Protocol B1971035

A PHASE 2, RANDOMIZED, CONTROLLED, OBSERVER-BLINDED STUDY CONDUCTED TO DESCRIBE THE IMMUNOGENICITY, SAFETY, AND TOLERABILITY OF A *NEISSERIA MENINGITIDIS* SEROGROUP B BIVALENT RECOMBINANT LIPOPROTEIN 2086 VACCINE (BIVALENT RLP2086) WHEN ADMINISTERED TO HEALTHY TODDLERS AGED 12 TO <18 MONTHS OR 18 TO <24 MONTHS

Statistical Analysis Plan
(SAP)

Version: 2.1

Date: 13-July-2017

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
CI	confidence interval
CRF	case report form
e-Diary	electronic diary
E-DMC	external data monitoring committee
GCP	Good Clinical Practice
GMC	geometric mean concentration
GMFR	Geometric Mean Fold Rise
GMT	geometric mean titer
HAV vaccine	Hepatitis A Virus vaccine
hSBA	serum bactericidal assay using human complement
ICD	informed consent document
IRC	Internal review committee
ISC	Independent Statistical Center
ITT	intent-to-treat
LLOQ	lower limit of quantitation
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
MnB	*Neisseria meningitidis* serogroup B
OMV	outer membrane vesicle
PDS	Pfizer Data System
RCDC	reverse cumulative distribution curve
rLP2086	recombinant lipoprotein 2086
rSBA	serum bactericidal assay using rabbit complement
SAE	serious adverse event
SAP	statistical analysis plan
SBA	serum bactericidal assay

1. AMENDMENTS FROM PREVIOUS VERSION(S)

Version	Date	Summary of Changes/Comments
Version 1.0	June 12, 2015	Initial Version
Version 2.0	June 16, 2016	• Modified evaluable population to be consistent with other MnB protocols. • Updated unblinding strategy according to protocol amendment • Clarified timing of primary analysis • Clarified the extent of sub group analyses for key endpoints • Provided details of when p-values and other between-group comparison statistics will be provided for safety endpoints. • Added additional endpoints for which the incidence rates will be summarized. • Modified reporting of AEs and RRIs occurring within 48 hours of blood draws from visit 8 to visit 13 to listings only.
Version 2.1	July 13, 2017	• Added criteria for when summaries within age strata may not be produced. • Added criteria of when a subgroup analysis may not be done. • Removed summaries by preferred term for some intervals and some endpoints. • Added 95% confidence interval for incidence rate. • Corrected typo of when severe reactions/events may be presented with between-group statistics.

2. INTRODUCTION

The purpose of this document is to provide further details about the statistical analysis methods specified in the study protocol B1971035. 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 immunological, and safety endpoints, followed by details about statistical methods. The windowing specifications, 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. Note that text taken directly from the protocol has been italicized.

2.1. Study Design

2.1.1. Description

The study is a Phase 2, randomized, active-controlled, observer-blinded, sponsor-unblinded, multicenter study in which approximately 396 healthy toddlers stratified by age, 12 to <18 months or 18 to <24 months old, will be randomly assigned in a 2:1 ratio to receive either 1 of 2 dose levels (60 µg or 120 µg) of bivalent rLP2086 or HAV a licensed pediatric vaccine /sterile saline solution for injection (0.85% sodium chloride) in a 0.5-mL dose.

The study will be conducted in 2 stages:

Stage 1 will assess vaccine immunogenicity, safety, and tolerability across 2 phases: a sentinel-enrollment phase and an expanded-enrollment phase. Within the sentinel-enrollment phase, 2 sentinel cohorts will be enrolled: one composed of subjects aged 12 to <15 months and the other of subjects aged 18 to <24 months. The expanded-enrollment phase will be open to subjects in 2 cohorts, one aged 12 to <18 months and the other aged 18 to <24 months.

