule the visit. This effort will be documented into the source data. Each study visit is described in detail below. An overview is found in Schedule of Assessment.

Each visit is assigned a time point and a window period within which the visit will be conducted. Deviations from the window periods are to be avoided; however in some exceptional situations, they are permitted at the discretion of the Principal Investigator in the interest of completing the study schedule and obtaining subject safety and immunogenicity evaluations.

5.1.1Visit 1: Randomization, assessment and immunization, Day 0

Adults aged 18-30 years, male and female with no significant medical history or active illness will be recruited by the clinical investigators.

Volunteers who consented and who have signed the informed consent will be screened for eligibility.

The following activities will be performed during the first visit:

- Informed consent form (ICF) will be provided:
    - The volunteer will be allowed sufficient time for questions and answers and reflection as to whether or not they wish to participate; no further activity will take place until signing of ICF;
- Inclusion and exclusion criteria will be evaluated;
- Demographic data (including sex, birthdate, race) will be recorded;
- The medical history (including potential concomitant medication) will be reviewed;
- The female participants will be informed that the study is not open to subjects whom may be or become pregnant. A urinary pregnancy test will be performed and enrollment only confirmed if negative. They will also be reminded of the need to use birth control measures in case of sexual intercourse.
- In all subjects a physical examination will be performed (subject's general appearance, skin, respiratory and cardiovascular function), as well as vital signs (blood pressure, heart rate, temperature, respiratory rate), weight, and height.

Relevant information will be recorded in the site records and in the screening log. Each subject will receive a screening number; no personally identifiable information (PII) will be included in the paper CRF. During this process, no information will be recorded in the CRF. Completion of the CRF will only start after randomization and treatment application.

Subjects remaining eligible for the study with no contraindication to treatment will then be randomized as described in 4.3 Randomization.

- 29 mL of blood will be collected from all subjects for:
    - Complete blood count;
    - Anti-PT and anti-FHA IgG antibodies by ELISA;
    - Anti-diphtheria and anti-tetanus IgG antibodies by ELISA;
    - Anti-PT neutralizing IgG;

    - Ficoll isolation and freezing of PBMCs with preservation and freezing of plasma aliquots;
- The treatment will be administered in a double-blind manner. All subjects will remain under close observation at the clinical trial unit (CTU) for 30 minutes;
- Subject will be given an emergency card with a phone number to call in case of questions or upon occurrence of any AE;
- The appointment for Visit 2 (Day 28) will be confirmed.

Subjects must not leave the study site before the solicited and unsolicited AE check list has been reviewed for the 30 minutes after vaccination. Ongoing AEs at time of discharge will be reported on the diary to be fully assessed over 24 hours by the subject and also for the following days. Should an AE be ongoing at time of discharge, a phone interview will be scheduled for the following day.

5.1.2Phone contact (Day 7 +/-1)

Study participants will be contacted by phone at Day 7 to confirm that adverse reactions have subsided. Should this not be the case, an unscheduled visit will be considered appropriate.

5.1.3Visit 2 (Day 28 +7)

Study participants will be contacted by phone or mail a few days before Visit 2 to confirm the appointment and be reminded of the need to bring the diary card.

The following activities will be performed during Visit 2:

- The diary card on solicited and unsolicited AEs, SAEs and concomitant medications will be discussed, verified and collected;
- AEs and SAEs will be recorded;
- 29 mL of blood will be collected from all subjects for:
    - Complete blood count;
    - Anti-PT and anti-FHA IgG antibodies by ELISA;
    - Anti-diphtheria and anti-tetanus IgG antibodies by ELISA;
    - Anti-PT neutralizing IgG;
    - Ficoll isolation and freezing of PBMC with preservation and freezing of plasma aliquots;
- The appointment for Visit 3 (Day 180) will be confirmed.

5.1.4Visit 3 (Day 180 +/- 30)

Study participants will be contacted by phone or mail a few days before Visit 3 to confirm the appointment.

The following activities will be performed during Visit 3:

- 29 mL of blood will be collected from all subjects for:
    - Complete blood count;
    - Anti-PT and anti-FHA IgG antibodies by ELISA;
    - Anti-diphtheria and anti-tetanus IgG antibodies by ELISA;
    - Anti-PT neutralizing IgG;
    - Ficoll isolation and freezing of PBMCs with preservation and freezing of plasma aliquots;
- The treatment will be administered in a double-blind manner. All subjects will remain under close observation at the clinical trial unit (CTU) for 30 minutes;
- A diary card for recording daily symptoms will be explained and distributed to all subjects;

- Subject will be given an emergency card with a phone number to call in case of questions or upon occurrence of any AE;
- The appointment for Visit 4 (Day 210) will be confirmed.

