Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals SA
Rue de l'Institut 89, 1330 Rixensart, Belgium

Primary Study vaccine and number	GlaxoSmithKline (GSK) Vaccines' Polygeline-free Tick-Borne Encephalitis Vaccine for adults (GSK3536859A)
Other Study vaccine(s)/product(s)	None
eTrack study number and Abbreviated Title	205847 (TBEV POLYGELINE FREE-025 EXT:021)
Investigational New Drug (IND) number	Not applicable
EudraCT number	2017-001356-59
Date of protocol	Final Version 1: 19 June 2017
Date of protocol amendment	Amendment 1 Final: 25 August 2017 Amendment 2 Final: 23 February 2018
Title	Long term immunogenicity up to 15 years after the first booster immunization with GSK Biologicals' *Encepur Adults* in adults who received 1 of 3 different primary vaccination schedules.
Detailed Title	A phase IV, open-label, single-center study to evaluate long term immunogenicity up to 15 years after the first booster immunization with *Encepur Adults* (Polygeline-free Tick-borne Encephalitis vaccine for adults) in adults who received 1 of 3 different primary vaccination schedules.
Co-ordinating author	PPD, Scientific writer, contractor for GSK Biologicals
Contributing authors **(Amended: 23 February 2018)**	• PPD, Clinical Research and Development Lead • PPD, Clinical and Epidemiology Research and Development Project Lead • PPD, Project statistician • PPD, Statistics

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Contributing authors	• PPD, Study Delivery Lead • PPD, Clinical Safety representative • PPD, Global Regulatory Affairs representative • PPD, Oversight Data Manager • PPD, ***Oversight Data Manager*** • PPD, Clinical Trial Supply Manager • PPD, Local Delivery Lead, PPD for GSK Biologicals • PPD, Clinical Readout Team Leader Clinical Laboratory Sciences • PPD, Global Patents

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Protocol Amendment 2 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	205847 (TBEV POLYGELINE FREE-025 EXT:021)
IND number	Not applicable
EudraCT number	2017-001356-59
Date of protocol amendment	Amendment 2 Final: 23 February 2018
Detailed Title	A phase IV, open-label, single-center study to evaluate long term immunogenicity up to 15 years after the first booster immunization with *Encepur Adults* (Polygeline-free Tick-borne Encephalitis vaccine for adults) in adults who received 1 of 3 different primary vaccination schedules.
Sponsor signatory	Ashwani Kumar Arora Clinical and Epidemiology Research and Development Project Lead
Signature	_____
Date	_____

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Protocol Amendment 2 Rationale

Amendment number:	Amendment 2

Rationale/background for changes:

This protocol has been amended to clarify that:

- New subject identification numbers will be assigned in the study with an established link to the subject identification numbers used in the previous V48P7, V48P7E1 and V48P7E2 studies
- Despite there being no randomization in the study; Source DataBase for Internet Randomization (SBIR) is being used for allocation of the study treatment and management of study vaccines.

Additionally, the exact assay name and details of the tick-borne encephalitis neutralization test has been presented.

Protocol Amendment 2 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' study vaccine(s) and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccine(s), and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	205847 (TBEV POLYGELINE FREE-025 EXT:021)
EudraCT number	2017-001356-59
Date of protocol amendment	Amendment 2 Final: 23 February 2018
Detailed Title	A phase IV, open-label, single-center study to evaluate long term immunogenicity up to 15 years after the first booster immunization with *Encepur Adults* (Polygeline-free Tick-borne Encephalitis vaccine for adults) in adults who received 1 of 3 different primary vaccination schedules.
Investigator name	_____
Signature	_____
Date	_____

For internal use only

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals
Rue de l'Institut 89, 1330 Rixensart, Belgium

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Sponsor Study Contact for Reporting of a Serious Adverse Event

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 8.4.2.

SYNOPSIS

Detailed Title	A phase IV, open-label, single-center study to evaluate long term immunogenicity up to 15 years after the first booster immunization with *Encepur Adults* (Polygeline-free Tick-borne Encephalitis vaccine for adults) in adults who received 1 of 3 different primary vaccination schedules.
Indication	Active immunization in individuals from 12 years of age against tick-borne encephalitis (TBE) virus. The vaccination is intended for residents or travellers in TBE-endemic areas, according to national recommendations.
Rationale for the study and study design	Tick-borne encephalitis (TBE) is a serious, acute, central nervous system infection caused by the tick-borne encephalitis virus (TBEV), a member of the genus *Flavivirus* which may result in death or long-term neurological sequelae in 35–58% of patients (WHO, 2011a). *Encepur Adults* is GSK Biologicals' vaccine which is licensed for use in vaccination against TBE. Active immunization with GSK Biologicals' TBE vaccine for adults is indicated for persons from 12 years of age or older who stay permanently or temporarily in TBE endemic areas, especially when pursuing outdoor activities. There are several different schedules registered for vaccination against TBE: • Conventional schedule: For the primary vaccination schedule the second dose is given 1-3 months after the first dose and third dose is given 9-12 months after the second dose. The second dose can be brought forward and given as early as 14 days after the first dose. The first booster dose is recommended to be given 3 years after completion of the primary vaccination schedule. • Rapid schedule: For the primary vaccination schedule the first, second and third dose are given on days 0, 7 and 21, respectively. The first booster dose is recommended to be given12-18 months after completion of the primary vaccination schedule. Independent of the primary schedule, all subsequent booster doses can be given 5 years after the last booster vaccination. Immunogenicity data collected so far in study V48P7E2 suggest that above 90% of individuals have neutralization titre (NT) ≥10 for at least 10 years after booster vaccination regardless of the type of primary vaccination schedule. This study is designed to continue the evaluation of antibody persistence through 11 to 15 years after first booster with TBE

vaccine, in order to learn more about the anti-TBEV antibodies' kinetics in the long term. This study will further investigate the booster response in subjects with a NT titre below 10 who will receive their second booster dose* in this study. These data will be utilized to guide recommendations for timing of the 2nd booster dose.

* Any booster given in this study will be the second that the subject has received (with regard to the follow-up of the previous study).

Participants of study V48P7E2 will be asked to participate in the current study. The aim of this study is to investigate the persistence of antibody response in adults up to 15 years after one *Encepur Adults* booster dose. Persistence of antibody response will be evaluated based on TBE-NT. Subjects with an NT titre below 10 at enrolment and subjects with a decrease of NT titre to below 10 during the study will be invited to receive a booster vaccination. Data collected from this study may allow the provision of better information to prescribers who administer *Encepur Adults,* so that they can appropriately adjust the time of booster vaccinations to individuals who received different primary vaccination schedules and who live in TBE endemic regions.

Objective(s)

Primary

- To evaluate the persistence of antibody response to a booster dose of *Encepur Adults* vaccine starting ≥11 years after the first booster administration and to continue following subjects up to 15 years after first booster administration.

Secondary

- To evaluate the immune response at 21 days after booster dose (boostability) in subjects with an NT titre below 10.
- To evaluate the safety of a booster dose with regard to SAEs collected after vaccination until study end.

Study design

- Experimental design: Phase IV, open-label, mono-centric study.
- Duration of the study: For each subject, the study will last less than 5 years.
  - Epoch 001: Persistence evaluation starting at Visit 23 (Year 11) and ending at Visit 27 or Visit 27.2.
- Primary completion Date (PCD): Visit 27

- End of Study (EoS): Last testing results released of samples collected at Visit 27 or Visit 27.2.
- Study groups: Same groups as in study TBEV POLYGELINE FREE (V48)-023 EXT:021.

Synopsis Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects*	Age	Epochs
			Epoch 001
C	51	25 years and above	x
R	46	25 years and above	x
AC	103	25 years and above	x

* Approximate number of subjects

C: Conventional schedule: Primary vaccination of 66 subjects in study V48P7 on Days 0, 28 (+10) and 300 (+21), booster vaccination of 55 subjects in study V48P7E1, no vaccination in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2).

R: Accelerated (or "rapid") schedule: Primary vaccination of 66 subjects in study V48P7 on Days 0, 7 (+3) and 21 (+7), booster vaccination of 9 subjects in study V48P7E1 (40 subjects received their booster vaccination before enrolment in study V48P7E1), no vaccination in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2).

AC: Accelerated conventional schedule: Primary vaccination of 133 subjects in study V48P7 on Days 0, 14 (+3) and 300 (+21), booster vaccination of 109 subjects in study V48P7E1, no vaccination in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2).

Synopsis Table 2 Study groups and treatment foreseen in the study*

Treatment name	Vaccine/Product name	Study Groups		
		C	R	AC
Encepur Adults	*Encepur Adults*	X	X	X

*Only subjects with an antibody titre below 10 at a scheduled visit.

- Vaccination schedule: Second booster vaccination six months (minus 60 days /plus 30 days) after the blood draw in subject with an NT titre below10.

  If a subject receives a booster dose in this study then he/she will not have to come for any future scheduled visits. This subject will be included in the sub-cohort of subjects who received a second booster dose. No further study procedures will be conducted after the blood sample collection 21 days post vaccination; however the subject will be followed up for safety until study conclusion (Refer Sections 8.3.1 and 8.3.2 in the protocol).
- Treatment allocation: Independent of the study group, vaccination six months (minus 60 days /plus 30 days) after the blood draw only in subjects with an NT titre below10.

- Sampling schedule: Blood samples to obtain neutralizing antibodies will be collected from all subjects at Visit 23 (Year 11), Visit 24 (Year 12 plus 15 days /minus 30 days), Visit 25 (Year 13 plus 15 days /minus 30 days), Visit 26 (Year 14 plus 15 days /minus 30 days) and Visit 27 (Year 15 plus 15 days /minus 30 days). Subjects who received a second booster vaccination will have an additional visit for a blood draw 21 days (plus 7 days) after the vaccination.
- Type of study: Extension of study TBEV POLYGELINE FREE (V48)-023 EXT:021 (V48P7E2).
- Data collection: Electronic Case Report Form (eCRF).
- Safety monitoring: SAEs related to study participation, or to a concurrent GSK medication/vaccine will be collected for all subjects as of informed consent obtained, for the entire duration of the study. For subjects who receive their second booster dose in this study, all SAEs will be collected for 1 month after vaccination. Post-study SAEs will be collected until study conclusion.

Number of subjects

A total of approximately 200 subjects who participated in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2), who received in a parent V48P7 study one of the following schedules: rapid (R), conventional (C), or accelerated conventional (AC) and a booster vaccination in study V48P7E1 or before study V48P7E1 (only R-schedule) and agreed to participate in this study are to be enrolled.

The number of subjects who will have a decrease of NT antibody titre to below 10 cannot be estimated. No information about the kinetics of NT antibodies until 15 years after booster vaccination with *Encepur Adults* is available from clinical studies or publications.

Endpoint(s)

Primary

The immunogenicity endpoints will be based on the TBE NT antibody levels in serum at year 11, 12, 13, 14 and 15 as measured by GSK Biologicals' NT.

Measures of immunogenicity are:

- Percentages of subjects with detectable TBE Neutralizing Antibody Titres ≥ 2 and ≥ 10 as measured by GSK Biologicals' NT.
- Geometric Mean Antibody Titres as measured by GSK Biologicals' NT calculated for each of the different schedule groups.

Endpoints will be summarized according to the immunization schedule received in the V48P7E1 study and further detailed by the following age subgroups: 25 to 49 years, ≥ 50 years and ≥ 60 years.

Secondary

The immunogenicity endpoints for subjects who received a second booster vaccination will be based on the TBE NT antibody levels in serum at 21 days after the booster vaccination as measured by GSK Biologicals' NT.

