Protocol B1971033

A PHASE 3 STUDY TO ASSESS THE PERSISTENCE OF hSBA RESPONSE UP TO 48 MONTHS AFTER COMPLETION OF A PRIMARY SERIES OF BIVALENT rLP2086, AND THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF A BOOSTER DOSE OF BIVALENT rLP2086

Statistical Analysis Plan
(SAP)

Version: 4.1

Date: 25-October-2017

[redacted]

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LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
CI	confidence interval
CRF	case report form
e-Diary	electronic diary
GMT	geometric mean titer
hSBA	serum bactericidal assay using human complement
ITT	intent-to-treat
LLOQ	lower limit of quantitation
MAE	medically-attended events
MAR	Missing at random
MCAR	Missing completely at random
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
MMRM	mixed effects model with repeated measurements
MnB	*Neisseria meningitidis* serogroup B
NDCMC	newly-diagnosed chronic medical conditions
RCDC	reverse cumulative distribution curve
rLP2086	recombinant lipoprotein 2086
RRI	research-related injuries
SAE	serious adverse event
SAP	statistical analysis plan

1. AMENDMENTS FROM PREVIOUS VERSION(S)

Version	Date	Summary of Changes/Comments
Version 1.0	March 5th 2012	Initial Version for Final Protocol Dated 14 December 2011 (05Jan2012 in GDMS)
Version 2.0	January 17th, 2014	Amended version for Protocol Amendment 2
Version 3.0	June 1, 2015	Amended version for Protocol Amendment 3
Version 4.0	June 5, 2017	Amended version for Protocol Amendment 4
Version 4.1	October 25, 2017	Amended version to clarify the language for mITT population in section 5.5. Removed the requirement for the creation of randomization listing in section 8.2.5.1

2. INTRODUCTION

The purpose of this document is to provide further details about the statistical analysis methods specified in the study protocol B1971033. This study is sponsored by Pfizer. A brief description of the study design and the study objectives are given. Subsequent sections include analysis populations, and the definitions of endpoints, followed by details about statistical methods. The list of tables/figures/listings, mock-up tables, listings, figures, and programming rules are prepared separately based on the methods described in this document. Any major deviations from the methods specified in this document and the protocol must be discussed in the clinical study report.

This SAP version is updated to reflect changes made in Protocol Amendment 4, dated April 18, 2017. Endpoints and analyses relating to the extension of the Booster Stage from 12 months to 26 months postbooster vaccination are specified. Subjects from primary study B1971012, Group 2 (0, 2, 6-schedule) and Group 3 (0, 6-schedule) will proceed to the 26 months postbooster vaccination visit.

In this document, text taken directly from the protocol has been italicized.

2.1. Study Design

This study consists of two stages. Stage 1 is a long-term serologic assessment in subjects enrolled in previously conducted Pfizer studies using the final formulation and dose of bivalent rLP2086 (primary studies). Subjects will attend up to 6 study visits (through 48 months after receiving last dose of investigational product) for collection of a 20-mL blood sample at each of these visits to evaluate the immunogenicity across the time. Protocol Amendment 3 added the Booster Stage in which a booster vaccination with a single dose of bivalent rLP2086 will be administered, plus two additional blood draws. To further evaluate the safety and immunogenic response to booster vaccination, Protocol Amendment 4 extended the Booster Stage from 12 months to 26 months following the booster dose. Subjects from primary study B1971012, Group 2 (0, 2, 6-schedule) and Group 3 (0, 6-schedule) will proceed to the 26 months postbooster vaccination visit.

An overview of the study design is provided in Table 1.

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Table 1. Overview of Study Design

	STAGE 1						BOOSTER STAGE				
Visit purpose	Blood draw	Blood draw	Blood draw	Blood draw	Blood draw	Blood draw	Booster vaccination	Blood draw	6-Month telephone call	Blood draw	Blood draw
Visit number	1	2	3	4	5	6	7	8	9	10	11
Approximate month	6	12	18	24	36	48	48	1 Month after booster	6 Months after booster	12 Months after booster	26 Months after booster
Vaccination							Bivalent rLP2086				
Blood draw	20 mL	20 mL	20 mL	20 mL	20 mL	20 mL		20 mL		20 mL	20 mL

2.2. Study Objectives

The primary objective of Stage 1 of this study is to describe the immunogenicity of bivalent rLP2086 as determined by hSBA-titers to 4 primary test strains at approximately 6, 12, 18, 24, 36, and 48 months after the last dose (second or third dose) of bivalent rLP2086 in the 'primary' study (a previously conducted Pfizer study using the final formulation and dose of bivalent rLP2086).