5.1.5Phone contact (Day 187 +/-1)

Study participants will be contacted by phone at Day 7 to confirm that adverse reactions have subsided. Should this not be the case, an unscheduled visit will be considered as appropriate.

5.1.6Visit 4 (Day 210 +7)

Study participants will be contacted by phone or mail a few days before Visit 4 to confirm the appointment and be reminded of the need to bring the diary card.

The following activities will be performed during Visit 4:

- The diary card on solicited and unsolicited AEs, SAEs and concomitant medications will be discussed, verified and collected;
- AEs and SAEs will be recorded;
- 29 mL of blood will be collected from all subjects for:
    - Complete blood count;
    - Anti-PT and anti-FHA IgG antibodies by ELISA;
    - Anti-diphtheria and anti-tetanus IgG antibodies by ELISA;
    - Anti-PT neutralizing IgG;
    - Ficoll isolation and freezing of PBMC with preservation and freezing of plasma aliquots;
- The appointment for Visit 5 (Day 365) will be confirmed.

5.1.7Visit 5 (Day 365 +/- 30)

Study participants will be contacted by phone or mail a few days before Visit 4 to confirm the appointment.

The following activities will be performed during the final Visit:

- 29 mL of blood will be collected from all subjects for:
    - Complete blood count;
    - Anti-PT and anti-FHA IgG antibodies by ELISA;
    - Anti-diphtheria and anti-tetanus IgG antibodies by ELISA;
    - Anti-PT neutralizing IgG;
    - Ficoll isolation and freezing of PBMC with preservation and freezing of plasma aliquots;
- Participants will be offered one dose of Revaxis® if needed for their protection.

5.2 Unscheduled visits

Subjects may contact the Clinical Investigator at any time for an unscheduled phone or on-site visit should they experience clinical symptoms or signs following treatment. At all unscheduled visits the following minimum will be performed:

- Questions concerning the history of the present illness as well as the subject's general health;
- Medically attended AEs and any possible SAEs will be recorded;
- Any concomitant medication will be noted (any other vaccination is forbidden during the study duration).

After eliciting the history of the present illness and performing any corresponding exams or laboratory tests, the Clinical Investigator will decide on the best course of treatment according to standard medical practice.

5.3 Study assessments

For each study subject, the blood volume taken per visit lies in the range of 29mL for immunologic assays. Blood will be taken on Day 0 and Day 180 (Baseline/vaccination days), as well as Day 28, Day 210 and Day 365 (to assess the response to immunization).

5.3.1 Safety tests

Safety tests will include:

- Vital signs evaluation: heart and respiratory rates, systolic and diastolic blood pressure and axillary body temperature at baseline;
- Local and systemic reactivity assessed 30 minutes after vaccination at the Plateforme de Recherche Clinique PGO;
- Local and systemic reactivity assessed through diary cards, during 7 days after immunization;
- Other adverse events or serious adverse events during 28 days after immunization;
- Study Participants will be able to contact us at any time in case of any adverse event.

5.3.2 Immunogenicity tests

The immunogenicity of both Pertagen® + Pertagen® and Revaxis® + Pertagen® will be assessed in all subjects by ELISA-based detection of antigen-specific human IgG in serum samples. Sera will be assessed by the pertussis toxin neutralization assays to characterize the functionality of anti-PT antibodies.

PBMCs will be frozen on site for subsequent analyses of the frequency and phenotype of specific B or T-cell responses before/after vaccination.

6 STUDY DISCONTINUATION

The study in its entirety may be discontinued prematurely with reasonable justification by the Principal Investigator (PI), Sponsor, Swissmedic or the Ethics Committee with oversight responsibilities at any time (see below), and/or individual subjects may terminate their participation prematurely, or have their participation be terminated by the Investigator.

6.1 Study termination

The PI, Sponsor, Swissmedic or the Ethics Committee with oversight responsibilities for the trial may terminate the trial at any time with reasonable justification. In the case of study termination, investigators will be informed of the procedures to be followed to ensure adequate consideration is given to the subject's safety.

The Investigator will be responsible for informing the Sponsor of the trial's termination within 24 hours, and, in accordance with art. 37 OClin, the Sponsor notifies Swissmedic within 7 calendar days.

6.2 Study withdrawal

In accordance with the principles of the current revision of the Declaration of Helsinki, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reason(s) for doing so. The Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. In addition the volunteer may withdraw/be withdrawn for any of the following reasons:

- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening); if the ineligibility is discovered after study application, the participant will be withdrawn from the study only in the event that the factor leading to ineligibility could directly jeopardize the health of the participant;
- Significant protocol deviation: failure to obtain informed consent prior to any study-specific test or procedure, or failure to follow protocol procedures that specifically relate to the primary safety and/or immunogenicity endpoints of the study;
- Volunteer non-compliance with study requirements;
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilized or a non-trial related causality has been assigned. Any volunteer who is withdrawn from the study may be replaced (see 6.3 Procedures for handling subjects with premature study discontinuation), if that is possible within the specified time frame (i.e., before the termination of study recruitment). The DSMB may recommend withdrawal of volunteers.