Measures of immunogenicity are:

- Percentages of subjects with detectable TBE Neutralizing Antibody Titres ≥ 2 and ≥ 10 as measured by GSK Biologicals' NT, overall and by study group.
- Geometric Mean Antibody Titres and Geometric Mean Ratios (GMRs) blood draw after/before booster as measured by GSK Biologicals' NT, overall and by study group.
- To complement the analysis of persistence, a thorough description of NT waning from year 1 after the first booster dose up to 15 years will be presented for the set of subjects completing the entire 15-year follow-up with no protocol deviations, including those receiving a second booster dose; for whom, a constant value of NT = 1 from the post booster visit will be used in the analysis.

Endpoints will be summarized according to the primary immunization schedule received in the parent study (V48P7).

The safety endpoint for subjects who will need a second booster vaccination will be based on SAEs collection after the second booster dose. In vaccinated subjects all SAEs will be collected for 1 month after vaccination. Depending on the timing of the booster dose this will be in the period year 11.5 (Visit 23.1), 12.5 (Visit 24.1), 13.5 (Visit 25.1), or 14.5 (Visit 26.1) or from year 15.5 (Visit 27.1) until 21 days after year 15.5 (Visit 27.2). Post-study SAEs will be collected until study conclusion (A post-study SAE is defined as any event that occurs outside of the SAE reporting period defined. Refer to Section 8.3.2 in the protocol for details on post-study SAE).

Measures of safety are:

- Incidence of serious adverse events.

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LIST OF ABBREVIATIONS

AC:	Accelerated Conventional
AE:	Adverse event
ANOVA:	Analysis of Variance
AP:	Analysis Plan
ATEAM:	Advanced Temperature Excursion Analysis and Management
C:	Conventional
CI:	Confidence Interval
CLS:	Clinical Laboratory Sciences
eCRF :	electronic Case Report Form
CSR:	Clinical Study Report
DNA	Deoxyribonucleic Acid
EoS:	End of Study
FAS:	Full Analysis Set
FDA:	Food and Drug Administration, United States
GCP:	Good Clinical Practice
GMR:	Geometric Mean Ratio
GMT:	Geometric Mean Titre
GSK:	GlaxoSmithKline
IB:	Investigator Brochure
ICF:	Informed Consent Form
ICH:	The International Conference on Harmonization
IDMC:	Independent Data Monitoring Committee
IEC:	Independent Ethics Committee
IM:	Intramuscular

IMP:	Investigational Medicinal Product
IND:	Investigational New Drug
IV:	Intravenous
LAR:	Legally Acceptable Representative
LSLV.	Last Subject Last Visit
LTFU:	Long Term Follow-up
MACDP:	Metropolitan Atlanta Congenital Defects Program
MedDRA:	Medical Dictionary for Regulatory Activities
MITT:	Modified Intention-to-treat
NT:	Neutralisation Test
PCD:	Primary Completion Date
PO	Per os, i.e., orally
PP:	Per protocol
PPS:	Per protocol Set
pIMD:	Potential Immune-Mediated Disease
R:	Rapid
RNA:	Ribonucleic Acid
SAE:	Serious Adverse Event
SBIR:	Source DataBase for Internet Randomization
SDV:	Source Document Verification
SmPC:	Summary of Product Characteristics
SPM:	Study Procedures Manual
TBE:	Tick-borne Encephalitis
TBEV:	Tick-borne Encephalitis Virus
WHO:	World Health Organisation

GLOSSARY OF TERMS

Adequate contraception: Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label for example:

- abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle,
- Combined oestrogen and progesterone oral contraceptives,
- injectable progestogen,
- implants of etenogestrel or levonorgestrel,
- Contraceptive vaginal ring,
- percutaneous contraceptive patches,
- intrauterine device or intrauterine system,
- male partner sterilisation prior to the female subject's entry into the study, and this male is the sole partner for that subject,

  The information on the male sterility can come from the site personnel's review of the subject's medical records; or interview with the subject on her medical history.
- male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository), and/or progesterone alone oral contraceptive.

Adequate contraception does not apply to subjects of child bearing potential with same sex partners, or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle.

Adverse event: Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event. In an open-label study, no blind is used. Both the investigator and the subject know the identity of the treatment assigned.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study (EoS): **(Synonym of End of Trial)**	For studies without collection of human biologicals samples or imaging data EoS is the Last Subject Last Visit (LSLV). For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after LSLV
Epoch:	An epoch is a set of consecutive timepoints or a single timepoint from a single protocol. Epochs are defined to support a main purpose which is either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the timepoints included in an epoch must be sufficient to fulfil the purpose of the epoch. Typical examples of epochs are screening, primary vaccinations, boosters, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety. An amendment should not aim at adding an epoch to an existing protocol: a new protocol should be initiated for additional epochs.
eTrack:	GSK's tracking tool for clinical trials.
Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.

Investigational vaccine: **(Synonym of Investigational Medicinal Product)**	A pharmaceutical form of an active ingredient being tested in a clinical trial, including a product with a marketing authorisation when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Menarche:	Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1-2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year without menses with an appropriate clinical profile at the appropriate age e.g. > 45 years.
Pharmacogenomics:	The International Conference on Harmonization (ICH) E15 Guidance for Industry defines pharmacogenomics as Study of variation of DNA and RNA characteristics as related to drug or treatment response. Pharmacogenetics, which is a subset of pharmacogenomics, is "the study of variations in DNA sequence as related to drug response." Pharmacogenomic biomarkers include germline (host) DNA and RNA as well as somatic changes (e.g., mutations) that occur in cells or tissues. Pharmacogenomic biomarkers are not limited to human samples but include samples from viruses and infectious agents as well as animal samples. The term pharmacogenomic experiment includes both the generation of new genetic or genomic (DNA and/or RNA) data with subsequent analysis as well as the analysis of existing genetic or genomic data to understand drug or treatment response (pharmacokinetics, safety, efficacy or effectiveness, mode of action). Proteomic and metabolomic biomarker research are not pharmacogenomics.

Primary completion date (PCD):	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical trial was concluded according to the pre-specified protocol or was terminated.
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study.
Randomisation:	Process of random attribution of treatment/schedule to subjects in order to reduce bias of selection.
Self-contained study:	Study with objectives not linked to the data of another study.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Study vaccine/product:	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/ product /placebo as a reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product.
Sub-cohort:	A group of subjects for whom specific study procedures are planned as compared to other subjects or a group of subjects who share a common characteristic (e.g. ages, vaccination schedule,…) at the time of enrolment.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s) or as a control.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.

Subset (Synonym of Immunosubset)	Selection of blood samples among all blood sample collected at given time point(s) for testing by specific assay
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.
Treatment number:	A unique number identifying a treatment to a subject, according to treatment allocation.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also any 'solicited' symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

TRADEMARKS

The following trademarks are used in the present protocol.

Note: In the body of the protocol (including the synopsis), the names of the vaccines will be written without the superscript symbol ™ or ® and in *italics*.

Trademarks of the GSK group of companies	Generic description
Encepur Adults	Polygeline-free Tick-Borne Encephalitis vaccine for adults

1. INTRODUCTION

1.1. Background

Tick-borne encephalitis (TBE) is a serious, acute central nervous system infection caused by the tick-borne encephalitis virus (TBEV), a member of the genus *Flavivirus* which may result in death or long-term neurological sequelae in 35–58% of patients [WHO, 2011a]. *Encepur Adults* is GSK Biologicals' vaccine which is licensed for use in vaccination against TBE. Active immunization with GSK Biologicals' TBE vaccine for adults is indicated for persons from 12 years of age or older who stay permanently or temporarily in TBE endemic areas, especially when pursuing outdoor activities.

1.2. Rationale for the study and study design

There are several different schedules registered for vaccination against TBE:

- Conventional schedule: For the primary vaccination schedule the second dose is given 1-3 months after the first dose and third dose is given 9-12 months after the second dose. The second dose can be brought forward and given as early as 14 days after the first dose. The first booster dose is recommended to be given 3 years after completion of the primary vaccination schedule.
- Rapid schedule: For the primary vaccination schedule the first, second and third dose are given on days 0, 7 and 21, respectively. The first booster dose is recommended to be given12-18 months after completion of the primary vaccination schedule.

Independent of the primary schedule, all subsequent booster doses can be given 5 years after the last booster vaccination. Immunogenicity data collected so far in study V48P7E2 suggest that above 90% of individuals have neutralization titre (NT) ≥10 for at least 10 years after booster vaccination regardless of the type of primary vaccination schedule.

This study is designed to continue the evaluation of antibody persistence through 11 to 15 years after first booster with TBE vaccine, in order to learn more about the anti-TBEV antibodies' kinetics in the long term. This study will further investigate the booster response in subjects with a NT titre below 10 who will receive their second booster dose* in this study. These data will be utilized to guide recommendations for timing of the 2nd booster dose.

* Any booster given in this study will be the second that the subject has received (with regard to the follow-up of the previous study).

1.3. Rationale for the study design

Participants of study V48P7E2 will be asked to participate in the current study. The aim of this study is to investigate the persistence of antibody response in adults up to 15 years after one *Encepur Adults* booster dose. Persistence of antibody response will be evaluated based on TBE-NT. Subjects with an NT titre below 10 at enrolment and subjects with a

decrease of NT titre to below 10 during the study will be invited to receive a booster vaccination. Data collected from this study may allow the provision of better information to prescribers who administer *Encepur Adults,* so that they can appropriately adjust the time of booster vaccinations to individuals who received different primary vaccination schedules and who live in TBE endemic regions.

1.4. Benefit : Risk Assessment

Please refer to the Prescribing Information for information regarding the summary potential risks and benefits of *Encepur Adults*.

The following section outlines the risk assessment and mitigation strategy for this study protocol:

1.4.1. Risk Assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational vaccine: *Encepur Adults*		
As with other vaccines there is a very rare risk of experiencing serious adverse events after vaccination with *Encepur Adults* such as severe allergic reactions (e.g. generalised urticaria, angioedema, stridor, dyspnoea, bronchospasm, hypotension)	A review of *Encepur Adults* safety data collected through spontaneous and solicited reports in the company safety databases concluded that the benefit-risk profiles of the vaccine continue to be positive.	Close monitoring of serious adverse events.
Study Procedures		
Risk of blood sampling	Blood sampling associated risk of syncope, dizziness, infection at the site after or during venepuncture.	Blood samples will be obtained by a trained professional and medical assistance will be available. The potential risk of feeling faint, or experiencing mild local pain, bruising, irritation or redness at the site where blood was taken, is mentioned in the ICF. The amount of blood to be taken for sampling will not be harmful to the subject's health.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Study design		
Study is designed to collect yearly blood draws that will be analysed with NT. A booster dose (if NT titre <10) is possible 6 months (4-7 months) after blood draw.	There are standard timelines to allow sample analysis and release of laboratory results (most likely 6 months), and analysis of the data that cannot be avoided. But in case laboratory results are available earlier we add a broad time window of minus 60 days /plus 30 days (i.e. vaccination may be possible 4-7 months after blood draw) so that subjects can receive their booster dose as soon as possible.	TBEV is transmitted through the bite of infected ticks. Ticks are active from spring to autumn. Blood sampling is expected to be in autumn at scheduled visits and sera will be tested within 4-7 months, so that a booster dose can be given to subjects with NT titre <10 the following year, i.e. most likely before the beginning of the high risk period.

1.4.2. Benefit Assessment

Benefits consideration may include:

- Appropriate timing for the administration of second booster dose; avoid administration of second booster dose if subject still has an NT titre ≥10.
- Potential benefit of receiving a booster vaccination with TBE vaccine under controlled conditions (after determination of individual antibody titres and a booster vaccination only if antibody titres decreased to below 10).
- Medical evaluations/assessments associated with this study (such as physical examination, control of antibody against TBE titre).