The primary objective of the Booster Stage of this study is to describe the immune response as measured by hSBA titers to 4 primary test strains 1 month following the last vaccination with bivalent rLP2086 in the primary study, before the booster vaccination, and 1 month, 12 months, and 26 months after a single booster dose of bivalent rLP2086.

The primary safety objective is to evaluate the safety profile of bivalent rLP2086 as measured by the incidence of local reactions, systemic events, AEs, serious adverse events (SAEs), newly diagnosed chronic medical conditions (NDCMCs), medically attended events, and immediate AEs following a booster vaccination of bivalent rLP2086.

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3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

Because of the timing of enrollment into the primary studies, subjects will reach the booster stage of Study B1971033 over an extended period. Up to three (3) analyses of the study will be conducted.

[redacted]

The first interim analysis will occur when all subjects who participated in the B1971012 primary study have Visit 8 (1 month after the booster vaccination) immunogenicity data available. The analysis will include all data from visits 1 through 8, for B1971012 subjects only (including data from visits 1 through 6 for any B1971012 subjects who did not receive the booster dose).

A second interim analysis *will* be conducted, when all subjects who participated in the B1971012 primary study, Group 3 (0, 6-schedule) have Visit 10 (12 months after the booster vaccination) immunogenicity data available. The analysis will include all data from visits 1 through 10, for B1971012 subjects only (including data from visits 1 through 6 for any B1971012 subjects who did not receive the booster dose).

The final analysis will be conducted once all immunogenicity data for all subjects enrolled in B1971033 are available, from all primary studies B1971010, B1971012 and B1971015. This includes data from visits 1 through 6 for any subject who did not receive the booster dose.

These criteria for analysis relate to primary strain immunogenicity data only. CCI [redacted]

Information from interim analyses will be used for internal decision making on further development of the vaccine, and will not modify the current study. Since the study is descriptive, there will be no type I error impact for these interim analyses.

Unblinding is not applicable to this study as the booster vaccination phase is open-label.

4. HYPOTHESES AND DECISION RULES

This Section is not applicable as it is a descriptive study and no hypothesis is tested.

5. ANALYSIS SETS

5.1. Full Analysis Set

The full analysis set will be the 'As Enrolled' population, which will include all of the subjects enrolled in this study.

5.2. Immunogenicity Analysis Set

Only subjects who were compliant with primary study eligibility criteria while enrolled in the primary study will be included in this study. Therefore, the modified intent-to-treat (mITT) population will be used for Stage 1 immunogenicity analyses. The mITT population will include subjects who have at least 1 valid and determinate assay result in Stage 1 of Study B1971033.

For booster stage time points, the primary immunogenicity analysis will be based on the evaluable population. For analyses including the 26 months postbooster vaccination time point, the analyses will use the subset of evaluable population for Booster stage who proceed to Visit 11.

The evaluable population includes subjects who:

[redacted]

1. Are eligible for the study (i.e., meet all Stage 1 and Booster Stage eligibility criteria).
2. Have received the scheduled investigational product as intended.
3. Have a prevaccination blood drawn prior to the booster vaccination, and have a postvaccination blood draw within 28-42 days after the booster vaccination.
4. Have valid and determinate assay result for the proposed analysis.
5. Have received no prohibited vaccines or treatment.
6. Have no other major protocol violations as determined by the sponsor's global medical monitor.

For above listed items, 1 through 4 will be computerized checks of the data, while items 5 and 6 will be determined by clinical review. A major protocol violation is a protocol violation that, in the opinion of the sponsor's global medical monitor would materially affect assessment of immunogenicity, e.g, subject receipt of a prohibited vaccine or medication that might affect immune response or a medication error with suspected decrease in potency of the vaccine. The global medical monitor from the sponsor will identify those subjects with protocol violations before any immunogenicity analysis is carried out.

Booster stage immunogenicity analyses will also be performed for the mITT population, which will include subjects who received the booster vaccination and who have at least 1 valid and determinate assay result in the booster stage. For analyses including the 26 months postbooster vaccination time point, the analyses will use the subset of mITT population for Booster stage who proceed to Visit 11.

5.3. Safety Analysis Set

The safety population for Stage 1 of the study will include all subjects who have at least 1 blood draw in the study. The safety population for the Booster Stage will include all subjects who received the booster vaccination and for whom safety data are available. For analyses including the 26 months postbooster vaccination time point, the analyses will use the subset of safety population for Booster stage who proceed to Visit 11.

5.4. Other Analysis Sets

Not applicable.