Further stopping rules

The Sponsor is entitled to terminate the study at any time for the following reasons:

- Negative criticism on the execution of the study. Upon signals that investigator did not react properly to complaints expressed by participants, violate the study protocol or perform unacceptably otherwise, the investigator(s) may be 'frozen' until further notice, to review the case;
- New data on the safety of the product under study that become known during the study that makes further use of the product undesirable, even in a controlled situation;
- Early results from the study that indicates failure or unsafety of the product under study;
- When investigators come into a situation that impedes the further progress of the study, the study may be discontinued if the investigator cannot be replaced or no other solution can be found.

6.3 Procedures for handling subjects with premature study discontinuation

In the absence of a medical contraindication or significant protocol violation, every effort will be made by the Investigator to keep the subject in the study. If a subject has to be withdrawn, all efforts will be made to complete and report the trial observations as thoroughly as possible. If a subject needs to be withdrawn, all efforts shall be made to follow safety of the subject as per protocol.

Subjects who withdraw or are withdrawn from the study prior to treatment application will be replaced. No follow-up of these subjects will be performed and no data will be analyzed. Subjects withdrawing or withdrawn after treatment application will not be replaced if study recruitment has already been terminated, and their data will be analyzed under the intention-to-treat (ITT) principle.

When a subject withdraws from the study before the planned end of the study period, all investigations scheduled for the end-of-study visit should be performed if the subject agrees. End-of-study evaluation will be completed at the time of the subject's withdrawal, with an explanation of the reason for this entered onto the respective "end-of-study" section of the CRF as follows:

- Adverse event (specify);
- Death;
- Protocol violation (specify);
- Medical condition (specify);
- Consent withdrawal, not due to AE;
- Other (specify).

6.4 Breaking the randomization code

The treatment codes will be held separately from the clinical investigators in charge of assessing the occurrence of adverse events and breaking the blind will be done only at study end.

Breaking the blind for a single subject will be considered when knowledge of the treatment assignment is deemed essential by the subject's physician for the subject's care.
Additionally, in the event of a SUSAR or a holding rule activation, the blind may be broken for the purposes of reporting to Swissmedic and/or determination of trial feasibility, respectively. Any intentional or unintentional breaking of the blind will be reported and explained at the end of the trial, irrespective of the reason for its occurrence. The procedure and timing for revealing the treatment assignments will be documented as well. All calls resulting in an unblinding event will be recorded and reported by the IWRS to the Medical Monitor and the Sponsor.

7 SAFETY ASSESSMENT

Safety will be assessed by the frequency, incidence, severity and nature of adverse events and serious adverse events arising during the study.

7.1 Adverse events

An AE is any untoward medical occurrence in a subject that may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

Each AE will be graded according to the tables for AE severity below (7.8 Assessment of severity). The following guidelines will be used to determine whether or not an AE is recorded in the study database:

- Solicited AE (*i.e.*, reactogenicity parameters) will be recorded on diary cards in the 7 days and recorded in the CRF in the 7 days following treatment;
- Unsolicited AE of all severities will be recorded on diary cards in the 7 days and recorded in the CRF in the 28 days following treatment;
- SAE (as defined in 7.3 Serious adverse events) will be recorded in the CRF in the 28 days following treatment.

7.2 Adverse reactions

An adverse reaction (AR) is any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the reporting Investigator as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as AR.

7.3 Serious adverse events

A SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention:

- Death;
- Life-threatening event (i.e., the subject was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more severe form, might have caused death;
- Persistent or significant disability or incapacity (i.e., substantial disruption of one's ability to carry out normal life functions);
- Hospitalization, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalization (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a SAE;
- An important medical event (that may not cause death, be life-threatening, or require hospitalization) that may, based upon appropriate medical judgment, jeopardize the subject and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive care in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization;
- Congenital anomaly or birth defect.

7.4 Serious adverse drug reaction (SADR)

An event that is expected or unexpected and is both serious and, in the opinion of the reporting investigator or Sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, based on the information provided, is considered a serious adverse drug reaction (SADR or SAR).

7.5 Suspected unexpected serious adverse reaction (SUSAR)

A SUSAR is an SAE that is unexpected and thought to be possibly, probably or definitely related to an IMP. No category of SAE has been defined as 'expected.'

7.6 Causality assessment

For every AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken. An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine.