1.4.3. Overall Benefit:Risk Conclusion

Taking into account the potential and/or known risks identified, the potential benefits support the conduct of this study.

2. OBJECTIVES

2.1. Primary objective

- To evaluate the persistence of antibody response to a booster dose of *Encepur Adults* vaccine starting ≥ 11 years after the first booster administration and to continue following subjects up to 15 years after first booster administration.

Refer to Section 10.1 for the definition of the primary endpoints.

2.2. Secondary objectives

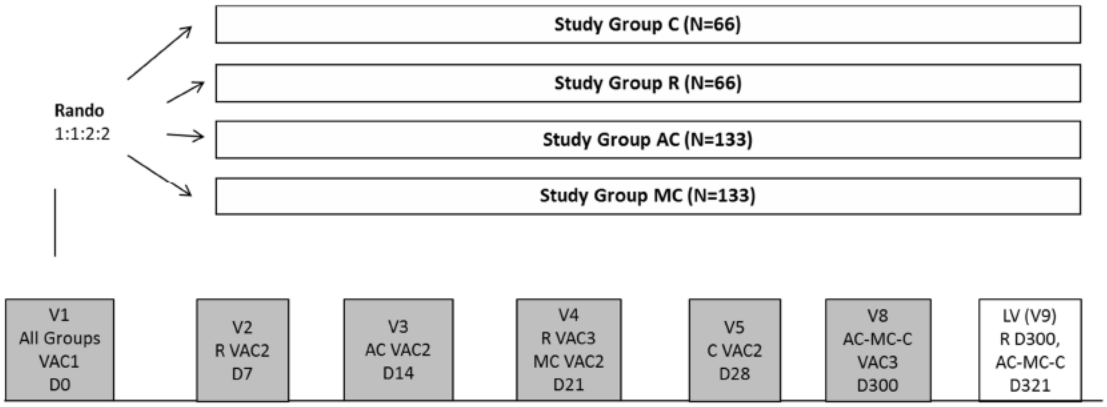
- To evaluate the immune response at 21 days after booster dose (boostability) in subjects with an NT titre below 10.
- To evaluate the safety of a booster dose with regard to SAEs collected after vaccination until study end.

Refer to Section 10.2 for the definition of the secondary endpoints.

3. STUDY DESIGN OVERVIEW

The study design of the current study along with the past 2 studies (primary and booster) is presented in the figures below. Figure 1 presents the primary study, Figure 2 presents booster study and Figure 3 presents the current long term follow-up study.

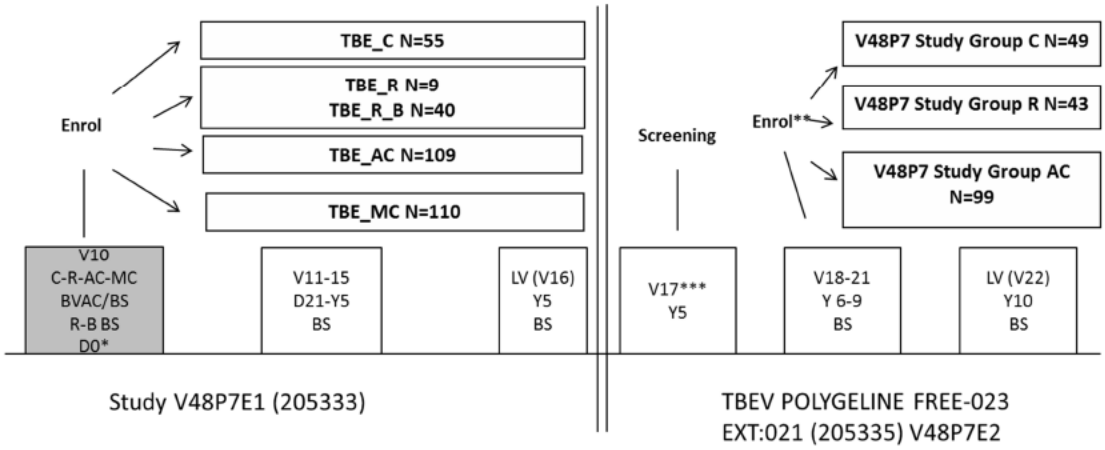
Figure 1 Study design of Studies V48P7



Study V48P7 (205332)

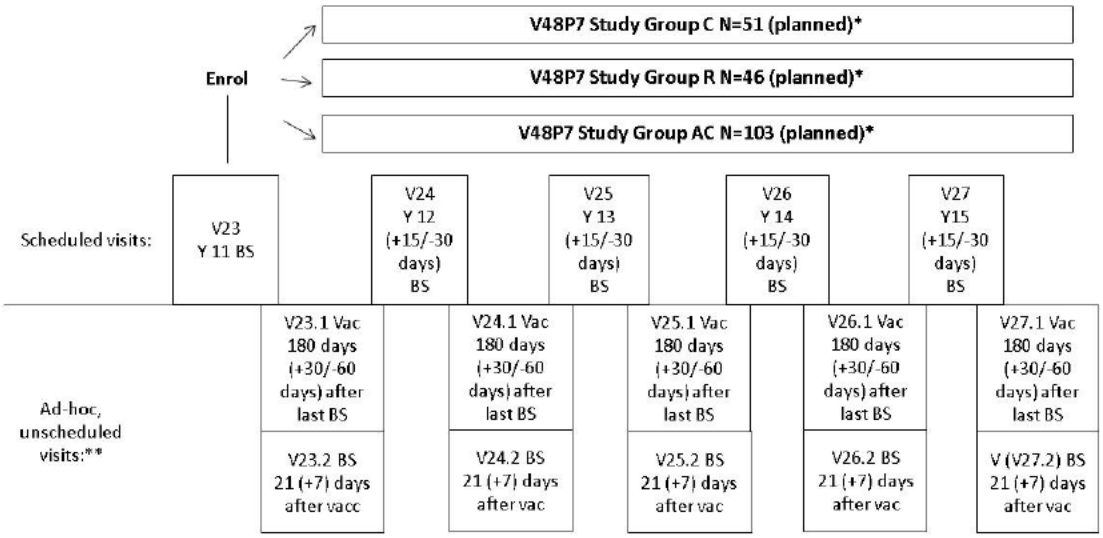
C = Conventional schedule; **R** = Rapid schedule; **AC** = Accelerated Conventional schedule; **MC** = Modified Conventional schedule; **Rando** = randomisation; **LV**= Last Visit; **V** = Visit; **D** = Day; **VAC1, VAC2, VAC3** = vaccination 1, 2 or 3 (indicated in grey);

Figure 2 Study design of study V48P7E1 and TBEV Polygeline-free-023 Ext:021 (V48P7E2)



TBE_R_B = R-schedule in V48P7 and Booster before enrollment in V48P7E1; **TBE_R** = R-schedule in V48P7 and Booster in V48P7E1; **TBE_C** = C-schedule in V48P7 and Booster in V48P7E1; **TBE_AC** = AC-schedule in V48P7 and Booster in V48P7E1; **TBE_MC** = MC-schedule in V48P7 and Booster in V48P7E1; **C** = Conventional schedule in study V48P7; **R** = Rapid schedule study V48P7; **AC** = Accelerated Conventional schedule study V48P7; **Enrol** = Enrolment; **LV**= Last Visit; **V** = Visit; **D** = Day; **Y** = Year; **BVAC** = Booster Vaccination; **BS** = Blood Sample
*Booster vaccination 3 years after primary vaccination in V48P7, except TBE R-B (vaccination before enrolment in V48P7E1 and BS only on Day 0)
**Study group MC was not followed, it is not a registered schedule; ***last V48P7E1 visit is screening visit

Figure 3 Study design of TBEV Polygeline-free-025 Ext:021



TBEV POLYGELINE FREE-025 EXT:021 (205847)

C = Conventional schedule in study V48P7; **R** = Rapid schedule study V48P7; **AC** = Accelerated Conventional schedule study V48P7; **Enrol** = Enrolment; VAC=Vaccination; **LV**= Last Visit; **V** = Visit; **D** = Day; **Y** = Year; **BS** = Blood Sample; SF = safety follow-up
* Approximate number of subjects planned for persistency, number of subjects with a NT titre below 10 during the study cannot be estimated.
**Some or all of these visits may not be needed, depending on necessary booster vaccination of subjects.

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 5.5), are essential and required for study conduct.

- Experimental design: Phase IV, open-label, mono-centric study.
- Duration of the study: For each subject, the study will last less than 5 years.
  - Epoch 001: Persistence evaluation starting at Visit 23 (Year 11) and ending at Visit 27 or Visit 27.2.
- Primary completion Date (PCD): Visit 27.

  Refer to GLOSSARY OF TERMS for the definition of PCD.
- End of Study (EoS): Last testing results released of samples collected at Visit 27 or Visit 27.2.

  Refer to GLOSSARY OF TERMS for the definition of EoS.
- Study groups: Same groups as in study TBEV POLYGELINE FREE (V48)-023 EXT:021

Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects*	Age	Epochs
			Epoch 001
C	51	25 years and above	x
R	46	25 years and above	x
AC	103	25 years and above	x

* Approximate number of subjects

C: Conventional schedule: Primary vaccination of 66 subjects in study V48P7 on Days 0, 28 (+10) and 300 (+21), booster vaccination of 55 subjects in study V48P7E1, no vaccination in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2).

R: Accelerated (or "rapid") schedule: Primary vaccination of 66 subjects in study V48P7 on Days 0, 7 (+3) and 21 (+7), booster vaccination of 9 subjects in study V48P7E1 (40 subjects received their booster vaccination before enrolment in study V48P7E1), no vaccination in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2).

AC: Accelerated conventional schedule: Primary vaccination of 133 subjects in study V48P7 on Days 0, 14 (+3) and 300 (+21), booster vaccination of 109 subjects in study V48P7E1, no vaccination in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2).

Table 2 Study groups and treatment foreseen in the study*

Treatment name	Vaccine/Product name	Study Groups		
		C	R	AC
Encepur Adults	*Encepur Adults*	X	X	X

*Only subjects with an antibody titre below 10 at a scheduled visit.

- Vaccination schedule: Second booster vaccination six months (minus 60 days /plus 30 days) after the blood draw in subject with an NT titre below10.

If a subject receives a booster dose in the study then he/ she will not have to come for any future scheduled visits. This subject will be included in the sub-cohort of subjects who received a booster dose. No further study procedures will be conducted after the blood sample collection 21 days post vaccination; however the subject will be followed up for safety until the study conclusion. Refer Sections 8.3.1 and 8.3.2.

- Treatment allocation: Independent from the study group, vaccination six months (minus 60 days /plus 30 days) after the blood draw only in subjects with an NT titre below 10.
- Sampling schedule: Blood samples to obtain neutralizing antibodies will be collected from all subjects at Visit 23 (Year 11), Visit 24 (Year 12 plus 15 days /minus 30 days), Visit 25 (Year 13 plus 15 days /minus 30 days), Visit 26 (Year 14 plus 15 days /minus 30 days) and Visit 27 (Year 15 plus 15 days /minus 30 days). Subjects who received a second booster vaccination will have an additional visit for a blood draw 21 days (plus 7 days) after the vaccination.
- Type of study: Extension of study TBEV POLYGELINE FREE (V48)-023 EXT:021 (V48P7E2).
- Data collection: Electronic Case Report Form (eCRF).
- Safety monitoring: SAEs related to study participation, or to a concurrent GSK medication/vaccine will be collected for all subjects as of informed consent obtained for the entire duration of the study. For subjects who will receive their second booster dose in this study, all SAEs will be collected for 1 month after vaccination. Post-study SAEs will be collected until study conclusion (Refer Section 8.3.2).