5.5. Treatment Misallocations

Treatment in this study is restricted to the Booster Stage. Subjects enrolled to the Booster Stage but not vaccinated will be excluded from the mITT immunogenicity population and safety analyses. Subjects vaccinated during the Booster Stage but not enrolled will be excluded from immunogenicity analyses but included in the safety population.

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5.6. Protocol Deviations

The following describes any protocol deviations that relate to the statistical analyses or populations:

5.6.1. Deviations Assessed Prior to Enrollment

Subjects who do not meet the inclusion / exclusion criteria will not be included in the Booster Stage evaluable immunogenicity population as specified in Section 5.2.

5.6.2. Deviations Assessed Post-Enrollment

The full list of protocol deviations (including protocol violations) for the study report(s) will be compiled prior to database closure for the first interim analysis. The subjects with major protocol deviations, as determined by Medical Monitor, will be excluded from the Booster Stage evaluable immunogenicity population.

6. ENDPOINTS AND COVARIATES

6.1. Immunogenicity Endpoints

The 4 primary test strains for hSBA testing are CCI *(A22 variant),* CCI *(A56 variant),* CCI *(B24 variant), and* CCI *(B44 variant). The lower limit of quantitation (LLOQ) for* CCI *(A22) is an hSBA titer equal to 1:16. The LLOQ for the other 3 primary test strains is an hSBA titer equal to 1:8.*

hSBA assays using the 4 primary test strains will be conducted using sera obtained at Visits 1 to 6 from all subjects entered into stage 1, including subjects who did not receive bivalent rLP2086 in the primary study (B1971015 Group 2).

6.1.1. Primary Immunogenicity Endpoints

Stage 1

- *Proportion of subjects with hSBA titers ≥ LLOQ for each of the 4 primary strains, at each blood draw visit in Stage 1 (Visits 1-6).*

Booster Stage

Proportions of subjects with hSBA titers ≥ LLOQ for each of the 4 primary strains at 1 month following the last vaccination received in the primary study, before the booster vaccination (Visit 6), and 1 month, 12 months, and 26 months (Visits 8, 10, and 11) following booster vaccination.

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6.2. Safety Endpoints

6.2.1. Primary Safety Endpoints

The following safety endpoints from the Booster Stage are considered primary.

- *Percentages of subjects reporting local reactions via the electronic diary (e-diary) by type (pain at the injection site, redness, and swelling) and by severity after a booster vaccination of bivalent rLP2086.*

[redacted]

- *Percentages of subjects reporting systemic events via the e-diary by type (fever, vomiting, diarrhea, headache, fatigue, chills, muscle pain other than muscle pain at the injection site, and joint pain) and by severity after a booster vaccination of bivalent rLP2086.*
- *Percentage of subjects reporting the use of antipyretic medication via the e-diary after a booster vaccination of bivalent rLP2086.*
- *Percentages of subjects with at least 1 AE occurring during the following time periods:*
  - *From Visit 7 to Visit 8*
- *Percentages of subjects with at least 1 SAE during the following periods:*
  - *From Visit 7 to Visit 8*
  - *From Visit 8 to Visit 9*
  - *From Visit 7 to Visit 9*
- *Percentages of subjects with at least 1 NDCMC occurring during the following time periods:*
  - *From the 6-month safety telephone call in the primary study to Visit 6 (Stage 1)*
  - *From Visit 7 to Visit 8*
  - *From Visit 8 to Visit 10*
  - *From Visit 7 to Visit 10*
  - *From Visit 8 to Visit 11 (only subjects proceeding to Visit 11)*
  - *From Visit 7 to Visit 11 (only subjects proceeding to Visit 11)*
- *Percentages of subjects with at least 1 medically attended event occurring during the following time periods:*
  - *From Visit 7 to Visit 8*
  - *From Visit 8 to Visit 9*
  - *From Visit 7 to Visit 9*
- *Percentage of subjects reporting at least 1 immediate AE after receiving the booster dose of bivalent rLP2086.*
- *Numbers of days subjects miss school or work because of AEs from Visit 7 through Visit 9.*

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6.2.3. Reactogenicity Data

Reactogenicity data is solicited adverse events (AEs). The reactogenicity data collected from the e-Diary will be: local reactions (redness, swelling, and pain at injection site), systemic events (vomiting, diarrhea, chills, headache, fatigue, muscle pain other than muscle pain at the injection site, and joint pain), temperature and use of antipyretic medication.

The e-Diary will record reactogenicity data from day 1 to day 7 following booster vaccination. The analysis interval for reactogenicity data will be 'any day 1-7' which includes data from day 1 to day 7. The day of vaccination is considered as day 1.