The degree of certainty with which an AE can be attributed to administration of the study vaccine(s) (or alternative causes, e.g. natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of one or more of the following:

related suspicion that there is a relationship between vaccine and AE (without determining the extent of probability); there is a reasonable possibility that the vaccine contributed to the AE

unrelated there is no suspicion that there is a relationship between vaccine and AE, there are other more likely causes and administration of the study vaccine is not suspected to have contributed to the AE

The causality assessment will be made on the basis of the available information at the reporting time point and apply to both AEs and SAEs. Assessment of causality can change according to follow-up information.

7.7 Reporting procedures for all adverse events

All AEs and SAEs occurring within the 28 days following treatment and observed by the Investigator or reported by the volunteer, whether or not attributed to study intervention, will be recorded in the CRF. All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this).

7.7.1 Reporting procedures for SAE

Following the occurrence of any SAE, the trial subject's randomization code may be broken if deemed necessary (see 6.4 Breaking the randomization code). The PI/Sponsor shall report the SAE to the CCER, BioNet-Asia Pharmacovigilance and Swissmedic, as soon as learning that the event meets the definition of a SAE and no later than 24h for fatal or life-threatening SAE and 7 calendar days for non-lethal SAE. The minimum information included in the initial SAE report will contain: subject's number, date of birth, description of the event, study vaccine (date of vaccination), reporter information and causality assessment.
Important follow-up information shall be reported when available. If relevant information is missing at the time of the initial SAE report, the reporter shall provide it in follow-up SAE report(s). A follow-up report shall contain new, updated or corrected information. The follow-up report shall describe whether the event has resolved or continues, if and how it was treated including documentation of all supportive actions taken, and whether the blind was broken or not.

7.7.2 Reporting procedures for SUSAR

Following the occurrence of a SUSAR, the trial subject's randomization code shall be broken (see 6.4 Breaking the randomization code) and the PI shall report the event to the CCER, BioNet-Asia Pharmacovigilance and Swissmedic within 24h of learning that the event meets the definition of a SUSAR.

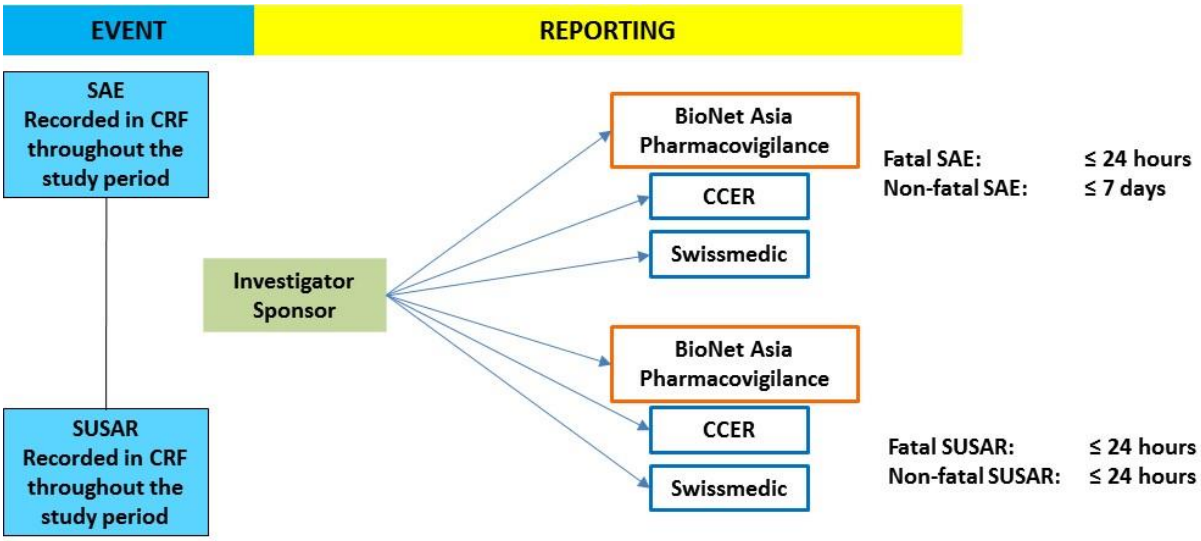

Figure 5. Workflow for reporting of serious adverse events and SUSAR

7.8 Assessment of severity

The severity of clinical and laboratory AE will be assessed according to the scales in Tables 5-7.

Table 5. Toxicity grading scale for local adverse events

Toxicity grading scale for local adverse events						
Local Reaction	**Grade 0**	**Grade 1a**[£] **Mild**	**Grade 1b**[†] **Mild**	**Grade 2** **Moderate**	**Grade 3** **Severe**	**Grade 4** **Potentially life-threatening**
Redness/Erythema*	None	<25 mm	25-50 mm	51-100 mm	>100 mm	Necrosis or exfoliative dermatitis
Swelling/Induration**	None	<25 mm	25-50 mm and does not interfere with activity	>50 mm or interferes with activity	Prevents daily activity	Necrosis
Pain	None	-	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization

[£]Grade 1a = when localized under the patch; [†]Grade 1b = mild but extending beyond the patch

*In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

**Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement. Adapted from the FDA's 2007 voluntary guidance: http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf.