4. STUDY COHORT

4.1. Number of subjects/centres

A total of approximately 200 subjects who participated in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2), who received in a parent V48P7 study one of the following schedules: rapid (R), conventional (C), or accelerated conventional (AC) and a booster vaccination in study V48P7E1 or before study V48P7E1 (only R-schedule) and agreed to participate in this study are to be enrolled.

The number of subjects who will have a decrease of NT antibody titre to below 10 cannot be estimated. There is no information about the kinetics of NT antibodies until 15 years after booster vaccination with *Encepur Adults* available from clinical studies or publications.

Table 3 Sub-cohorts

Sub-cohort name	Description	Estimated number of subjects
Cohort for persistence	Subjects will come to the yearly scheduled blood draw visit for investigation of 11 to 15 year persistence of NT titres.	Approximately 200
Sub-cohort of subjects who received a second booster dose	Subjects who have an NT titre below 10 at one of the scheduled visits will receive a second booster dose 6 months after this visit at an unscheduled visit. Subsequent data of these subjects will be analysed separately in a subgroup.	*

* Number of subjects who will need a second booster dose because their NT titres decreased to below 10 cannot be estimated.

Overview of the recruitment plan

At the time of the initiation of this long term follow-up study, the investigator will contact all subjects who participated in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2). The reason for non-participation in the long-term follow-up study will be documented in the site's screening log.

4.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

Inclusion criteria for all subjects

- Subjects who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., return for follow-up visits).
- Written informed consent obtained from the subject prior to performance of any study specific procedure.
- Subjects who have participated in study V48P7E2 and who received in the parent V48P7 study one of the following schedules: rapid (R), conventional (C), or accelerated conventional (AC) and a booster vaccination in study V48P7E1 or before study V48P7E1 (only R-schedule)

Additional inclusion criteria for subjects who will need a second booster dose

- Healthy subjects as established by medical history and clinical examination before entering into the study.
- Female subjects of non-childbearing potential may be enrolled in the study. Non-childbearing potential is defined as pre-menarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral ovariectomy or post-menopause.

Please refer to the GLOSSARY OF TERMS for the definition of menarche and menopause.

- Female subjects of childbearing potential can receive the booster vaccine in the study, if the subject: has practiced adequate contraception for 30 days prior to vaccination, and has a negative pregnancy test on the day of vaccination, and has agreed to continue adequate contraception for 2 months after booster administration.

  Please refer to the GLOSSARY OF TERMS for the definition of adequate contraception.

4.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity regulatory acceptability of the study, or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

Each subject must not be:

- Unwilling or unable to give written informed consent to participate in the study.
- Perceived to be unreliable or unavailable to complete the study.

Each subject must not have:

- Clinical conditions representing a contraindication to blood draws.
- Any other clinical condition that, in the opinion of the investigator, might interfere with the results of the study or pose additional risk to the subject due to participation in the study.
- Received an investigational or non-registered medicinal product within 30 days prior to informed consent.
- Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational vaccine/product (pharmaceutical product or device).
- Levels of NT<10 in V48P7E2 study.
- Previous vaccination against TBE or other *Flavivirus* diseases with other TBE (comparator vaccines) and *Flavivirus* vaccines (e.g. Yellow fever vaccine, Dengue fever vaccine, Japanese encephalitis vaccine) before, during and after completion of the V48P7E2 and before starting TBEV POLYGELINE FREE-025 EXT 21 study.
- Primary immunization with TBE vaccine in the parent study V48P7 according to the modified conventional (MC) schedule.
- History of confirmed TBE infection.
- Known exposure (documented infection) to other Flaviviruses.

Each subject who will receive the second booster vaccination in this study additionally to the exclusion criteria above must not have:

- Hypersensitivity, including allergy, to any component of vaccines, medicinal products or medical equipment whose use is foreseen in this study.
- Clinical conditions representing a contraindication to intramuscular vaccination.
- Progressive, unstable or uncontrolled clinical conditions.
- Abnormal function of the immune system resulting from: Clinical conditions. Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to informed consent. This will mean prednisone ≥20 mg/day (for adult subjects) or equivalent. Inhaled and topical steroids are allowed. Administration of antineoplastic and immuno-modulating agents or radiotherapy within 90 days prior to vaccination.
- Received immunoglobulins or any blood products within 180 days prior to vaccination.
- Administration of long-acting immune-modifying drugs at any time during the study period (e.g. infliximab).
- Acute disease and/or fever at the day of booster vaccination.
  - Fever is defined as temperature ≥38.0°C / 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
  - Subjects with minor illness such as mild diarrhea or mild upper respiratory tract infection without fever (oral temperature <38.0°C) may be vaccinated at the discretion of the investigator.
- Expected general decrease in immune response, (eg, those who had sustained major injury or undergone recent major surgical operations, were undernourished, or had disorders involving a decreased immune response).
- Organic brain disturbances, including seizure disorders.
- Progressive neurological disorders.
- Suffered febrile or afebrile convulsions.
- Serious chronic illness (such as insulin dependent diabetes, cancer, autoimmune diseases).
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine or chemically related substances.
- Individuals who received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to vaccination in this study or who are planning to receive any vaccine within 28 days from the study vaccines.
- Pregnant.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for GCP, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

The study has been designed and will be conducted in accordance with the ICH Harmonised Tripartite Guideline for clinical investigation of medicinal products in the paediatric population (ICH E11) and all other applicable ethical guidelines.

GSK will obtain favourable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject informed consent
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written informed consent must be obtained from each subject prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IEC.

5.2. Subject identification and randomisation

A total of 200 subjects who participated in study TBEV POLYGELINE FREE (V48)-023 EXT 021 (V48P7E2), who received in a parent V48P7 study one of the following schedules: R, C and AC and a booster vaccination in study V48P7E1 or before study V48P7E1 (only R-schedule) and agreed to participate in this study are to be enrolled.

The subjects will have new identification numbers which will be pre-assigned with a link established to the subject identification numbers used in the previous V48P7, V48P7E1 and V48P7E2 studies. There is no randomization in the study as only the number of subjects who will have a NT antibody titre of below 10 will receive vaccination. ***However allocation of the study treatment and management of study vaccines will be done through Source DataBase for Internet Randomization (SBIR).*** **(Amended 23 February 2018)**

For details on the sub-cohorts refer to Section 4.1.

5.2.1. Treatment allocation to the subject (who may receive vaccination in the study)

The treatment numbers will be allocated by product.

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will ***access SBIR*** at Visit 23.1/ Visit 24.1/ Visit 25.1/ Visit 26.1/ Visit 27.1***. Upon providing the subject identification number, the randomisation system will provide the treatment number to be used for the dose.*** **(Amended 23 February 2018)**

The number of the administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

When SBIR is not available, please refer to the SBIR user guide or the Study Procedures Manual for specific instructions. **(Amended 23 February 2018)**

5.3. Method of blinding

The study is open-label.

The laboratory in charge of the laboratory testing will be blinded to the treatment, subject and visit number, and codes will be used to link the subject, visit and study (without any link to the treatment attributed to the subject) to each sample.

5.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

5.5. Outline of study procedures

Table 4 List of study procedures on scheduled visits

Epoch	Epoch 001				
Type of contact	**Visit 23**	**Visit 24**	**Visit 25**	**Visit 26**	**Visit 27**
Timepoint (s)	**Year 11 (132 months)**	**Year 12 (144 months)**	**Year 13 (156 months)**	**Year 14 (168 months)**	**Year 15 (180 months)**
Sampling timepoint(s)	**LTFU**	**LTFU**	**LTFU**	**LTFU**	**LTFU**
Informed consent	●				
Check inclusion / exclusion criteria	●				
Collect demography data	●				
Medical history	●	●	●	●	●
Physical examination	●	●	●	●	●
Review of subject's continued eligibility	●	●	●	●	●
Measure/record height	●				
Measure/record weight	●	●	●	●	●
Laboratory Assays					
Blood sampling for antibody determination (~10 ml)	●	●	●	●	●
Safety assessments					
Record any concomitant medications/vaccinations	●	●	●	●	●
Record any intercurrent medical conditions		●	●	●	●
Recording of SAEs and pregnancies #		●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●	●	●	●
Study Conclusion					●

Note: Visits numbers are consecutive numbers from previous studies, timepoints are indicated starting after enrolment in study V48P7E1. Results of the immunogenicity testing will be checked yearly in order to establish if antibody titre is below 10; if below this value subject will receive their second booster dose in this study, Table 5).

LV: Last Visit. LTFU: Long-term follow-up.

Will only be collected from subjects who received a second booster vaccination in this study, all SAEs will be collected for 1 month after vaccination.

Table 5 List of study procedures for ad hoc and additional visits

Timepoint(s)	Year 11		Year 12		Year 13		Year 14		Year 15	
Type of contact	**Visit 23.1**	**Visit 23.2**	**Visit 24.1**	**Visit 24.2**	**Visit 25.1**	**Visit 25.2**	**Visit 26.1**	**Visit 26.2**	**Visit 27.1**	**Visit 27.2**
	ad hoc visit 6 months after scheduled visit 23	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 24**	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 25**	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 26**	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 27**	**21 days after ad hoc visit**
Sampling timepoint(s)		**post-vacc**		**post-vacc**		**post-vacc**		**post-vacc**		**post-vacc**
Check inclusion / exclusion criteria	•		•		•		•		•	
Medical history	•		•		•		•		•	
Physical examination	•		•		•		•		•	
Urine pregnancy test	•		•		•		•		•	
Check contraindications and warnings and precautions to vaccination	•		•		•		•		•	
Pre-vaccination body temperature	•		•		•		•		•	
Measure/record weight	•		•		•		•		•	
Vaccination										
Vaccine administration	•		•		•		•		•	
Date of vaccine administration	•		•		•		•		•	
30 minutes post booster injection assessment	•		•		•		•		•	
Laboratory Assays										
Blood sampling for antibody determination (~10 ml)		•		•		•		•		•
Safety assessments										
Record any concomitant medications/vaccinations	•		•		•		•		•	

Timepoint(s)	Year 11		Year 12		Year 13		Year 14		Year 15	
Type of contact	**Visit 23.1**	**Visit 23.2**	**Visit 24.1**	**Visit 24.2**	**Visit 25.1**	**Visit 25.2**	**Visit 26.1**	**Visit 26.2**	**Visit 27.1**	**Visit 27.2**
	ad hoc visit 6 months after scheduled visit 23	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 24**	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 25**	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 26**	**21 days after ad hoc visit**	**ad hoc visit 6 months after scheduled visit 27**	**21 days after ad hoc visit**
Sampling timepoint(s)		**post-vacc**		**post-vacc**		**post-vacc**		**post-vacc**		**post-vacc**
Record any intercurrent medical conditions	•		•		•		•		•	
Recording of SAEs and pregnancies #	•	•	•	•	•	•	•	•	•	•
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	•	•	•	•	•	•	•	•	•	•
Study Conclusion										•

Note: Visits numbers are consecutive numbers from previous studies, timepoints are indicated starting after enrolment in study V48P7E1. Results of the immunogenicity testing will be checked yearly in order to establish if antibody titre is below 10; if below this value, the subject will be included in the sub-cohort of subjects who received a booster dose, and booster dose of vaccine will be offered).

Will only be collected from subjects who received a second booster vaccination in this study, all SAEs will be collected for 1 month after vaccination.

LV: Last Visit. LTFU: Long-term follow-up.