Table 2. Analysis Interval for Reactogenicity Data

#	Analysis Interval	Analysis Population	Interval Start date (inclusive)	Interval Stop (inclusive)
1	Vaccination	Booster Stage Safety	Vaccination date	Vaccination date + 6 days (or until resolved day)

6.2.3.1. Local Reaction Endpoints

The local reactions collected from the e-Diary will be: pain at injection site, redness, and swelling.

Local Reaction Presence (Proportion of subjects reporting each local reaction)

For each local reaction, the derivation of whether or not the specific reaction occurred on each day and 'any day 1-7' will be made. The derivation of this variable is given in the table below.

Table 3. Derived Variables for Local Reactions

Variable	Yes (1)[a]	No (0)[b]	Missing (.)
Any day 1-7	Subject reports the reaction as 'mild' 'moderate' or 'severe' on any day 1-7	Subject reports the reaction as 'none' on all 7 days or as a combination of 'none' and missing on all 7 days.	Subject reports the reaction as missing on all 7 days.

a. For redness and swelling, 'mild', moderate', and 'severe' categories were based on the caliper size reported from the e-Diary

b. For redness and swelling, 'none' means 0-4 caliper units reported in the e-Diary

[redacted]

Maximum Severity for Local Reaction

A caliper is used to measure the redness or swelling area. Caliper units are converted to centimeters according to 1 caliper unit = 0.5 centimeters. The grading of local reactions is listed below in Table 4 and Table 5.

Table 4. Grading of Redness and Swelling

Grades	Measurement
None	0 to 2.0 cm (0 to 4 caliper units)
Mild	2.5 to 5.0 cm (5 to 10 caliper units)
Moderate	5.5 to 10.0 cm (11 to 20 caliper units)
Severe	>10.0 cm (>20 caliper units)

Table 5. Grading of Pain

Grades	
Mild	Does not interfere with activity
Moderate	Interferes with activity
Severe	Prevents daily activity

The maximum severity (highest grading) of each local reaction within 7 days of vaccination will be derived as follows:

= •, if values are missing for all days 1-7;

= 0, if the subject reports all reactions as 'None' or a combination of missing and none for all days 1-7

= *highest grade* (maximum severity) within 7 days of vaccination, if the answer is not 'None' for at least 1 day;

Duration and Onset Day of Each Local Reaction

For subjects experiencing any local reaction (or those with derived reaction presence in Table 3), the onset day (first day of the local reaction reported via e-Diary relative to the vaccination) and the maximum duration (last day of reaction –first day of reaction +1) will be derived. Resolution of the event is the last day in which the event is recorded in the e-Diary or the date the event ends if it is unresolved during the subject diary-recording period, unless chronicity is established.

In summary, the following variables will be derived for local reactions:

1. Each local reaction on each day (up to day 7) after booster vaccination

2. Each local reaction on 'any day 1-7' after booster vaccination
3. Maximum severity of each local reaction on 'any day 1-7' after booster vaccination
4. Maximum duration of each local reaction after booster vaccination
5. Any local reaction on 'any day 1-7' after booster vaccination

6.2.3.2. Systemic Events Endpoints

The systemic events reported via e-Diary will be: vomiting, diarrhea, chills, headache, fatigue, muscle pain other than muscle pain at the injection site, and joint pain. See the table below for the severity scales of each systemic event.

Table 6. Grading of Other Systemic Events

	Grade 1 (Mild)	**Grade 2** (Moderate)	**Grade 3** (Severe)
Vomiting	1 to 2 times in 24 hours	> 2 times in 24 hours	Requires IV hydration
Diarrhea	2 to 3 loose stools in 24 hours	4 to 5 loose stools in 24 hours	6 or more loose stools in 24 hours
Headache	Does not interfere with activity	Some interference with activity	Prevents daily routine activity
Fatigue	Does not interfere with activity	Some interference with activity	Prevents daily routine activity
Chills	Does not interfere with activity	Some interference with activity	Prevents daily routine activity
Muscle pain (other than muscle pain at the injection site)	Does not interfere with activity	Some interference with activity	Prevents daily routine activity
Joint pain	Does not interfere with activity	Some interference with activity	Prevents daily routine activity

Abbreviation: IV=intravenous.

For each systemic event, the following variables will be available similar to local reactions:

1. Each systemic event on each day (up to day 7) after booster vaccination
2. Each systemic event on 'any day 1-7' after booster vaccination
3. Maximum severity of each systemic event on 'any day 1-7' after booster vaccination
4. Maximum duration of each systemic event after booster vaccination
5. Any systemic event (including fever) on 'any day 1-7' after booster vaccination

The derivation of these variables is similar to the derivation of the variables for local reactions (Section 6.2.3.1).