Table 6. Toxicity grading scale for Vital signs

Vital signs					
Observation	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Fever (axillary)	38.0° - 39.0°C	>39.0°C - 40.0°C	>40.0°C for ≤24 hours	>40.0°C for ≥24 hours	Death
Tachycardia (bpm)*	Asymptomatic, intervention not indicated	Symptomatic, non-urgent medical intervention indicated	Urgent medical intervention indicated	Life-threatening consequences, urgent intervention indicated	Death
Bradycardia (bpm)**	Asymptomatic, intervention not indicated	Symptomatic, medical intervention indicated	Severe, medically significant, urgent medical intervention indicated	Life-threatening consequences, urgent intervention indicated	Death
Hypertension**	Prehypertension (systolic BP 120 - 139 mm Hg or diastolic BP80 - 89 mm Hg)	Stage 1 hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 - 99 mm Hg); medical intervention indicated; recurrent or persistent (≥24h); symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously WNL; monotherapy indicated	Stage 2 hypertension (systolic BP ≥160 mm Hg or diastolic BP ≥100 mm Hg); medical intervention indicated; more than one drug or more intensive therapy than previously used indicated	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated	Death
Hypotension	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated	Death

Definitions	
Fever	A disorder characterized by elevation of the body's temperature above the upper limit of normal.
Tachycardia	A disorder characterized by a dysrhythmia with a heart rate greater than 100 beats per minute.
Bradycardia	A disorder characterized by a dysrhythmia with a heart rate less than 60 beats per minute.
Hypertension	A disorder characterized by a pathological increase in blood pressure; a repeatedly elevation in the blood pressure exceeding 140 over 90 mm Hg.
Hypotension	A disorder characterized by a blood pressure that is below the normal expected for an individual in a given environment.

*Taken after ≥10 minutes at rest; **when resting heart rate is between 60 - 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subjects, for example, conditioned athletes.

From CTCAE v4 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)

Table 7. Toxicity grading scale for systemic AEs excluding the vital signs listed above

Toxicity grading scale for systemic adverse events				
Systemic sign/symptom	**Grade 1** **Mild**	**Grade 2** **Moderate**	**Grade 3** **Severe**	**Grade 4** **Potentially life-threatening**
Headache	No interference with activity	Some interference with activity	Significant; prevents daily activity	Medical consultation and/or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	Medical consultation or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	Medical consultation and/or hospitalization
Arthralgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	Medical consultation and/or hospitalization
Chills	No interference with activity	Some interference with activity	Significant; prevents daily activity	Medical consultation and/or hospitalization
Sweats	No interference with activity	Some interference with activity	Significant; prevents daily activity	Medical consultation and/or hospitalization
Subjective Fever	No interference with activity	Some interference with activity	Significant; prevents daily activity	Medical consultation and/or hospitalization
Nausea	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Medical consultation and/or hospitalization
Vomiting	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Medical consultation and/or hospitalization
Abdominal Pain	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Medical consultation and/or hospitalization
Diarrhea	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Medical consultation and/or hospitalization
Other systemic symptoms	No interference with activity	Some interference with activity	Prevents daily activity	Medical consultation and/or hospitalization

From the FDA's 2007 voluntary guidance:
(http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf)

7.9 Summary of risk mitigation strategies

The following risk mitigation strategies have been identified and will be implemented.

Table 8. Risk mitigation strategies

Individual risk	Risk evaluation	Identified risk mitigation strategies	Suggested implementation
Local allergic reaction	Low risk. No known allergen included.		Local cortical cream or antihistaminic treatment for symptom relief
Anaphylactic reaction	Low risk. No known allergen included.	Exclusion of subjects with history of severe vaccine allergic reactions	Surveillance of all subjects on site for 30 minutes after immunization.
Strong / sustained inflammatory reactions	Low risk: low reactogenicity of pertussis antigens.	Paracetamol, NSAID	Monitoring (Day 7 phone call) + emergency phone numbers.
			Indications on use of paracetamol and NSAID for symptom relief
Direct pathogenicity of rPT	Low risk: reduced toxicity (by a factor of 10^5- 10^6) compared to native PT	Exclusion of potentially vulnerable populations (young children, pregnant women, etc.)	None additional needed

8 STATISTICAL ANALYSIS

8.1 Sample size calculation

This is a phase II/III randomized, double-blind controlled study to compare the safety and immunogenicity of one compared to two doses of acellular pertussis vaccines including genetically-detoxified PT in adults previously immunized with chemically inactivated acellular pertussis vaccines. The sample size for this study is based on the previous study using the rPT vaccine in teenagers in Geneva (Blanchard Rohner et al. CID 2019). Where applicable, 2-sided test at the 5% significance level will be utilized with no multiplicity adjustment. P-values will be considered exploratory only. One hundred volunteers will be randomized in total, with 50 in each group.