Whenever possible the investigator should arrange study visits within the interval provided in Table 6. Of note, Visit 23 is the baseline visit for this study and it will need to be considered as the driver for the calculation of the interval for all subsequent visits as highlighted in the grey shaded rows of Table 6 .

Table 6 Intervals between study visits

Interval	Optimal length of interval
Scheduled visits	
Visit 23→Visit 24	1 year
Visit 23 → Visit 25	**2 years**
Visit 24→Visit 25	*1 year*
Visit 23 → Visit 26	**3 years**
Visit 25→Visit 26	*1 year*
Visit 23 → Visit 27	**4 years**
Visit 26→Visit 27	*1 year*
Unscheduled visits*	
Visit 23→Visit 23.1	6 months
Visit 23.1→Visit 23.2	21 days
Visit 24→Visit 24.1	6 months
Visit 24.1→Visit 24.2	21 days
Visit 25→Visit 25.1	6 months
Visit 25.1→Visit 25.2	21 days
Visit 26→Visit 26.1	6 months
Visit 26.1→Visit 26.2	21 days
Visit 27→Visit 27.1	6 months
Visit 27.1→Visit 27.2	21 days

Note: visits out of range can lead to elimination from per protocol analyses.
* Only subject with an NT antibody titre below 10 at a scheduled visit will receive 6 months later a booster vaccination (all .1 visits) and will have an additional blood draw 21 days after this vaccination (all .2 visits)..

5.6. Detailed description of study procedures

Scheduled visits:

5.6.1. Informed consent

The signed informed consent of the subject must be obtained before study participation. Refer to Section 5.1 for the requirements on how to obtain informed consent.

5.6.2. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

5.6.3. Collect demographic data

Record demographic data such as date of birth, sex and race in the subject's eCRF.

5.6.4. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to each visit as specified in Table 4, in the eCRF.

5.6.5. Physical examination

Perform a physical examination of the subject, including assessment of oral body temperature and resting vital signs: systolic/diastolic blood pressure and heart rate prior to each visit as specified in Table 4. Collected information needs to be recorded in the eCRF.

Treatment of any abnormality observed during physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

The height of the subject will be measured at the first visit (Visit 23). The weight of the subject will be measured at all annual visits. The information will be recorded in the eCRF.

5.6.6. Review of subject's continued eligibility

At each annual study visit, review subject's continued eligibility in the study by checking inclusion/exclusion criteria.

If the subject is eligible to continue, study procedures are to be followed as outlined in Table 4 and the next visit is to be scheduled.

5.6.7. Sampling

Refer to the Module on Biospecimen Management in the SPM for detailed instructions for the collection, handling and processing of the samples.

5.6.7.1. Blood sampling for immune response assessments

Blood samples will be taken during certain study visits as specified in Section 5.5 List of Study Procedures.

- A volume of at least approximately 10 mL of whole blood (to provide ca. 5mL of serum) should be drawn from all subjects included in the immunogenicity sub-cohort (Sub-cohort of subjects who received a second booster dose) for each analysis of humoral immune response at each pre-defined time point. After centrifugation, serum samples should be kept at –20°C/ –4°F or below until shipment. Refer to the SPM for more details on sample storage conditions.

5.6.8. Check and record concomitant medication/vaccination and intercurrent medical conditions

Concomitant medication/vaccination must be checked and recorded in the eCRF as described in Section 6.7.

Intercurrent medical conditions must be checked and recorded in the eCRF as described in Section 6.8.

5.6.9. Recording of SAEs and pregnancies

- Refer to Section 8.3 for procedures for the investigator to record SAE and pregnancies. Refer to Section 8.4 for guidelines and how to report SAE and pregnancy reports to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

Unscheduled visits (For subjects with NT<10):

5.6.10. Check inclusion and exclusion criteria

Refer Section 5.6.2.

5.6.11. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to each visit as specified in Table 5, in the eCRF

5.6.12. Physical examination

Perform a physical examination of the subject, including assessment of oral body temperature and resting vital signs: systolic/diastolic blood pressure and heart rate at visits specified in Table 5. Collected information needs to be recorded in the eCRF.

If the investigator determines that the subject's health on the day of vaccination temporarily precludes vaccination, the visit will be rescheduled.

Treatment of any abnormality observed during physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

The weight of the subject will be measured and the information will be recorded in the eCRF at each vaccination visit.

5.6.13. Pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study vaccine administration. The study vaccine may only be administered if the pregnancy test is negative.

Note: Pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

5.6.14. Check contraindications, warnings and precautions to vaccination

Contraindications, warnings and precautions to vaccination must be checked at the beginning of the vaccination visit. Refer to Sections 6.5 and 6.6 for more details.

5.6.15. Assess pre-vaccination body temperature

The oral body temperature of each subject needs to be measured prior to any study vaccine administration. If the subject has fever [fever is defined as temperature ≥38.0°C/100.4°F regardless the location of measurement] on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 6).

5.6.16. Study group and treatment number allocation

Study group and treatment number allocation will be performed as described in Section 5.2. The number of the administered treatment must be recorded in the eCRF. **(Amended 23 February 2018)**

5.6.17. Study Vaccine administration

- After completing all prerequisite procedures prior to vaccination, if found eligible, one dose of study vaccine will be administered intramuscularly (IM) in the deltoid of the non-dominant arm (refer to Section 6.3 for detailed description of the vaccine administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccine administration, the visit will be rescheduled within the allowed time for this visit (refer to Table 6 and Figure 3). The date of the administration of the vaccine should be recorded in the eCRF.
- The subjects will be observed closely for at least 30 minutes following the administration of the vaccine, with appropriate medical treatment readily available in case of anaphylaxis.

5.6.18. Sampling

Refer Section 5.6.7.

5.6.19. Check and record concomitant medication/vaccination and intercurrent medical conditions

Refer Section 5.6.8.

5.6.20. Recording of SAEs and pregnancies

Refer Section 5.6.9.

5.6.21. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness
- complete the Study Conclusion screen in the eCRF.

5.7. Biological sample handling and analysis

Please refer to the SPM for details on biospecimen management (handling, storage and shipment).

Samples will not be labelled with information that directly identifies the subject but will be coded with a unique sample identifier.

- Collected samples will be used for protocol mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.
- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in Czechia and will only be performed once an independent Ethics Committee or Review Board has approved this research.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject.

Refer also to the Amendment 2 Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

If additional testing is performed, the marker priority ranking given in Section 5.7.4 may be changed.

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

5.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the per-protocol analysis (See Section 10.4 for the definition of analysis sets to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

5.7.2. Biological samples

Table 7 Biological samples

Sample type	Quantity	Unit	Timepoint	Sub-cohort Name*
Blood	Approximately 10	mL	Visit 23, 24, 25, 26, 27	Cohort for persistence
Blood	Approximately 10	mL	Visit 23.2 or 24.2 or 25.2 or 26.2 or 27.2	Sub-cohort of subjects who received a second booster dose

* Refer to Section 4.1 for sub-cohort description

5.7.3. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis.

Antibodies against TBEV will be determined using a virus neutralisation test (NT). The assay will be performed at a GSK Biologicals' laboratory using standardised and validated procedures (refer to Table 8).

Table 8 Humoral Immunity (Antibody determination) (Amended 23 February 2018)

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**
Serum	Anti TBE ***Antibody***	NT	GSK Biologicals***	***1/dilution***	***2***	GSK Biologicals***

* Assay cut-off and unit might be subject to change during the course of the study (e.g. in case of requalification, revalidation or standardization). In this case, this will be documented in the clinical report.
**Refer to APPENDIX B for the laboratory addresses.
*** GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium; Marburg, Germany

Additional exploratory testing on the vaccine and/or on the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

5.7.4. Biological samples evaluation

5.7.4.1. Immunological read-outs

Table 9 Immunological read-outs (Amended 23 February 2018)

Blood sampling timepoint		Subset /Sub-cohort Name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Visit 23	Year 11	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 23.2	Year 11 + 6 months + 21 days	Subjects with second booster on Visit 23.1**	**	Anti TBE ***Antibody*** *	1
Visit 24	Year 12	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 24.2	Year 12 + 6 months + 21 days	Subjects with second booster on Visit 24.1**	**	Anti TBE ***Antibody*** *	1
Visit 25	Year 13	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 25.2	Year 13 + 6 months + 21 days	Subjects with second booster on Visit 25.1**	**	Anti TBE ***Antibody*** *	1
Visit 26	Year 14	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 26.2	Year 14 + 6 months + 21 days	Subjects with second booster on Visit 26.1**	**	Anti TBE ***Antibody*** *	1
Visit 27	Year 15	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 27.2	Year 15 + 6 months + 21 days	Subjects with second booster on Visit 27.1**	**	Anti TBE ***Antibody*** *	1

*The results of this testing might potentially impact the subject medical care.
**Only subject with an NT antibody titre below 10 at a scheduled visit will receive 6 months later a second booster vaccination and will have an additional blood draw 21 days after this vaccination.

5.7.5. Immunological correlates of protection

Neutralization assay:

The presence of circulating antibodies to the virus at or above locally agreed concentrations (for example, an NT titre of ≥10, evaluated with the Holzmann NT, Vienna) is commonly considered to be a surrogate marker of protection [WHO, 2011b]. However, systematic clinical studies that substantiate this assumption are not available [WHO, 2011b].

According to the test procedure of the GSK Biologicals' NT, the lower limit for detectable neutralizing TBE antibodies is a titre of 2. The lower working range is defined by technical conditions, since the lowest starting dilution is a 1:2 dilution). In order to identify a clinically meaningful threshold of NT titre for GSK in-house NT, an evaluation of TBE NT antibody titres in 19 subjects with evidence for a naturally acquired TBE immunity was performed [Zent, 2004]. This approach was based on the following consideration: TBE disease is assumed to provide a life-long lasting immunity. That is, no reports of re-occurrence of TBE disease have been reported to date.

Therefore, it can be assumed that antibody titres in subjects with a naturally acquired TBE immunity, but without manifest TBE disease are clinically meaningful. Retrospective analysis of basic NT titres of 19 subjects who tested TBE NT positive prior to their 1st TBE vaccination revealed an NT titre of approximately 10 as a lower confidence limit of the GMT (NT). Therefore, a titre of 10 has been considered as a more conservative threshold than 2 for interpretation of results of the GSK Biologicals' in-house NT.

The investigator is encouraged to share the immunological assay results for non-responders with the study subjects/subjects' parent(s)/LAR(s).

For the subjects identified as non-responders, it remains the responsibility of the investigator in charge of the subject's clinical management to determine the medical need for re-vaccination and to re-vaccinate the subjects as per local/regional practices.

Note: Non-responders in this study refer to subjects with an NT antibody titre below 1:10. Subjects with an NT antibody titre below 10 at a scheduled visit will be offered another booster vaccination in this study. If the subject has an exclusion criterion against study vaccination, it remains the responsibility of the investigator in charge of the subject's clinical management to determine the medical need for re-vaccination and to re-vaccinate the subject as per local/regional practices outside the frame of the study.

The date of the administration of the booster dose of *Encepur Adults* has to be recorded in the eCRF. The investigator should inform the subjects of the post-booster antibody response. In case of a suboptimal response after the administered booster dose, the subject may receive another booster outside the scope of the study.

6. STUDY VACCINE AND ADMINISTRATION

6.1. Description of study vaccine

The candidate vaccine to be used has been developed and manufactured by GSK Biologicals.

The Quality Control Standards and Requirements for the candidate vaccine are described in separate Quality Assurance documents (e.g. release protocols, certificate of analysis) and the required approvals have been obtained.

The vaccines are labelled and packed according to applicable regulatory requirements.