Within the study age cohort of 12 to <18 months, an additional subset of subjects aged 12 to <15 months is identified for the sentinel phase of the study. The designation of a subset of subjects aged 12 to <15 months aims to ensure that the sentinel cohort includes adequate enrollment of this youngest age cohort to support assessment of post-vaccination reactions and AEs prior to the start of enrollment of the expanded-enrollment phase.

Stage 2 will assess the duration of the immune response to bivalent rLP2086; hence, only those subjects randomly assigned to bivalent rLP2086 (irrespective of dose level) will be eligible for Stage 2.

Bivalent rLP2086 will be administered on a Month 0, 2, and 6 schedule.

HAV vaccine is administered at Months 0 and 6 and has been selected as the vaccine control so that subjects randomly assigned to the control group receive a benefit from participating in the study. HAV vaccine has a well-established tolerability profile and will provide protection to subjects who may become at increased risk for hepatitis A viral infection either during future travel or during other exposures. As the recommended schedule for HAV vaccine is 2 doses 6 months apart, saline will be given at Month 2 to maintain the study blind.

2.1.2. Sentinel- and Expanded-Enrollment Plan, Stage 1

2.1.2.1. Sentinel Enrollment, Stage 1

Two (2) sentinel cohorts, one aged 12 to <15 months and the other aged 18 to <24 months, will each simultaneously and independently enroll approximately 33 subjects. Stopping rules apply for sentinel cohorts (see Section 7.9 Stopping Rules of the protocol*).*

60-µg Sentinel Cohort

For each 60-µg dose-level sentinel cohort, approximately 33 subjects will be randomly assigned in a 2:1 ratio to receive either 60 µg of bivalent rLP2086 or HAV vaccine/saline. Stopping rules apply for sentinel cohorts (see Section 7.9 Stopping Rules of the protocol*).*

Within the 60-µg sentinel cohort of each age group, when the last subject has received Vaccination 1 and has attended the Day 7 follow-up visit, all post–Vaccination 1, 7-day electronic diary (e-diary) and AE data (summary safety data) will be tabulated for review by the sponsor's IRC.

Following completion of the data review(s) for the 60-μg sentinel cohort, a decision will be made by the IRC whether to proceed to the 120-μg sentinel cohort.

If the IRC finds the 60-μg summary safety data to be acceptable, and no further review is warranted, the subjects in the 60-μg sentinel cohort will continue through the vaccine series.

If the 60-μg summary safety data are unacceptable for the older-age sentinel cohort, then vaccinations will be halted for both of the 60-μg sentinel cohorts and enrollment into the study will stop.

If the summary safety data 60-μg dose are acceptable for the older-age sentinel cohort but not the younger-age sentinel cohort, then vaccination and enrollment will cease for the younger-age sentinel cohort only but enrollment may commence for the 120-μg dose-level older-age sentinel cohort only.

120-μg Sentinel Cohort

For each 120-μg dose-level sentinel cohort, approximately 33 subjects will be randomly assigned in a 2:1 ratio to receive either 120 μg of bivalent rLP2086 or HAV vaccine/saline. Stopping rules apply for sentinel cohorts (see Section 7.9 Stopping Rules of the protocol*).*

Within the 120-μg sentinel cohort of each age group (if applicable), when the last subject has received Vaccination 1 and has attended the Day 7 follow-up visit, all post–Vaccination 1, 7-day e-diary and AE data (summary safety data) will be tabulated for review by the sponsor's IRC.

Following completion of the sentinel data review(s) of the 120-μg dose, either the 60-μg dose or the 120-μg dose will be selected for each age cohort by the IRC for use in the expanded-enrollment phase of Stage 1.

If the IRC finds the summary safety data for the 120-μg dose to be acceptable, and no further review is warranted, the subjects in the 120-μg sentinel cohort will continue through the vaccine series. Younger-age sentinel subjects will not progress to the 120-μg dose level before the safety data of the 120-μg dose level have been found to be acceptable in the older-age sentinel cohort. If the 120-μg summary safety data are unacceptable in the younger-age sentinel cohort but acceptable in the older-age sentinel cohort, then a) vaccination and enrollment at the 120-μg dose level will cease in the younger-age sentinel cohort, but vaccination and expanded enrollment may continue in the younger-age sentinel cohort at the 60-μg dose level, and b) vaccination and expanded enrollment may continue at the 120-μg dose level for the older-age sentinel cohort.