8.2 Safety analysis

The safety analysis will include all randomized subjects who have received a dose of study vaccine.
The overall percentage of subjects with at least one spontaneously reported adverse event, with date of onset up to 28 days after vaccination will be tabulated with exact 95% confidence intervals, by type of adverse event; by severity; and by causality. They will be displayed by vaccine group as both frequencies and percentages on the ITT data set.
All reported adverse events that start post-vaccination will be tabulated. If a given disease is already reported as ongoing at the first visit on the medical history pages, it will be counted and tabulated as a vaccine emergent adverse event only if it worsens after the immunization with the study vaccine.
Serious adverse events and discontinuation due to adverse event(s) will be described in detail by vaccine group.

8.3 Immunogenicity analyses

The primary immunogenicity endpoint will assess the immune response to two doses of Pertagen®, as compared to one dose, by measuring the Day 28 geometric mean concentration (GMC) of neutralizing antibodies to PT after immunization.
The other immunogenicity end-points are listed in Section 2.2 of this protocol.

The following descriptive statistics will be provided for each variable: number of subjects, percentages, mean, geometric mean, standard deviation, median, minimum, maximum, and range.

Immunogenicity endpoints and confidence bounds will be calculated for each group and compared (using Chi-square or Fisher's exact test for binary endpoints, and Wilcoxon-Mann-Whitney's test or Kruskal-Wallis test for quantitative endpoints). All tests will be two-sided.

9 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

9.1 Investigator procedures

Approved site-specific SOPs will be used at clinical and laboratory sites.

9.2 Monitoring

Monitoring will be performed according to ICH Good Clinical Practice (GCP) by the Centre de Recherche Clinique (CRC) of the University Hospital of Geneva. Following a Monitoring Plan and written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The CTU will provide direct access to all trial-related source data, documents and reports for the purpose of monitoring and inspection by local and regulatory authorities.

9.3 Modification to protocol

No substantial amendments to this protocol will be made without consultation with BNA. HUG/Sponsor will promptly inform BNA of any proposed amendment to the Protocol, in writing, and allow BNA to review and comment on the proposed amendment. However, for the avoidance of doubt, HUG/Sponsor shall have overall responsibility for finalizing the Protocol in accordance with the Applicable Requirements.

The Principal Investigator is responsible for ensuring that changes to an approved trial, during the period for which regulatory and EC approval has already been given, are not initiated without regulatory and EC review and approval except to eliminate apparent immediate hazards to the subject.

9.4 Protocol and GCP deviation

Any deviations from the protocol and GCP will be documented in a protocol deviation form and filed in the trial master file.

9.5 Trial progress

The progress of the trial will be overseen by the Principal Investigator.

9.6 Publication policy

The investigators shall have the right, consistent with academic standards, to publish or present the results of the research work performed as described in this protocol. The investigators agree to appropriately acknowledge the source of the product and information as well as the contribution of any scientist from BNA, as appropriate. The Principal Investigator agrees that submission for publication of any results relating to this study may be undertaken only after the sponsor and other Consortium members have had thirty (30) working days to review the manuscript (fifteen (15) days for abstracts).

10 ETHICAL AND REGULATORY ASPECTS

10.1 Declaration of Helsinki

Study investigators will ensure that this study is conducted according to the principles of the latest revision of the Declaration of Helsinki (Fortaleza, Brazil, October 2013).

10.2 ICH Guidelines for Good Clinical Practice (GCP)

Study investigators will ensure that this study is conducted in full conformity with the ICH Good Clinical Practice (GCP), the requirements of the Swiss Human Research Act (HRA; 810.30, 2011) and the Swiss ordinance on clinical trials (ClinO; 810.305, 2013), and local regulatory requirements.

10.3 Study registration

The study will be registered in www.clinicaltrials.gov as soon as we have all authorizations to start the study.

10.4 Categorization of study

This Clinical Trial is under Category C, as it is evaluating the effects of a medicinal product that is authorized in other countries but not yet in Switzerland.

10.5 Ethics committee and regulatory agency review

The protocol, proposed ICF, and all other study documents, will be submitted to the Geneva Canton ethics committee and Swissmedic for approval. The Principal Investigator will submit and, where necessary, obtain approval from the EC and Swissmedic for all subsequent substantial amendments to the protocol and ICF.

