GlaxoSmithKline	**Statistical Analysis Plan**
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.
eTrack study number and Abbreviated Title	205847 (TBEV POLYGELINE FREE-025 EXT:021)
Scope:	All data pertaining to the above study.
Date of Statistical Analysis Plan	Final: 04-Sep-2017
Co-ordinating author:	PPD
Reviewed by:	PPD (Clinical and Epidemiology Project Lead) PPD (Clinical Research and Development Lead) PPD (Lead statistician) PPD (Lead statistical analyst) PPD (Scientific writer) PPD (Regulatory Affair) PPD (SERM physician) PPD (Public disclosure representative)
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APP 9000058193 Statistical Analysis Plan Template (Effective date: 01 April 2017)

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LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
CI	Confidence Interval
eCRF	Electronic Case Report Form
CSR	Clinical Study Report
CTRS	Clinical Trial Registry Summary
EL.U/ml	ELISA unit per milliliter
Eli Type	Internal GSK database code for type of elimination code
ELISA	Enzyme-linked immunosorbent assay
ES	Exposed Set
FAS	Full Analysis Set
GMC	Geometric mean antibody concentration
GMT	Geometric mean antibody titer
GSK	GlaxoSmithKline
IU/ml	International units per milliliter
LL	Lower Limit of the confidence interval
MedDRA	Medical Dictionary for Regulatory Activities
N.A.	Not Applicable
NT	Neutralization test
PD	Protocol Deviation
PPS	Per Protocol Set
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
SUSAR	Suspected Unexpected Serious Adverse Reactions
TBE	Tick-borne Encephalitis
TBEV	Tick-borne Encephalitis virus
TFL	Tables Figures and Listings
TOC	Table of Content
UL	Upper Limit of the confidence interval

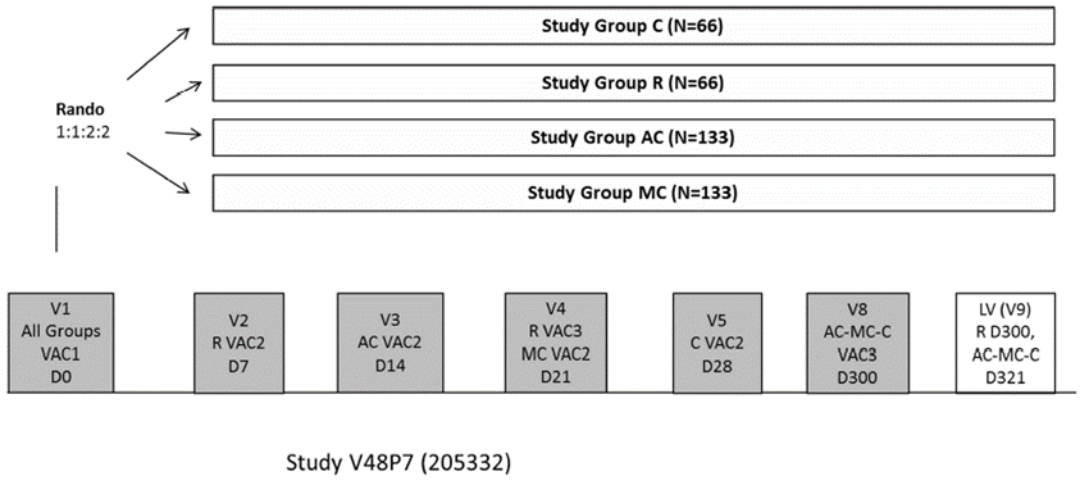
1. DOCUMENT HISTORY

Date	Description	Protocol Version
04-SEP-2017	Final version	Final – 25-AUG-2017

2. STUDY DESIGN

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.