If the summary data for the 120-μg dose are unacceptable in the older-age sentinel cohort, vaccination and enrollment for the sentinel cohort at the 120-μg dose level will cease. Further expanded enrollment and vaccination, however, may continue at the 60-μg dose level.

2.1.2.2. Expanded Enrollment, Stage 1

The expanded-enrollment stage will enroll approximately 132 subjects each into 2 age groups (12 to <18 months and 18 to <24 months), randomly assigned in a 2:1 ratio to the selected dose level of bivalent rLP2086 or HAV vaccine/saline. Randomization will be substratified in the younger age group only: 12 to <15 months and 15 to <18 months. The selected dose level in the expanded-enrollment phase may differ between age groups (12 to <18 months and 18 to <24 months), but not within the substratum (12 to <15 months and 15 to <18 months) of the younger age group.

2.1.2.3. Enrollment Plan, Stage 2

Those subjects who received all 3 study vaccinations of bivalent rLP2086 and who were randomly assigned to bivalent rLP2086 at either dose level may progress to the open-label Stage 2. Subjects randomly assigned to HAV vaccine/saline will not enter Stage 2. To facilitate this transition, the subject's parent(s)/legal guardian will be notified of the subject's randomization allocation at prior to entry into Stage 2.

2.1.3. Schedule of Activities

The Schedule of Activities provides an <u>overview</u> of the protocol visits and procedures. Refer to the Study Procedures and Assessments sections of the protocol for detailed information on each procedure and assessment required for compliance with the protocol.

The investigator may schedule visits (unplanned visits) in addition to those listed on the Schedule of Activities, in order to conduct evaluations or assessments required to protect the well-being of the subject.

Schedule of Activities for Stage 1

Visit Identifier	1	2	3	4[a]	5	6	7	8	9	10
Time Period	Month 0	Week 1	Month 1	Month 2	Month 3	Month 6	Month 7	Month 12	Month 18	End of Stage 1
Visit Description	Vaccination 1	Post–Vaccination 1 Follow-up Visit	Telephone Contact	Vaccination 2	Post–Vaccination 2 Blood Draw	Vaccination 3	Post–Vaccination 3 Blood Draw	6-Month Follow-up Visit[b]	Antibody Persistence Blood Draw[b]	Telephone Contact
		Vaccination Phase						Follow-up Phase		
Visit Window	Day 1 (Baseline)	7 to 10 Days After Visit 1	25 to 35 Days After Visit 1	42 to 70 Days After Visit 1	28 to 42 Days After Visit 4	105 to 126 Days After Visit 4	28 to 42 Days After Visit 6	168 to 196 Days After Visit 6	337 to 365 Days After Visit 6	-
Informed consent[c]	X									
Review eligibility criteria	X									
Confirm continued eligibility		X	X	X	X	X	X	X	X	
Demography[d]	X									
Medical history[e]	X									
Physical examination[f]	X									
Vaccination History	X									
Brief physical examination[g]				X		X				
Heart rate, respiratory rate	X			X		X				
Axillary temperature	X			X		X				
Obtain 5-mL blood sample	X[h]				X		X	X	X	
Randomization	X									
Vaccination & 30-minute observation[i]	X			X		X				