10.6 Declaration of interest

The Principal Investigator and all co-investigators declare no conflict of interest in the concept and execution of this study.

10.7 Informed consent

Written, informed consent must be obtained before a volunteer can be enrolled, as described in 4.2.1 Informed consent. The risks and benefits incurred by the volunteer's participation will be stated clearly in the ICF and discussed directly with the volunteer before any enrollment can occur.

10.8 Benefits and risks to the participant

All the participants will receive an efficient pertussis immunization with a BNA® aP (Pertagen®) and Revaxis®, to ensure the recall of immunity against diphtheria, tetanus and the 3 pertussis antigens. Vaccine-induced immunity will be confirmed by serological analyses.
The risks to the participant are the potential side effects of the vaccine, as described above, as well as the pain and bruising that may occur as a result of the blood draws described above.
Participants will receive a compensation of 200 CHF for the inconvenience (time, blood draws, etc.).

10.9 Subject confidentiality

All data will be coded: subject data will be identified by a unique study number containing no personally identifiable information (PII) in the CRF and database. A separate confidential file containing

PII will be stored in a secured (locked) location in accordance with data protection requirements. Only the sponsor study investigators, the study monitor, the EC and regulatory authorities will have access to the records. Photographs taken of vaccination sites (if required, with the subject's written, informed consent) will not include the subject's face and will be identified by the date, trial code and subject's unique identifier. Photographs will be stored as confidential records, as above. Again, with the subject's written consent, this material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

11 DATA HANDLING AND RECORD KEEPING

11.1 Data handling and management

The Principal Investigator will have the responsibility for overseeing the receiving, entering, cleaning, querying, analyzing and storing all data that accrue from the study by designated persons. Data handling and management will be performed by the Plateforme de Recherche Clinique PGO. Throughout regular data collection and monitoring, clinical data reported on CRFs and/or relevant serological results will be integrated into a trial database using TeleForm (http://www.cardiff-teleform.com/). This includes safety data and outcome data.

For each set of data, quality control and triggers to computerized logic and/or consistency checks will be systematically applied in order to detect errors or omissions. After integration of all corrections in the complete set of data, the database will be locked and saved before being released for final statistical analyses. Each step of this process will be monitored through the implementation of individual passwords and/or regular backups in order to maintain appropriate database access and to guarantee database integrity.

11.2 Record keeping

The Investigator will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The Principal Investigator, co-investigators and clinical research nurses will have access to records. Investigators will permit authorized representatives of the Sponsor, monitors, as well as ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of monitoring, quality assurance reviews, audits and evaluation of the study safety and progress.

11.3 Source data and case report forms (CRFs)

All protocol-required information will be collected in source documents and/or entered into CRFs by study investigators. Source documents are original documents, data, and records from which the subject's CRF data are obtained. For this study, these will include, but are not limited to, subject consent form, clinical notes (medical history, concomitant medication, copy of vaccination records, vital signs, physical examination, AE data), laboratory data (pregnancy test for females, hematology and immunology results), diaries, and correspondence. All source data and CRFs will be stored securely.

The information to be recorded in the CRF is listed in detail in Box 1. The CRF accompanies this study protocol.

Box 1. Subject information to be recorded in the CRF.

Demographic information and habits:

- Sex
- Age
- Ethnicity

Medical history and physical findings:

- Past medical and surgical history
- Any current medical conditions
- Occurrence of AE, solicited and unsolicited

- Concomitant medications and other therapy
- The contents of the subject diary card (Days 0 – 7)
- Physical examination findings

Results of laboratory and other analyses:

- Results of urine pregnancy screens, if applicable
- In the event of an AE, the results of any additional laboratory testing as determined clinically indicated and appropriate

Note: The collection of information on ethnicity is routine practice in vaccine studies, as ethnicity may influence vaccine responses (Haralambieva *et al*. 2013 and 2014, Avnir *et al*. 2016)

11.4 Data protection and ownership

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party. As delineated in the *Collaboration Agreement* between the Sponsor and Bionet Asia, the data generated throughout the course of the study will be owned by the Sponsor.

11.5 Retention and destruction of study data and biological material

Study data and biological material will be stored for 15 years after study termination. Thereafter, biological samples will be destroyed according to usual biosafety/laboratory, which include documentation of each sample and its destruction. There is currently no plan for further use of samples (biobanking).

12 FINANCING AND INSURANCE

12.1 Financing

This investigator-driven study is funded by private foundations and available research funds of the Centre for Vaccinology. BNA will provide the Study vaccine (Pertagen®) and assistance at no charge. A contract between HUG and BioNet-Asia Co., Ltd. is finalized and will be communicated as soon as signed.

There will be no costs to the institution.

12.2 Insurance

Study insurance will be provided by the Sponsor. The finalized insurance policy will be solicited following reception of Ethical Authorization and forwarded as soon available – prior to study initiation.