6.2.3.3. Temperature

Oral temperature will be collected in the e-Diary for 7 days (day 1 to day 7) after booster vaccination. The highest temperature for each day will be recorded in the e-Diary. The protocol defines fever as a temperature ≥38.0°C. Fever will be categorized as 38.0°C to ≤38.4°C, 38.5°C to ≤38.9°C, 39.0°C to ≤40°C, and >40°C for a given day per Protocol.

Similar to the derivations of systemic events and local reactions, fever will be derived for:

1. Fever on each day (up to day 7) after booster vaccination
2. Fever on 'any day 1-7' after booster vaccination
3. Highest Fever (maximum severity) on 'any day 1-7' after booster vaccination
4. Maximum duration of fever after booster vaccination

Temperatures <35.0°C and >42.0°C will be excluded from the analysis due to the likelihood of subject recording error.

6.2.3.4. Use of Antipyretic Medication

The use and type of antipyretic medication will be recorded in the e-Diary for 7 days (day 1 to day 7) after each vaccination.

The following variables will be derived:

1. Use of antipyretic medication on each day (up to day 7) after booster vaccination
2. Use of antipyretic medication on 'any day 1-7' after booster vaccination
3. Maximum duration of Use of antipyretic medication after booster vaccination
4. Onset day of antipyretic use relative to booster vaccination

6.2.4. Safety Data Collected through CRF

The relationship between (S)AEs and the investigational product (bivalent rLP2086 vaccine) will be characterized as related or not related as determined by investigators and as described in the protocol. The severity of AEs will be characterized as mild, moderate, and severe.

Adverse events will be categorized according to MedDRA.

6.2.4.1. Analysis Intervals

There will be up to 6 analysis intervals for safety data collected via case report form (CRF).

Table 7. Analysis Intervals for AE, SAE, Medically Attended Events (MAEs) and Newly Diagnosed Major Conditions (NDCMC)

#	Analysis Interval	Analysis Population	Interval Start date (inclusive)	Interval Stop (inclusive)	Safety Data
1	Stage 1	Stage 1 Safety	Enrollment	Visit 7 date – 1 day	NDCMC, AE
2	Vaccination Phase	Booster Stage Safety	Booster vaccination date (Visit 7)	Visit 8 date – 1 day	AE, SAE, MAE, NDCMC
3	Follow-up Phase	Booster Stage Safety	Visit 8 date	Visit 9 date (Visit 10 date for NDCMC)	SAE, MAE, NDCMC
4	Throughout the Booster Stage	Booster Stage Safety	Booster vaccination date (Visit 7)	Visit 9 date (Visit 10 date for NDCMC)	SAE, MAE, NDCMC, autoimmune and neuroinflammatory conditions, Days missed school/work
5	Throughout the Extended Booster Stage	Booster Stage Visit 11 Subset	Visit 7 date	Visit 11	NDCMC
6	Extended Follow-up Phase	Booster Stage Visit 11 Subset	Visit 8 date	Visit 11	NDCMC

AE = Adverse Events, SAE = Serious Adverse Events, MAE = Medically Attended Events, NDCMC = Newly Diagnosed Chronic Medical Conditions

6.2.4.2. Immediate Adverse Events

Any AE that occurred within the first 30 minutes after the investigational product administration will be classified as an immediate AE. Immediate AEs are a subset of AEs.

6.2.4.3. Research-Related Injuries

Research-related injuries (RRI) occurring within of 48 hours of a blood draw will be collected for all Stage 1 visits, and at Visit 10 and Visit 11 in the Booster Stage.

6.3. Other Endpoints

6.3.1. E-Diaries Completion

For any given day, an e-diary will be transmitted and considered as complete if all expected data (the 3 local reactions, the 8 systemic reactions (including fever), and the use of antipyretics) are available. If any of the items in the e-Diary is missing on a specific day, the e-Diary will not be transmitted and the e-Diary data will be missing for all items. When all the day for the day is complete within the e-diary, the data is transmitted to a centralized database.

The following e-Diary compliance variables will be derived as follows:

1. Compliance per day: the numerator is the number of subjects who completed (transmitted) the e-diary on a given day (day 1 to day 7) and the denominator is the total number of subjects who receive the vaccination.

2. At least 1 day: the numerator is the number of subjects who completed (transmitted) the e-diary on any 1 day and the dominator is the total number of subjects who received a vaccination.