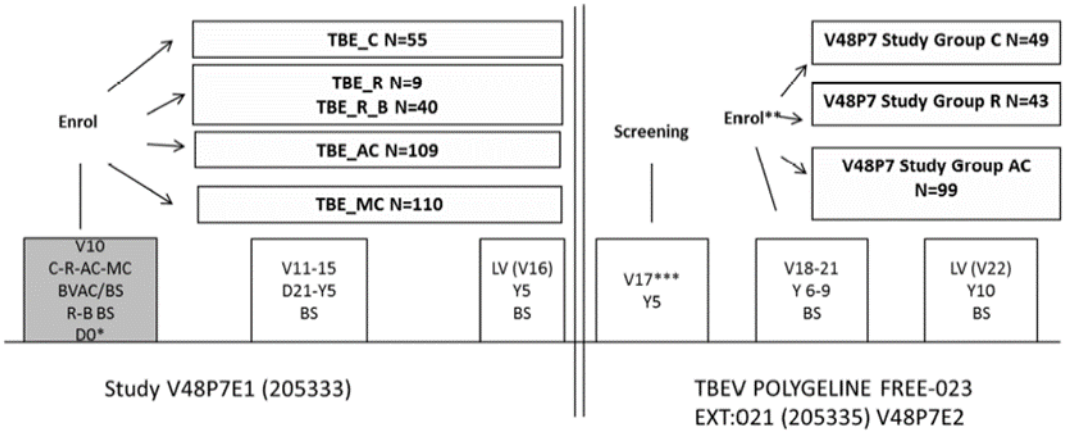
Figure 1 Study design of Studies V48P7



Study V48P7 (205332)

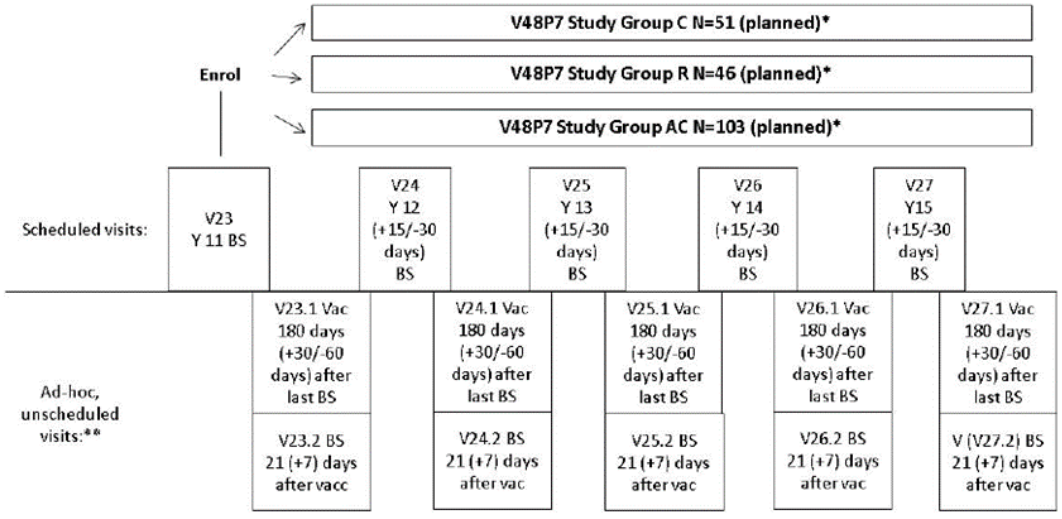
C = Conventional schedule; **R** = Rapid schedule; **AC** = Accelerated Conventional schedule; **MC** = Modified Conventional schedule; **Rando** = randomisation; **LV**= Last Visit; **V** = Visit; **D** = Day; **VAC1, VAC2, VAC3** = vaccination 1, 2 or 3 (indicated in grey);

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



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 of 205847 "TBEV POLYGELINE FREE-025 EXT:021" protocol Amendment 1 Final: 25 August 2017), 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.
- 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)-023EXT:021

3. OBJECTIVES

3.1. 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.

3.2. Secondary objectives

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

4. ENDPOINTS

4.1. Primary Endpoints

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 V48P7 study and further detailed by the following age subgroups: 25 to 49 years, ≥ 50 years and ≥ 60 years.

4.2. Secondary Endpoints

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 within the previous clinical studies and current study; for those ones, a constant value of NT = 1 from the post booster visit will be used in the analysis. To this end, percentages of subjects with detectable TBE Neutralizing Antibody Titres ≥ 2 and ≥ 10 and GMTs will be presented by study group.

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 administration of the 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 of 205847 "TBEV POLYGELINE FREE-025 EXT:021" protocol Amendment 1 Final: 25 August 2017 for details on post-study SAEs)

Measures of safety are:

- Incidence of serious adverse events.