12.3 Compensation of subjects

A compensation of 200 CHF will be offered to the subjects after completing all 5 visits.
All study visits and related assays will be financed by the Sponsor, with no charge to the subjects or their healthcare insurance carriers.

REFERENCES

Avnir, Y., et al., IGHV1-69 polymorphism modulates anti-influenza antibody repertoires, correlates with IGHV utilization shifts and varies by ethnicity. Sci Rep, 2016. 6: p. 20842.
Acosta AM, DeBolt C, Tasslimi A *et al*. Tdap vaccine effectiveness in adolescents during the 2012 Washington State pertussis epidemic. Pediatrics 2015;135(6):981-989.

Althouse BM, Scarpino SV. Asymptomatic transmission and the resurgence of Bordetella pertussis. BMC Med 2015;13:146.

Black S. Epidemiology of pertussis. Pediatr Infect Dis J 1997;16(4 Suppl):S85-S89.

Buasri W, Impoolsup A, Boonchird C *et al*. Construction of Bordetella pertussis strains with enhanced production of genetically-inactivated Pertussis Toxin and Pertactin by unmarked allelic exchange. BMC Microbiol 2012;12:61.

Centers for Disease Control and Prevention. From the Centers for Disease Control and Prevention. Resurgence of pertussis--United States, 1993. MMWR Morb Mortal Wkly Rep 1993;42:952-960.

Cherry JD. Pertussis in the preantibiotic and prevaccine era, with emphasis on adult pertussis. Clin Infect Dis 1999;28 Suppl 2:S107-S111.

Christie CD, Marx ML, Marchant CD, Reising SF. The 1993 epidemic of pertussis in Cincinnati. Resurgence of disease in a highly immunized population of children. N Engl J Med 1994;331(1):16-21.

Di Tommaso A, De Magistris MT, Bugnoli M, Marsili I, Rappuoli R, Abrignani S. Formaldehyde treatment of proteins can constrain presentation to T cells by limiting antigen processing. Infect Immun 1994; 62:1830–4.

Garcia-Corbeira P, Dal-Re R, Aguilar L, Garcia-de-Lomas J. Seroepidemiology of Bordetella pertussis infections in the Spanish population: a cross-sectional study. Vaccine 2000;18(21):2173-2176.

Blanchard Rohner, G., et al. (2019). "Boosting Teenagers With Acellular Pertussis Vaccines Containing Recombinant or Chemically Inactivated Pertussis Toxin: A Randomized Clinical Trial." Clin Infect Dis 68(7): 1213-1222.

Greco D, Salmaso S, Mastrantonio P, Giuliano M, Tozzi AE, Anemona A, et al.A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. N Engl J Med 1996; 334:341–8.

Guris D, Strebel PM, Bardenheier B *et al*. Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990-1996. Clin Infect Dis 1999;28(6):1230-1237.

Haralambieva, I.H., et al., Associations between race, sex and immune response variations to rubella vaccination in two independent cohorts. Vaccine, 2014. 32(17): p. 1946-53.

Haralambieva, I.H., et al., Race and sex-based differences in cytokine immune responses to smallpox vaccine in healthy individuals. Hum Immunol, 2013. 74(10): p. 1263-6.

Ibsen PH. The effect of formaldehyde, hydrogen peroxide and genetic detoxification of pertussis toxin on epitope recognition by murinemonoclonal antibodies. Vaccine 1996; 14:359–68.

Keitel WA, Muenz LR, Decker MD *et al*. A randomized clinical trial of acellular pertussis vaccines in healthy adults: dose-response comparisons of 5 vaccines and implications for booster immunization. J Infect Dis 1999;180(2):397-403.

Podda A, Carapella De LE, Titone L *et al*. Immunogenicity of an acellular pertussis vaccine composed of genetically inactivated pertussis toxin combined with filamentous hemagglutinin and pertactin in infants and children. J Pediatr 1993;123(1):81-84.

Rappuoli R. The vaccine containing recombinant pertussis toxin induces early and long-lasting protection. Biologicals 1999;27(2):99-102.

Salmaso S, Mastrantonio P, Tozzi AE, Stefanelli P, Anemona A, Ciofi degli Atti ML, et al. Sustained efficacy during the first 6 years of life of 3-component acellular pertussis vaccines administered in infancy: the Italian experience. Pediatrics2001; 108:E81.

World Health Organization. Pertussis vaccines: WHO position paper. Wkly Epidemiol Rec 2010;85(40):385-400.

World Health Organization. Revised guidance on the choice of pertussis vaccines: July 2014. Wkly Epidemiol Rec 2014a;89(30):337-340.

World Health Organization. WHO SAGE pertussis working group: Background paper SAGE April 2014. 2014b