Table 10 Study vaccine

Vaccine name	Formulation	Presentation	Volume to be administered	Number of doses
Encepur Adults	TBE, K23 strain=1.5µg; $Al(OH)_3$	Whitish, turbid suspension for injection in a pre-filled syringe	0.5 mL	1

6.2. Storage and handling of study vaccine

The study vaccine must be stored at the respective label storage temperature conditions in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded. Refer to the Module on Clinical Trial Supplies in the SPM for more details on storage of the study vaccine.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of +2.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form (Advanced Temperature Excursion Analysis and Management [ATEAM]). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor.

Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccine.

6.3. Dosage and administration of study vaccine

Table 11 Dosage and administration

Type of contact and timepoint	Study group	Treatment name	Volume to be administered	Route[1]	Site	
					Location	Laterality[2]
Visit 23.1	all study groups[3]	*Encepur Adults*	0.5 ml	IM	Deltoid	Non-dominant
Visit 24.1	all study groups[3]	*Encepur Adults*	0.5 ml	IM	Deltoid	Non-dominant
Visit 25.1	all study groups[3]	*Encepur Adults*	0.5 ml	IM	Deltoid	Non-dominant
Visit 26.1	all study groups[3]	*Encepur Adults*	0.5 ml	IM	Deltoid	Non-dominant
Visit 27.1	all study groups[3]	*Encepur Adults*	0.5 ml	IM	Deltoid	Non-dominant

[1]Intramuscular (IM)
[2]The non-dominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the non-dominant arm, an injection in the dominant arm may be performed.
[3]Only subjects with an NT antibody titre below 10 at a scheduled visit will receive 6 months later at an unscheduled visit a second booster vaccination.

6.4. Replacement of unusable vaccine doses

In addition to the vaccine doses provided for the planned number of subjects (including over-randomisation when applicable), at least 50% additional vaccine doses will be supplied to replace those that are unusable.

6.5. Contraindications to subsequent vaccination

The following events constitute absolute contraindications to administration of *Encepur Adults*. If any of these events occur during the study, the subject must not receive a dose of vaccine but may continue other study procedures at the discretion of the investigator.

- Anaphylaxis following any previous administration of vaccine.
- Pregnancy (see Section 8.2.1).
- Any condition that in the judgment of the investigator would make intramuscular injection unsafe.

The following events constitute contraindications to administration of *Encepur Adults* at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol, or withdrawn at the discretion of the investigator.

- Acute disease on the day of booster vaccination (acute disease means moderate or severe illness with or without fever). The vaccine may be administered to subjects with minor illness such as mild diarrhoea or mild upper respiratory tract infection with oral temperature <38.0°C).

6.6. Warnings and precautions

Refer to the approved product label/package insert.

6.7. Concomitant medications/products and concomitant vaccinations

At each study visit, the investigator or delegate should question the subject about any medications/products taken and vaccinations received by the subject.

6.7.1. Recording of concomitant medications/products and concomitant vaccinations

The following concomitant medication(s)/product(s)/vaccine(s) must be recorded in the eCRF.

For all subjects:

- Relevant medications/products:
  - Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to all blood draws. This will mean prednisone ≥ 20 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.
  - Administration of antineoplastic and immuno-modulating agents or radiotherapy within 90 days prior to all blood draws.
  - Received immunoglobulins or any blood products within 180 days prior to blood draw.
  - Administration of long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab).
  - Information on the vaccinations administered since the completion of the V48P7E2 study and prior to enrolment into this study.
- Any concomitant medications/products/vaccines relevant to a SAE to be reported as per protocol or administered during the study period for the treatment of a SAE. In addition, concomitant medications relevant to SAEs need to be recorded on the expedited Adverse Event report.

For subjects who will need an unscheduled second booster vaccination the following concomitant medications/products/vaccinations should also be recorded:

- All concomitant medications/products, except vitamins and dietary supplements, administered during the period starting 7 days before booster vaccination and up to 28 days after booster vaccination.
- Any concomitant vaccination administered in the period starting 28 days before the booster dose of study vaccine and ending at the last study visit.

- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

  E.g. an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring [fever is defined as temperature ≥ 38.0°C/100.4°F regardless of the location of measurement]. The preferred location for measuring temperature in this study will be the oral cavity.

- Any concomitant medications/products/vaccines listed in Section 6.7.2.

6.7.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from per-protocol analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the per-protocol analysis. See Section 10.4 for analyses sets to be analysed.

For all subjects:

- Any investigational or non-registered product (drug or vaccine) other than the study vaccine used during the study period.
- Immunosuppressants or other immune-modifying drugs defined as follows:
  - Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to all blood draws. This will mean prednisone ≥ 20 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.
  - Administration of antineoplastic and immuno-modulating agents or radiotherapy within 90 days prior to all blood draws.
- Received immunoglobulins or any blood products within 180 days prior to blood draw.
- Administration of long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab).

Additionally for subjects who will need a second booster vaccination:

- Other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to vaccination in this study or who are planning to receive any vaccine until the end of the study.

  *In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organised by the public health authorities, outside the routine immunisation program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Prescribing Information and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.

6.8. Intercurrent medical conditions that may lead to elimination of a subject from per-protocol analyses

At each study visit subsequent to the first study visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

Subjects may be eliminated from the per-protocol set for immunogenicity if, during the study, they incur a condition that has the capability of altering their immune response (e.g. HIV infection) or are confirmed to have an alteration of their initial immune status.

7. HEALTH ECONOMICS

Not applicable.

8. SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol.

Each subject will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

8.1. Safety definitions

8.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study vaccine administration even though they may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.

- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study vaccine or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with study vaccine administration.
- Significant failure of expected pharmacological or biological action.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Pre-existing conditions or signs and/or symptoms present in a subject prior to the study vaccination. These events will be recorded in the medical history section of the eCRF.

8.1.2. Definition of a serious adverse event

A SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalisation or prolongation of existing hospitalisation,

   Note: In general, hospitalisation signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting. Complications that occur during hospitalisation are also considered AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.

   Hospitalisation for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.

d. Results in disability/incapacity, OR

   Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza like illness, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect in the offspring of a study subject.

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

8.1.3. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g. clinical chemistry, haematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to Sections 8.1.1 and 8.1.2). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

8.1.3.1. Potential immune-mediated diseases

Not applicable.

8.2. Events or outcomes not qualifying as adverse events or serious adverse events

8.2.1. Pregnancy

Female subjects who become pregnant after the vaccination may continue the study at the discretion of the investigator.

While pregnancy itself is not considered an AE or SAE, any adverse pregnancy outcome or complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an AE or a SAE.

Note: The pregnancy itself should always be recorded on an electronic pregnancy report.

The following should always be considered as SAE and will be reported as described in Sections 8.4.1 and 8.4.3:

- Spontaneous pregnancy loss, including:
  - spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
  - ectopic and molar pregnancy
  - stillbirth (intrauterine death of foetus after 22 weeks of gestation).

  Note: the 22 weeks cut-off in gestational age is based on WHO-ICD 10 noted in the EMA Guideline on pregnancy exposure [EMA, 2006]. It is recognized that national regulations might be different.
- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect (as per [CDC MACDP] guidelines) identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the foetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the study vaccine will be reported to GSK Biologicals as described in Section 8.4.3. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

8.3. Detecting and recording serious adverse events and pregnancies

8.3.1. Time period for detecting and recording serious adverse events and pregnancies

In this study, only serious adverse events (SAEs) and pregnancies will be collected.

For vaccinated subjects:

- All pregnancies after vaccination until study end. See section 8.4 for instructions on reporting of pregnancies.
- All SAEs will be collected for 1 month after vaccination.

In addition to the above-mentioned reporting requirements and in order to fulfil international reporting obligations, SAEs that are related to study participation (i.e. protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged from the study.

An overview of the protocol-required reporting periods for SAEs and pregnancies is given in the table below.

Table 12 Reporting periods for collecting safety information

Event	scheduled BD Visit 23*	scheduled BD Visit 24, 25, 26 or 27	unscheduled V Visit 23.1, 24.1, 25.1 or 26.1	unscheduled BD 21 days after vaccination visit: Visit 23.2, 24.2, 25.2 or 26.2	Study conclusion
SAEs leading to withdrawal from the study					
SAEs related to study participation or concurrent GSK medication/vaccine					
SAEs[1]					
Pregnancies[2]					

*Informed consent obtained before events described below are collected.
V: vaccination; BD: blood draw
[1]In vaccinated subjects all SAEs will be collected for 1 month after vaccination.
[2] In vaccinated subjects all pregnancies after vaccination until study end

8.3.2. Post-Study adverse events and serious adverse events

A post-study SAE is defined as any event that occurs outside of the SAE reporting period defined in Table 12. Investigators are not obligated to actively seek SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study vaccine, the investigator will promptly notify the Study Contact for Reporting SAEs.

8.3.3. Evaluation of adverse events and serious adverse events

8.3.3.1. Active questioning to detect serious adverse events

As a consistent method of collecting AEs, the subject should be asked a non-leading question such as:

'*Have you felt different in any way since receiving the vaccine or since the previous visit?*'

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

8.3.3.2. Assessment of adverse events

8.3.3.2.1. Assessment of intensity

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgement.

Every effort should be made by the investigator to evaluate safety information reported by a subject for an underlying diagnosis and to capture this diagnosis as the event in the AE page. In other words, the practice of reporting only symptoms (e.g., "cough" or "ear pain") are better reported according to the underlying cause (e.g., "asthma exacerbation" or "otitis media").

The severity of events reported on the Adverse Events eCRF will be determined by the investigator as:

Mild: transient with no limitation in normal daily activity.
Moderate: some limitation in normal daily activity.
Severe:unable to perform normal daily activity.

8.3.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between study vaccine and the occurrence of each AE/SAE using clinical judgement. In case of concomitant administration of multiple vaccines/products, if possible, the investigator should specify if the AE could be causally related to a specific vaccine/product administered (i.e investigational, control/placebo or co-administered vaccine). When causal relationship to a specific vaccine(s)/product(s) cannot be determined the investigator should indicate the AE to be related to all products.

Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study vaccine will be considered and investigated. The investigator will also consult the SmPC and/or Prescribing Information for marketed products to determine his/her assessment.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the Expedited Adverse Events Report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Causality of all other AEs should be assessed by the investigator using the following question:

Is there a reasonable possibility that the AE may have been caused by the study vaccine?

YES	:	There is a reasonable possibility that the study vaccine contributed to the AE.
NO	:	There is no reasonable possibility that the AE is causally related to the administration of the study vaccine. There are other, more likely causes and administration of the study vaccine is not suspected to have contributed to the AE.

If an event meets the criteria to be determined as ‘serious’ (see Section 8.1.2), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine, if applicable.
- Erroneous administration.
- Other cause (specify).

8.3.3.3. Assessment of outcomes

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

8.4. Reporting of serious adverse events and pregnancies

8.4.1. Prompt reporting of serious adverse events and pregnancies to GSK Biologicals

SAEs that occur in the time period defined in Section 8.3 will be reported promptly to GSK within the timeframes described in Table 13, once the investigator determines that the event meets the protocol definition of a SAE.

Pregnancies that occur in the time period defined in Section 8.3 will be reported promptly to GSK within the timeframes described in Table 13, once the investigator becomes aware of the pregnancy.