3. At least 2 days: the numerator is the number of subjects who completed (transmitted) the e-diary on any 2 days and the dominator is the total number of subjects who received a vaccination.

4. At least 3 days: the numerator is the number of subjects who completed (transmitted) the e-diary on any 3 days and the dominator is the total number of subjects who received a vaccination.

5. At least 4 days: the numerator is the number of subjects who completed (transmitted) the e-diary on any 4 days and the dominator is the total number of subjects who received a vaccination.

6. At least 5 days: the numerator is the number of subjects who completed (transmitted) the e-diary on any 5 days and the dominator is the total number of subjects who received a vaccination.

7. At least 6 days: the numerator is the number of subjects who completed (transmitted) the e-diary on any 6 days and the dominator is the total number of subjects who received a vaccination.

8. All 7 days: the numerator is the number of subjects who completed (transmitted) the e-diary on all 7 days and the denominator is the total number who received a vaccination.

6.3.2. Demographic, Medical History, and Baseline Characteristics Variables

The demographic variables are gender, race, ethnicity, and age at time of first vaccination in the primary study. Age will be derived based on birthday. For example, if the first vaccination date is one day before the subject's 13th birthday, the subject is 12 years old.

Medical history will be categorized according to the Medical Dictionary of Regulatory Activities (MedDRA).

Physical exam will be recorded as normal, abnormal, and not done.

6.4. Covariates

The covariates are mainly based on the 'primary' studies' characteristics.

- Age at first vaccination (10-14 years vs 15-18 years)
- Gender (Female, Male)

7. HANDLING OF MISSING VALUES

7.1. Immunogenicity Data

The primary immunogenicity analyses for the study will be based on the observed, determinate observations, which is under the assumption of missing completely at random (MCAR (Scott and Hsu)[1]). Missing data will not be imputed for analysis. As a sensitivity analysis, a mixed-effects model with repeated measurements (MMRM) will be utilized for the immunogenicity analysis, which assumes the missingness is at random (MAR).

The hSBA LLOQ are listed in Table 8.

Table 8. LLOQ for hSBA

Strain	LLOQ
CCI [redacted]	1:16
CCI [redacted]	1:8
CCI [redacted]	1:8
CCI [redacted]	1:8

For the calculation of GMT, values below the LLOQ will be set to 0.5*LLOQ for the primary analysis.

8. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

8.1. Statistical Methods

Unless otherwise explicitly stated, descriptive statistics for continuous variables are: n, mean, median, standard deviation, minimum and maximum. Descriptive statistics for categorical variables are: n, percentage, and total (N).

8.1.1. Analyses for Continuous Data

For each of the 4 primary test strains, hSBA titers measured at each blood sampling time point will be logarithmically transformed for analysis and geometric mean hSBA titers will be computed at each blood sampling time point along with 2-sided 95% confidence intervals (CIs). The CIs will be constructed by back transformation of the confidence limits computed for the mean of the logarithmically transformed assay data based on Student's t distribution.

The hSBA GMT will be summarized for the primary immunogenicity populations within each primary study as well as by the subgroups as presented in Section 6.4. A mixed model with repeated measurements adjusted with covariates (age at first vaccination, gender, dose group, visit, and dose group by visit interaction) will also be used to estimate the GMTs at different time points.

The empirical reverse cumulative distribution curves (RCDCs) of hSBA titer will be presented graphically for each of the 4 primary strains, and at each sampling time point.

8.1.2. Analyses for Binary Endpoints

All of the binary immunogenicity endpoints will be descriptively summarized along with exact 2-sided 95% confidence intervals (or Clopper-Pearson confidence limits) for the proportion.

The exact confidence interval for proportion will be computed using the F distribution. If r equals the number of responses and n equals the number of subjects, then it follows that $p = r / n$ is the estimate of the proportion of responses. An exact 95% confidence interval can be computed by solving the following 2 equations. For the lower limit p_L, use: $p_L = \frac{rF_L}{(rF_L + (n - r + 1))}$ and for the upper limit p_U, use $p_U = \frac{(r+1)F_U}{(n-r)+(r+1)F_U}$

where F_L is the quantile from the F distribution for α=0.025, with numerator degrees of freedom equal to 2r and denominator degrees of freedom equal to 2(n-r+1). F_U is the quantile from the F distribution for α=0.975, with numerator degrees of freedom equal to 2(r+1) and denominator degrees of freedom equal to 2(n-r). When r equals 0, F_L should be set equal to 1.0 so p_L equals 0. When r equals n, F_U should be set equal to 1.0 so p_U equals 1. The confidence interval using the F distribution is described in Collett [2] (1991).