5. ANALYSIS SETS

5.1. Definition

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 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 TBEV (documented diagnosis of TBEV infection) or other Flaviviruses,
- documented TBEV 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.

f. Safety Population

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

5.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each sets.

5.2.1. Elimination from Exposed Set (ES)

Not applicable.

5.2.2. Elimination from Per-protocol analysis Set (PPS)

5.2.2.1. Excluded subjects

A subject will be excluded from the PPS analysis under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data
150/1040	Administration of concomitant vaccine(s) forbidden in the protocol
130/1070	Subjects got vaccinated with the correct vaccine but containing a lower volume
140/1070	Vaccination not according to protocol
140/1080	Vaccine temperature deviation
140/1090	Expired vaccine administered
200/2010	Protocol violation (inclusion/exclusion criteria)
230/2040	Administration of any medication forbidden by the protocol
270/2090	Subjects did not comply with blood sample schedule
110/2100	Serological results not available post-vaccination
112/2120	Obvious incoherence or abnormality or error in data

Codes referring to vaccination errors or issues are only applicable to those subjects receiving a booster dose at ad-hoc unscheduled visits.

5.2.2.2. Right censored Data

Not applicable.

5.2.2.3. Visit-specific censored Data

Data from visit x will be censored for the PPS analysis under the following conditions.

Code	Condition under which the code is used
270.x/2090.X	Subjects did not comply with blood sample schedule at visit x

5.3. Important protocol deviation not leading to elimination from per-protocol analysis set

Not applicable.

6. STATISTICAL ANALYSES

6.1. Demography

6.1.1. Analysis of demographics/baseline characteristics planned in the protocol

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

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

6.1.2. Additional considerations

In addition to what planned in the protocol, subject distribution by booster visit will be provided per vaccine schedule and overall.

6.2. Exposure

Subjects exposed to vaccination will be those who will receive the second booster dose, only if their values go below 10.

6.2.1. Analysis of exposure planned in the protocol

Not applicable.

6.2.2. Additional considerations

Not applicable.

6.2.3. Efficacy/Effectiveness

Not applicable.

6.2.4. Analysis of efficacy planned in the protocol

Not applicable.

6.2.5. Additional considerations

Not applicable.

6.3. Immunogenicity

6.3.1. Analysis of immunogenicity planned in the protocol

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.

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 schedule 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.

  SAS code

  The SAS statements to calculate the adjusted GMCs will be similar to:

```
PROC glm;
  BY vaccinegroup;
  CLASS group;
  MODEL log_Ab=group;
  LSMEANS group / tdiff stderr;
RUN;
```

Between-group ratios of GMTs will be calculated by including additional SAS statements within PROC GLM, similar to:

```
ESTIMATE 'Group 1 - Group 2' group 1 -1;
```

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.

  SAS code

  The SAS statements to calculate the adjusted GMCs will be similar to:

```
PROC glm;
  BY vaccinegroup;
  CLASS group;
  MODEL log_Ab=group;
  LSMEANS group / tdiff stderr;
RUN;
```

  Between-group ratios of GMTs will be calculated by including additional SAS statements within PROC GLM, similar to:

```
ESTIMATE 'Group 1 - Group 2' group 1 -1;
```

- 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 prior to the booster visit (i.e. at the previous scheduled clinic visit) will be considered as the denominator for the calculation of the ratio.

Analyses will be conducted on the PPS-2 (and repeated on the FAS-2, if appropriate).

6.3.2. Additional considerations

All analyses will be repeated for each age subgroup.

- 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 within the previous clinical studies and current study; for those ones, a constant value of NT = 1 from the post booster visit will be used in the analysis.
- Analysis of boostability will be presented both by time-point (i.e. for each unscheduled visit separately) and aggregated (i.e. all visits considered together).
- Depending on the amount of missing data and as a form of sensitivity analysis, a repeated measure model could be used to evaluate the GMTs persistence.

6.4. Analysis of safety

6.4.1. Analysis of safety planned in the protocol

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

For vaccinated subjects:

- All pregnancies after vaccination until study end.
- All SAEs will be collected for 1 month after vaccination.
- SAEs leading to study withdrawal

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.