Table 13 Timeframes for submitting serious adverse event and pregnancy to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*‡	paper/electronic Expedited Adverse Events Report	24 hours*	paper/electronic Expedited Adverse Events Report
Pregnancies	2 weeks*	paper pregnancy notification report/electronic pregnancy report	2 weeks*	paper pregnancy follow-up report/electronic pregnancy report

* Timeframe allowed after receipt or awareness of the information.
‡ The investigator will be required to confirm review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE

8.4.2. Contact information for reporting serious adverse events, pregnancies and AESIs

Study Contact for Reporting SAEs and pregnancies
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs and pregnancies
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** Outside US & Canada sites: Fax: +32 2 656 51 16 or +32 2 656 80 09 Email address: Rix.CT-safety-vac@gsk.com

8.4.3. Completion and transmission of SAE reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

8.4.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designate) must complete, then date and sign a paper Expedited Adverse Events Report and fax it to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designate) must complete the electronic Expedited Adverse Events Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

8.4.4. Completion and transmission of pregnancy reports to GSK Biologicals

Once the investigator becomes aware that a subject is pregnant, the investigator (or designate) must complete the required information onto the electronic pregnancy report WITHIN 2 WEEKS.

Note: Conventionally, the estimated gestational age (EGA) of a pregnancy is dated from the first day of the last menstrual period (LMP) of the cycle in which a woman conceives. If the LMP is uncertain or unknown, dating of EGA and the estimated date of delivery (EDD) should be estimated by ultrasound examination and recorded in the pregnancy report.

8.4.5. Updating of SAE and pregnancy information after removal of write access to the subject's eCRF

When additional SAE or pregnancy information is received after removal of the write access to the subject's eCRF, new or updated information should be recorded on the appropriate paper report, with all changes signed and dated by the investigator. The updated report should be faxed to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within the designated reporting time frames specified in Table 13.

8.4.6. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 8.4.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the study vaccine and unexpected. The purpose of the report is to fulfil specific regulatory and GCP requirements, regarding the product under investigation.

8.5. Follow-up of serious adverse events and pregnancies

8.5.1. Follow-up of serious adverse events

8.5.1.1. Follow-up during the study

After the initial SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs; refer to Table 13).

All SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the last visit of the subject.

8.5.1.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- with SAEs or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper/ electronic Expedited Adverse Events Report and/or pregnancy report as applicable.

GSK Biologicals may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognised follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

8.5.2. Follow-up of pregnancies

Pregnant subjects will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether full-term or premature, information on the status of the mother and child will be forwarded to GSK Biologicals using the paper pregnancy follow-up report/electronic pregnancy report and the Expedited Adverse Events Report if applicable. Generally, the follow-up period doesn't need to be longer than six to eight weeks after the estimated date of delivery.

Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is a SAE, it should always be reported as SAE.

8.6. Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of a SAE should be recorded in Expedited Adverse Event Report of the subject's eCRF (refer to Section 6.7).

8.7. Subject card

Study subjects must be provided with the address and telephone number of the main contact for information about the clinical study.

The investigator (or designate) must therefore provide a "subject card" to each subject. In an emergency situation this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator.

Subjects must be instructed to keep subject cards in their possession at all times during the study duration.

8.8. Holding rules and safety monitoring

Not applicable.

9. SUBJECT COMPLETION AND WITHDRAWAL

9.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol is considered to have completed the study.

9.2. Subject withdrawal

Withdrawals will not be replaced.

9.2.1. Subject withdrawal from the study

From an analysis perspective, a 'withdrawal' from the study refers to any subject who did not come back for the concluding visit foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Unsolicited non-serious adverse event.
- Solicited adverse event
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event*.
- Moved from the study area.
- Lost to follow-up.
- Other (specify).

*In case a subject is withdrawn from the study because he/she has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject, in the eCRF.

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of a SAE/AE until resolution of the event (see Section 8.5.1.2).

9.2.2. Subject withdrawal from study vaccine

Not applicable.

9.3. Extension study

During the study conclusion visit, the investigator may ask each subject if they are interested to participate in a booster study/long-term study. If a subject is not interested in participating in the booster study/long-term study the reason for refusal will be documented in the subject's eCRF.

10. STATISTICAL METHODS

10.1. Primary Endpoint(s)

The immunogenicity endpoints will be based on the TBE NT antibody levels in serum at year 11, 12, 13, 14 and 15 as measured by GSK Biologicals' NT.

Measures of immunogenicity are:

- Percentages of subjects with detectable TBE Neutralizing Antibody Titres ≥ 2 and ≥ 10 as measured by GSK Biologicals' NT.
- Geometric Mean Antibody Titres as measured by GSK Biologicals' NT calculated for each of the different schedule groups.

Endpoints will be summarized according to the immunization schedule received in the V48P7E1 study and further detailed by the following age subgroups: 25 to 49 years, ≥ 50 years and ≥ 60 years.

10.2. Secondary Endpoint(s)

The immunogenicity endpoints for subjects who received a second booster vaccination will be based on the TBE NT antibody levels in serum at 21 days after the booster vaccination as measured by GSK Biologicals' NT.

Measures of immunogenicity are:

- Percentages of subjects with detectable TBE Neutralizing Antibody Titres ≥ 2 and ≥ 10 as measured by GSK Biologicals' NT, overall and by study group.
- Geometric Mean Antibody Titres and Geometric Mean Ratios (GMRs) blood draw after/before booster as measured by GSK Biologicals' NT, overall and by study group.
- To complement the analysis of persistence, a thorough description of NT waning from year 1 after the first booster dose up to 15 years will be presented for the set of subjects completing the entire 15-year follow-up with no protocol deviations, including those receiving a second booster dose; for those ones, a constant value of NT = 1 from the post booster visit will be used in the analysis.

Endpoints will be summarized according to the primary immunization schedule received in the parent study (V48P7).

The safety endpoint for subjects who will need a second booster vaccination will be based on SAEs collection after the second booster dose. In vaccinated subjects all SAEs will be collected for 1 month after vaccination. Depending on the timing of the booster dose this will be in the period year 11.5 (Visit 23.1), 12.5 (Visit 24.1), 13.5 (Visit 25.1), or 14.5 (Visit 26.1) or from year 15.5 (Visit 27.1) until 21 days after year 15.5 (Visit 27.2). Post-study SAEs will be collected until study conclusion (Refer Section 8.3.2 for details on post-study SAEs)

Measures of safety are:

- Incidence of serious adverse events.

10.3. Determination of sample size

The sample size in this study is determined by the number of eligible subjects still being followed from the original study, V48P7 in a subset of the vaccine groups from that study. In the parent study, sample size for each vaccine group was not based on power calculations, but was considered sufficient when compared with studies using the same vaccine and based on the fact that the primary objective was to be evaluated by summary statistics, not formal hypotheses testing.

10.4. Analysis Sets

Definition of populations:

a. All Enrolled Population

- all subjects who:
  have signed an informed consent and have been enrolled

b. Full Analysis Set-1 (FAS-1)/Modified Intention-to-treat-1 (MITT-1) population, Immunogenicity

- all subjects in the enrolled population who:
  provide at least one evaluable serum sample

c. Full Analysis Set-2 (FAS-2)/Modified Intention-to-treat (MITT) population, Immunogenicity

- all subjects in the enrolled population who receive one booster dose during the trial and provide at least one evaluable serum sample after booster dose

d. Per Protocol Set/ Per protocol-1 (PP-1) population, Immunogenicity

- all subjects in the FAS-1/MITT-1 Immunogenicity population who:
  provide evaluable serum samples at the relevant time points and have no major protocol violation as defined in the Statistical Analysis Plan (SAP).

e. Per Protocol Set/ Per protocol-2 (PP-2) population, Immunogenicity

- all subjects in the FAS-2/MITT-2 Immunogenicity population who:
  provide evaluable serum samples after booster dose and have no major protocol violation as defined in the Statistical Analysis Plan (SAP).

  A major deviation is defined as a protocol deviation that is considered to have a significant impact on the immunogenicity result of the subject.

  Examples of major deviations would include:

- confirmed exposure to TBE (documented diagnosis of TBE infection) or other Flaviviruses,
- documented TBE or *Flavivirus* vaccination other than indicated during the course of the study.

The main populations for immunogenicity analyses will be the PPS. FAS will be supplied as sensitivity analyses.

Subjects lost to follow up after having received their first booster dose in the V48P7E1 study will not be part of any set of analysis.

f. Safety Population

- The safety population will include all subjects who will receive a booster vaccination in this study.

10.5. Derived and transformed data

- Immunogenicity
  - The cut-off value is defined by the laboratory before the analysis and is described in Section 5.7.3.
  - A seronegative subject is a subject whose titre is below the cut-off value.
  - A seropositive subject is a subject whose titre is greater than or equal to the cut-off value.
  - The GMTs calculations are performed by taking the anti-log of the mean of the log concentration/titre transformations. Values to be used for the antibody concentrations/titres below the assay cut-off will be described in the Statistical Analysis Plan (SAP).
  - Handling of missing data: for a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will be replaced only if values before and after are available. In such an occurrence, the following rule will apply:
  - The missing value will be imputed to be equal to the lowest one between the previous and the subsequent available values (conservative approach).
  - Subjects receiving a booster dose because their NT <10 will be kept in the analysis of persistence until year 15 with an NT imputed value equal to 1.

  In case of significant non-compliance of study procedures for reporting symptoms, the analysis plan will be reassessed to ensure more accurate reporting of study data by further analysis.

10.6. Analysis of Demographic and Baseline Characteristics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age, height and weight at enrolment will be calculated overall and by vaccine group.

Distributions of subjects by sex and ethnic origin will be summarized overall and by vaccine schedule as per parent study assignment.

10.7. Analysis of immunogenicity endpoints

10.7.1. Description of Response Variables

The primary analysis population for immunogenicity is the per protocol set and if, in any study group, the percentage of subjects with serological results excluded from the PP set is at least 10%, a second analysis will be performed on the FAS. All analyses entail the calculation of summary statistics by vaccination schedule and age stratification, together with their 95% confidence intervals.

10.7.2. Statistical Methods for Immunogenicity Variables

Long-term immunogenicity (persistence).

- Percentages of subjects with neutralizing antibody titres ≥ 2 and ≥ 10 as measured by NT assay will be tabulated by vaccine schedule (as assigned from the parent study) together with the associated two-sided 95% Clopper-Pearson confidence intervals (CIs). The vaccine group difference in the percentage of subjects with neutralizing antibody titres ≥ 2 and ≥ 10 will be calculated using a binomial distribution. The associated confidence interval for these differences will be constructed using the Miettinen-Nurminen method.
- GMTs with the associated 95% CIs will be computed for each vaccine schedule (as assigned from the parent study), by taking the exponential of the corresponding log10-transformed (least squares) means and 95% confidence intervals, from an ANOVA model with group as fixed factor. Vaccine schedule differences along with 95% CIs will also be computed.

Subjects with antibody levels below 10 at a given visit (and that will therefore receive a booster dose) will be kept in the analysis of persistence also for the subsequent years with a value imputed to half of the detection limit, i.e. 1.

Analyses will be conducted on the PPS-1 (and repeated on the FAS-1, if appropriate).

Immune response to booster dose (boostability):

- Percentages of subjects with neutralizing antibody titres ≥ 2 and ≥ 10 at 21 days after administration of the booster dose, as measured by NT will be tabulated by vaccine schedule (as assigned from the parent study) together with the associated two-sided 95% Clopper-Pearson CIs.

- GMTs with the associated 95% CIs at 21 days after administration of the booster dose, will be computed for each vaccine schedule (as assigned from the parent study), by taking the exponential of the corresponding log10-transformed (least squares) means and 95% confidence intervals, from an ANOVA model with group as fixed factor.
- GMRs with the associated 95% CIs after/before booster as measured by GSK Biologicals' NT calculated for each of the different schedule groups.

Computationally, the antibody value as recorded 6 months prior to the booster visit (i.e. at the previous scheduled clinic visit) will be considered as baseline.