8.2. Statistical Analyses

8.2.1. Analysis of Primary Endpoints

The immunogenicity data will be analyzed within each primary study. The primary analysis will include the summary tables on the primary immunogenicity endpoint (proportion of subjects with hSBA titer ≥ LLOQ) at each blood sampling timepoint in the primary analysis populations (mITT for Stage 1, evaluable population for Booster Stage at Visits 8 and 10) for each of the 4 primary test strains.

The analysis for immunogenicity endpoints including Visit 11 will use the subset of evaluable population for Booster stage subjects who proceed to Visit 11.

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8.2.3. Sensitivity Analyses

A mixed-effects model with repeated measurement will be utilized to assess the effect of covariates in which both baseline and post-booster titers are modeled as dependent variables for each primary test strain. This model is using maximum likelihood estimation, therefore, it also serves as a sensitivity analyses on missing data for the GMT. To account for the intrasubject correlation among the repeated measures, an unstructured covariance matrix will be used. In case the model does not converge, further covariance structures will be explored (eg. First-order autoregressive, compound symmetry). The model will take the form

Log (hSBA) = Initial Study Group + gender + age at first vaccination +visit+ Group* visit. The intercept will be set as random effect.

In addition to Type III analysis output, least squares GMTs at each visit will be summarized for each strain.

These analyses will only be applied to mITT population, using ½ LLOQ to impute the hSBA values below LLOQ, for the primary strains only.

8.2.4. Safety Analyses

The safety data will be pooled across the primary studies and presented by dose groups ((0,2,6-schedule), (0,1,6-schedule), (0,6-schedule), (0,2-schedule), (0,4-schedule)).

8.2.4.1. Reactogenicity Data

The derived variables for the presence of each local reaction, each systemic event, fever, and use of antipyretic medication at each day and 'any day 1-7', and any local reaction and any systemic events at 'any day 1-7' will be summarized with number and proportion of subjects reporting such event. The summaries will include 95% CIs. Clopper-Pearson's method will be used to calculate the 95% CI.

The maximum severity of each local reaction, systemic events, and fever at 'any day 1-7' will be summarized with number and proportion of subjects reporting such maximum severity of the event. The summaries will include 95% Clopper-Pearson's CI.

For local reactions and systemic events, including fever and use of antipyretic medication, the maximum duration of the event will be summarized. The number of unknown durations will also be summarized.

All of these summaries will be summarized using the Booster Stage safety population, according to the vaccine received.

A listing will be provided for all of the severe reactogenicity data.

[redacted]

8.2.4.2. Safety Data Collected through CRF

8.2.4.2.1. SAE

The percentage of subjects reporting at least one SAE, and the number of episodes will be summarized for each of the analysis intervals (Section 6.2.4.1). 95% exact confidence interval for the percentages will be provided. Similarly, the percentage of subjects reporting at least one related SAE and total # of related SAEs reported by each group will be provided.

In addition, for the analysis intervals of Booster vaccination phase, Booster follow-up phase and Throughout the Booster Stage (Visit 7 to Visit 9), the SAEs will be summarized by each preferred term (MedDRA) with percentage and number of events for each group.

All of the SAE will be listed with subject ID, event onset, the most recent vaccination, days of event relative to vaccination, duration of the event, and management and outcome.

8.2.4.2.2. Newly Diagnosed Chronic Medical Condition (NDCMC)

These data will be summarized, by preferred term, for the Stage 1 interval and Throughout the Booster Stage. NDCMC are collected through Visit 10 (Visit 11 for subset of subjects proceeding to the 26 months postbooster vaccination visit). Summaries will be presented based on the analysis intervals outlined in Section 6.2.4.1. A subset of 'related' may also be summarized similarly if numbers are sufficient.

8.2.4.2.3. Neuroinflammatory and Autoimmune Conditions

A list of preferred terms (MedDRA), to include all of the neuroinflammatory and autoimmune conditions, will be provided by the medical monitor prior to the database lock. These events can be SAE, or AE.

A listing of adverse events matching these preferred terms will be provided.

8.2.4.2.4. Medically Attended Events (MAE)

A medically attended event is defined as a nonserious AE that results in an evaluation at a medical facility. The percentage of subjects with at least one MAE in each of the Booster Stage intervals will be summarized, including 95% exact confidence intervals for the percentages.

8.2.4.2.5. Immediate AE

The number and percentage of subjects reporting adverse events during the protocol specified first 30-minute observation period following booster vaccination will be summarized. These summaries will include 95% Clopper-Pearson CIs. The immediate AE categorized as SAE (defined as immediate AE and also meet the definition of SAE) will be listed.