Event	scheduled BD Visit 23*	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

6.4.2. Additional considerations

6.4.2.1. Exclusion of implausible solicited Adverse Event

Not applicable.

6.4.2.2. Solicited Adverse Events

Not applicable.

Safety completeness analysis

Not applicable.

6.4.2.3. Unsolicited Adverse Events

This analysis applies to all SAEs 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 SAEs, 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 SAEs, as well as SAEs 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 an SAE 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

6.4.2.4. Combined Solicited and Unsolicited Adverse Events

Not applicable.

6.4.2.5. Clinical Safety Laboratory Investigations

Not applicable.

6.4.2.6. Concomitant Medication

Medications will be coded using the GSKDRUG dictionary.

The frequencies and percentages of subjects reporting concomitant medications will be tabulated by vaccine schedule and separated for scheduled and unscheduled visit

7. ANALYSIS INTERPRETATION

Comparative analyses will be descriptive with the aim to characterise the difference in immunogenicity between groups. No formal statistical comparison will be made.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Annual listings reporting subjects NT titres will be produced and made available to the investigators. Every year, any subject whose NT titre falls below 10 will be offered a second booster vaccination with *Encepur Adults*.

Final analysis will be conducted after completion of the immunogenicity evaluation at year 15, when the CSR will be produced.

A clinical study report containing all data will be written at study end.

Description	**Analysis ID**	**Disclosure Purpose (CTRS=public posting, SR=study report, internal)**	**Dry run review needed (Y/N)**	***Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)**	**Reference for TFL**
Immunogenicity analysis year 11	IMM11y	Internal	N	No	16.2.6.1
Immunogenicity analysis year 12	IMM12y	Internal	N	No	16.2.6.1
Immunogenicity analysis year 13	IMM13y	Internal	N	No	16.2.6.1
Immunogenicity analysis year 14	IMM14y	Internal	N	No	16.2.6.1
Final analysis year 15	Final	Study report	Y	Yes	All tables and listings in the TOC

8.2. Statistical considerations for interim analysis

The annual evaluations will simply have an informative purpose, evaluating on a yearly basis the subjects who will need a second booster dose. No further statistical considerations need to be applied.

9. CHANGES FROM PLANNED ANALYSES

Not applicable.

10. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

The following group names will be used in the TFLs, to be in line with the T-domains:

Group order in tables	Group label in tables	Group definition for footnote
1	C	Conventional
2	R	Rapid
3	AC	Accelerated Conventional

The following sub-group names for age class will be used in the TFLs

Sub-group order in tables	Sub-group label in tables	Sub-group definition for footnote
1	25-49Y	25-49 years old subjects
2	>=50Y	>=50 years old subjects
3	>=60Y	>=60 years old subjects

11. ANNEX 1 STANDARD DATA DERIVATION RULE AND STATISTICAL METHODS

11.1. Statistical Method References

The exact two-sided 95% CIs for a proportion within a group will be the Clopper-Pearson exact CI [Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika*. 1934;26:404-413].

The standardised asymptotic two-sided 95% CI for the group difference in proportions is based on the method described in the following paper:Robert G. Newcombe, interval estimation for the difference between independent proportions: comparison of eleven methods, *Statist Med.* 1998; 17, 873-890]. The standardised asymptotic method used is the method six.

11.2. Standard data derivation

NV legacy

The incomplete date derivations are as follow for an event with start and stop date:

For an incomplete start date, if the day is missing while the month and year are available:

- when the month and year are identical to a complete stop date then the start date is imputed to the stop date.
- when the month and year are not identical to a complete stop date then the start day is imputed to the first day of the month.

For an incomplete start date, if the day and month are missing while the year is available:

- when the year is identical to a complete stop date then the start date is imputed to the stop date.
- when the year is not identical to a complete stop date then the start day/month is imputed to 01Jan.

For an incomplete stop date, if the day is missing while the month and year are available:

- when the month and year are identical to a complete start date then the stop date is imputed to the start date.
- when the month and year are not identical to a complete start date then the stop day is imputed to the first day of the month.

For an incomplete stop date, if the day and month are missing while the year is available:

- when the year is identical to a complete start date then the stop date is imputed to the start date.
- when the year is not identical to a complete start date then the start day/month is imputed to 31dec.