All analyses will be repeated for each age subgroup.

10.8. Analysis of Safety (Endpoints) and Tolerability

Number and percentages of SAEs will be summarized only for subjects receiving a second booster vaccination in this study.

10.8.1. Analysis of Extent of Exposure

Not applicable.

10.8.2. Analysis of Solicited Local, Systemic and Other Adverse Events

Not applicable.

10.8.3. Analysis of Unsolicited Serious Adverse Events

This analysis applies to all serious adverse events occurring during the study and related to the study participation or to a concurrent GSK medication/vaccine; additionally, for subjects receiving a booster dose, all serious adverse events, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded in AE CRF, with a start date on or after the date of booster dose. The original verbatim terms used by investigators to identify adverse events in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The serious adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. All reported serious adverse events, as well as serious adverse events judged by the investigator as at least possibly related to study vaccine, will be summarized according to system organ class and preferred term within system organ class. When a serious adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Separate summaries will be produced for the following categories:

- Serious adverse events.
- Serious adverse events that are possibly or probably related to vaccine.

- Serious Adverse event leading to withdrawal.
- Deaths

Data listings of all serious adverse events and pregnancies will be provided by subject. In addition, serious adverse events in the categories above will be provided as listed data.

10.9. Interpretation of analyses

Comparative analyses will be descriptive with the aim to characterise the difference in immunogenicity between groups. These descriptive analyses should not be interpreted.

10.10. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

10.10.1. Sequence of analyses

Annual analyses will be performed at year 11, 12, 13, and 14 for internal purpose only: no annual clinical study report (CSR) will be produced. The final analysis will be conducted after completion of the immunogenicity evaluation at year 15, when the CSR will be produced. All analyses will take into consideration preceding evaluations. At each year, any subject whose NT titre falls below 10 will be offered a second booster vaccination with *Encepur Adults* in this study.

An integrated clinical study report containing all data will be written and made available to the investigators.

10.10.2. Statistical considerations for interim analyses

The annual evaluations will simply have an informative purpose, hence no statistical penalties will be applied.

11. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, public disclosure requirements and publications must be fulfilled.

11.1. electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database

or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with an electronic format in read only mode of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

11.2. Subject Diary

Not applicable

11.3. Study Monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst other items, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

11.4. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures, otherwise, the minimum retention period will default to 25 years after completion of the study report.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

11.5. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

11.6. Posting of information on publicly available clinical trial registers and publication policy

GSK assures that the key design elements of this protocol will be posted on the GSK website and in publicly accessible database(s) such as clinicaltrials.gov, in compliance with the current regulations.

GSK also assures that results of this study will be posted on the GSK website and in publicly accessible regulatory registry(ies) within the required time-frame, in compliance with the current regulations. The minimal requirement is to have primary endpoint summary results disclosed at latest 12 months post primary completion date (PCD) and to

have secondary endpoint disclosed at latest 12 months after the last subject last visit (LSLV) as described in the protocol.

As per EU regulation, summaries of the results of GSK interventional studies (phase I-IV) in adult population conducted in at least one EU member state will be posted on publicly available EMA registers within 12 months of EoS (as defined in the protocol) in the concerned EU member state. However, where, for scientific reasons detailed in the protocol, it is not possible to submit a summary of the results within one year in the concerned EU member state, the summary of results shall be submitted as soon as it is available. In this case, the protocol shall specify when the results are going to be submitted, together with a justification.

GSK also aims to publish the results of these studies in searchable, peer reviewed scientific literature and follows the guidance from the International Committee of Medical Journal Editors.

11.7. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

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

11.8. Data Sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non- GSK studies, to investigate further about the study product(s) and other product(s), and /or the disease/condition under investigation and related diseases and conditions.

12. COUNTRY SPECIFIC REQUIREMENTS

Not applicable

13. REFERENCES

Centers for Disease Control and Prevention Metropolitan Atlanta Congenital Defects Program (CDC MACDP) guidelines. Birth defects and genetic diseases branch 6-digit code for reportable congenital anomalies; http://www.cdc.gov/ncbddd/birthdefects/documents/MACDPcode0807.pdf

EMA Guideline on the exposure to medicinal products during pregnancy: need for post-authorization data (Doc. Ref. EMEA/CHMP/313666/2005) 'adopted at Community level in May 2006); *http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500011303.pdf*

WHO Background Document on Vaccines and Vaccination against tick-borne Encephalitis (TBE). WHO 2011(a) http://www.who.int/immunization/sage/6_TBE_backgr_18_Mar_net_apr_2011.pdf Accessed 17 February 2017

WHO. Vaccines against tick-borne encephalitis: WHO position paper. *WHO Weekly epidemiological record* 2011(b) Jun; 86 (24):241–256.

Zent O, Schwarz TF, Plentz A, Banzhoff A, Jilg W. TBE booster immunization in adults--first experience with a new tick-borne encephalitis (TBE) vaccine, free of protein-derived stabilizer. *Int J Med Microbiol* 2004 Apr; 293 Suppl 37: 134-8.

APPENDIX A LABORATORY ASSAYS

The TBE-virus neutralization test (TBE-NT) is a functional assay that detects in human serum neutralizing antibodies that are directed against TBEV.

Heat-inactivated sera are serially diluted 2-fold in 96well microtiter plates and incubated with equal volumes of TBE-virus (isolate K23). Each dilution is tested 4-fold in a human lung carcinoma cell line (A549) for infectious TBE-virus. After incubation the cell layers in each well are screened microscopically for cytopathic effects. The neutralizing antibody titer is expressed as the neutralizing dose that protects 50% of the cell cultures calculated according to Kaerber.

The serum starting dilution is 1:2. Seropositivity is defined as a titer of $\geq$2 (lower limit of detection: titer of 2).

APPENDIX B CLINICAL LABORATORIES

Table 14 GSK Biologicals' laboratories

Laboratory	Address
GSK Biological's Clinical Laboratory Sciences, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart – Belgium
GSK Biological's Clinical Laboratory Sciences, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium
GSK Vaccines GmbH Clinical Laboratory Sciences, Marburg, Germany	Emil-von-Behring-Str. 76 35041 Marburg Germany

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals SA Vaccines R &D **Protocol Amendment 1**	
eTrack study number and Abbreviated Title	205847 (TBEV POLYGELINE FREE-025 EXT:021)
EudraCT number	2017-001356-59
Amendment number:	Amendment 1
Amendment date:	25 August 2017
Co-ordinating author:	PPD, Scientific writer, contractor for GSK Biologicals

Rationale/background for changes:

Following feedback from CA and EC, this protocol has been amended to provide clarifications about:

- End of study (EoS): this can be either the last Visit due for "immune persistence" evaluation (V27) or last Visit due for the "boostability" evaluation in case of subresponder(s) to be re-vaccinated (V27.2).
- Duration of the study: "less of 5 years" is now used consistently throughout the protocol and with the ICF.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

The following statement in the copyright statement has been deleted as it is not relevant (new copyright statement is in place):

~~Unauthorised copying or use of this information is prohibited.~~

In Synopsis, Study design and Section 3 Study design overview:

- Duration of the study: For each subject, the study will last ~~approximately~~ ***less than*** 5 years.
  - Epoch 001: Persistence evaluation starting at Visit 23 (Year 11) and ending at ***Visit 27 or*** Visit 27.2.
- End of Study (EoS): Last testing results released of samples collected at ***Visit 27 or*** Visit 27.2.

GlaxoSmithKline Biologicals SA Vaccines R &D **Protocol Amendment 2**	
eTrack study number and Abbreviated Title	205847 (TBEV POLYGELINE FREE-025 EXT:021)
EudraCT number	2017-001356-59
Amendment number:	Amendment 2
Amendment date:	23 February 2018
Co-ordinating author:	PPD, Scientific writer, contractor for GSK Biologicals
Contributing author:	• PPD, ***Oversight Data Manager***
Rationale/background for changes: **This protocol has been amended to clarify that:** • New subject identification numbers will be assigned in the study with an established link to the subject identification numbers used in the previous V48P7, V48P7E1 and V48P7E2 studies • Despite there being no randomization in the study; Source DataBase for Internet Randomization (SBIR) is being used for allocation of the study treatment and management of study vaccines. Additionally, the exact assay name and details of the TBE-neutralization test has been presented.	

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

<u>In Section 5.2 Subject identification and randomisation:</u>

~~The subjects will have the same identification numbers as the previous studies.~~ ***The subjects will have new identification numbers which will be pre-assigned with a link established to the subject identification numbers used in the previous V48P7, V48P7E1 and V48P7E2 studies.*** There is no randomization in the study as only the number of subjects who will have a NT antibody titre of below 10 will receive vaccination. ***However allocation of the study treatment and management of study vaccines will be done through Source DataBase for Internet Randomization (SBIR).***

<u>In Section 5.2.1 Treatment allocation to the subject (who may receive vaccination in the study)</u>

The treatment numbers will be allocated by product.

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will ~~take~~, ***access SBIR*** at Visit 23.1/ Visit 24.1/ Visit 25.1/ Visit 26.1/ Visit 27.1. ~~,the lowest treatment number for the vaccine dose to be used for the subject.~~ ***Upon providing the subject identification number, the randomisation system will provide the treatment number to be used for the dose.***

The number of the administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

When SBIR is not available, please refer to the SBIR user guide or the Study Procedures Manual for specific instructions.

In Section 5.6 Detailed description of study procedures; sub section 5.6.16 Study group and treatment number allocation

~~Not applicable.~~

Study group and treatment number allocation will be performed as described in Section 5.2. The number of the administered treatment must be recorded in the eCRF.

In Section 5.7.3 Laboratory assays

Table 8 Humoral Immunity (Antibody determination)

System	Component	Method	Kit / Manufacturer	Unit*	Cut-off*	Laboratory**
Serum	Anti TBE ***Antibody***	NT	GSK Biologicals***	- ***1/dilution***	~~1:2~~ ***2***	GSK Biologicals***

* Assay cut-off and unit might be subject to change during the course of the study (e.g. in case of requalification, revalidation or standardization). In this case, this will be documented in the clinical report.
**Refer to APPENDIX B for the laboratory addresses.
*** GSK Biologicals laboratory refers to the Clinical Laboratory Sciences (CLS) in Rixensart, Belgium; Wavre, Belgium; Marburg, Germany

In Section 5.7.4.1 Immunological read-outs

Table 9 Immunological read-outs

Blood sampling timepoint		Subset /Sub-cohort Name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Visit 23	Year 11	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 23.2	Year 11 + 6 months + 21 days	Subjects with second booster on Visit 23.1**	**	Anti TBE ***Antibody*** *	1
Visit 24	Year 12	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 24.2	Year 12 + 6 months + 21 days	Subjects with second booster on Visit 24.1**	**	Anti TBE ***Antibody*** *	1
Visit 25	Year 13	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 25.2	Year 13 + 6 months + 21 days	Subjects with second booster on Visit 25.1**	**	Anti TBE ***Antibody*** *	1
Visit 26	Year 14	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 26.2	Year 14 + 6 months + 21 days	Subjects with second booster on Visit 26.1**	**	Anti TBE ***Antibody*** *	1
Visit 27	Year 15	All subjects	200	Anti TBE ***Antibody*** *	1
Visit 27.2	Year 15 + 6 months + 21 days	Subjects with second booster on Visit 27.1**	**	Anti TBE ***Antibody*** *	1

*The results of this testing might potentially impact the subject medical care.
**Only subject with an NT antibody titre below 10 at a scheduled visit will receive 6 months later a second booster vaccination and will have an additional blood draw 21 days after this vaccination.