8.2.4.2.6. AE

The AEs will be analyzed similar to SAE, except that non-serious AEs (except MAEs and non-serious NDCMCs) are only collected during Stage 1 and the Booster vaccination phase.

If non-serious AEs during the Booster follow-up phase were recorded in the database (excluding MAEs and non-serious NDCMCs), these data will be listed separately.

8.2.4.2.7. Multiple Events per Subject (Incidence Rates)

The frequency distribution on number of events reported by each subject will be summarized for each of the following safety variables:

1. Any AE during the Booster vaccination phase

2. Any SAE throughout the booster stage

For each subject, the number of events reported per year will be estimated by summarizing the total number of events reported over the observation period and standardizing it to an annual rate by multiplying by 365 and dividing by days on study. The overall incidence rate (including 95% confidence intervals) for each of the safety variables will be reported by dose group.

8.2.4.3. RRIs Occurring within 48 hours after Blood Draw

For all Stage 1 visits, and at Visit 10 and Visit 11 in the Booster Stage, research-related injuries (RRI) collected within of 48 hours of a blood draw will be collected. *A medically important RRI is any untoward medical occurrence that, results in death, is life-threatening (immediate risk of death), requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, results in congenital anomaly/birth defect. Medical and scientific judgment is exercised in determining whether an injury is an important medical event.* The percentage of subjects with at least one RRI in Stage 1 and Booster Stage will be summarized descriptively.

8.2.4.4. Death

Any death data will be listed.

8.2.5. Analyses of Study Conduct

8.2.5.1. Subject Disposition, Vaccination Administration, Blood Samples

The number and percentage of subjects who are enrolled, withdraw during Stage 1, complete Stage 1, receive the booster vaccination, withdraw during the Booster Stage and complete the Booster Stage will be summarized. The reasons for withdrawal will also be tabulated.

For each blood draw, the number and percentage of subjects providing blood samples within the protocol-specified time frame, as well as before and after the specified time frame, will be tabulated.

The timing of booster vaccination relative to first vaccination in the primary study will be summarized.

The number and percentage of subjects included in each analysis population will be summarized.

The ITT population will be used to generate these tables. All of the summary tables will be presented for the total population.

A listing of subjects' non-compliant vaccine administration will be provided. The protocol violators will also be listed. A listing of subjects that withdrew and subjects who withdrew due to AEs will be provided.

Listings for the subjects with flags to be included or excluded in each analysis population will be provided. The reason for not including a subject in a specific analysis population will be listed.

8.2.5.2. Demographic, Medical History, and Baseline Characteristics

Standard summary reports will be provided. Demographic summaries may also be generated for different analysis sets as defined in Section 5.

8.2.5.3. E-Diary Completion

Variables defined in Section 6.3.1 will be summarized for each group using descriptive statistics. The denominator for the e-Dairy compliance rates will be the total number of subjects who receive the vaccination.

8.2.5.4. Representativeness of Subjects from Primary Studies

For each of the primary studies, there will be a descriptive comparison between subjects enrolled in B1971033 and the remaining subjects, within the appropriate primary study groups, to assess representativeness. The endpoints summarized in this way will include:

- Demographic characteristics (age at first vaccination, gender, race);
- Proportions of subjects with hSBA titer ≥LLOQ at 1 month after the second and third doses of rLP2086, from the primary studies;
- hSBA GMTs at 1 month after the second and third doses of rLP2086, from the primary studies; for each of the 4 primary test strains, along with 2-sided 95% Cis;
- Proportions of subjects reporting at least one AE and at least one SAE, after 2 doses and 3 doses of rLP2086, during the primary studies.

8.2.6. Subgroup Analysis

The primary immunogenicity endpoints, and the CCI [redacted] specified in Section 6.1 and Section 6.2.1 may be descriptively summarized within each primary study by age, gender as described in Section 6.4. The safety endpoints (including reactogenicity data and AE) may be summarized by pooling the safety data across the primary studies and presented by dose groups.

Any p-values and 95% CI provided for the subgroup analyses are considered as descriptive and no multiplicity adjustment will be made on these analyses. No formal inferences will be made between the subgroups.

[redacted]

9. REFERENCES

[1] Scott, J.A. & Hsu, H. (2011): Missing Data Issues at the FDA Center for Biologics Evaluation and Research, *Journal of Biopharmaceutical Statistics*, 21:2, 196-201.

[2] Collett D. *Modeling Binary Data.* London: Chapman & Hall; 1991.