Schedule of Activities for Stage 1

Visit Identifier	1	2	3	4[a]	5	6	7	8	9	10
Time Period	Month 0	Week 1	Month 1	Month 2	Month 3	Month 6	Month 7	Month 12	Month 18	End of Stage 1
Visit Description	Vaccination 1	Post–Vaccination 1 Follow-up Visit	Telephone Contact	Vaccination 2	Post–Vaccination 2 Blood Draw	Vaccination 3	Post–Vaccination 3 Blood Draw	6-Month Follow-up Visit[b]	Antibody Persistence Blood Draw[b]	Telephone Contact
	Vaccination Phase							Follow-up Phase		
Visit Window	Day 1 (Baseline)	7 to 10 Days After Visit 1	25 to 35 Days After Visit 1	42 to 70 Days After Visit 1	28 to 42 Days After Visit 4	105 to 126 Days After Visit 4	28 to 42 Days After Visit 6	168 to 196 Days After Visit 6	337 to 365 Days After Visit 6	-
Record nonstudy vaccinations		X	X	X	X	X	X	X	X	X
Provide e-diary, thermometer, measuring tape, and caliper	X			X		X				
Review and collect e-diary		X			X		X			
Assess reactogenicity[j]	Days 1 to 7			Days 1 to 7		Days 1 to 7				
Record the use & type of antipyretic medication	Days 1 to 7			Days 1 to 7		Days 1 to 7				
(S)AE collection as appropriate	X[k]	X	X	X[k]	X	X[k]	X	X		
Record AEs / research-related injury[l]									X	X
Record concomitant medications used to treat AEs	X	X	X	X	X	X	X	X		
Complete study visit AE checklist[m]		X	X	X	X	X	X	X		

Schedule of Activities for Stage 1

Visit Identifier	1	2	3	4[a]	5	6	7	8	9	10
Time Period	Month 0	Week 1	Month 1	Month 2	Month 3	Month 6	Month 7	Month 12	Month 18	End of Stage 1
Visit Description	Vaccination 1	Post–Vaccination 1 Follow-up Visit	Telephone Contact	Vaccination 2	Post–Vaccination 2 Blood Draw	Vaccination 3	Post–Vaccination 3 Blood Draw	6-Month Follow-up Visit[b]	Antibody Persistence Blood Draw[b]	Telephone Contact
	Vaccination Phase							Follow-up Phase		
Visit Window	Day 1 (Baseline)	7 to 10 Days After Visit 1	25 to 35 Days After Visit 1	42 to 70 Days After Visit 1	28 to 42 Days After Visit 4	105 to 126 Days After Visit 4	28 to 42 Days After Visit 6	168 to 196 Days After Visit 6	337 to 365 Days After Visit 6	-
Issue memory aid							X	X[n]	X[n]	
Notification of randomization group[o]										X

a. See Section 6.1.11 of the protocol for Subjects who do not complete Stage 1 on internal review committee (IRC)/external data monitoring committee (EDMC) recommendations of the protocol.
b. Relative to Vaccination 3.
c. Must be obtained prior to any protocol-required procedures.
d. Date of birth, sex, race, and ethnicity.
e. Chronic conditions and/or medical history of significance, including relevant surgical procedures, allergies (drug/nondrug).
f. General appearance, skin, head, eyes, nose, ears, throat, heart, lungs, abdomen, extremities, neurologic, musculoskeletal, lymph nodes, height (or length for nonwalking subjects) and weight.
g. General appearance, ears, throat, heart, and lungs.
h. Blood must be collected before vaccination and only after eligibility has been confirmed.
i. Injection performed by unblinded administrator; acute reactions assessed by blinded observer. Location of vaccination should be noted in the source, the CRF and the e-diary.
j. Between visits, review the e-diary data online at frequent intervals. Contact the parent(s)/legal guardian in order to obtain stop dates for any local reactions, systemic events, or use of antipyretic medication that was ongoing on the last day that the electronic diary (e-diary) was completed.
k. For additional reporting period details for AEs, SAEs, newly diagnosed chronic medical conditions (NDCMCs) and medically attended events (MAEs), see Section 8 of the protocol
l. Occurring within 48 hours after blood draw.
m. Checklist includes questions regarding NDCMC and MAEs/AEs.
n. If required.
o. An End– of– Stage 1 telephone call will take place after the subject has completed Visit 9 and following sponsor release of randomization codes.
Abbreviations: AE = adverse event; e-diary = electronic diary; MAE = medically attended event; NDCMC = newly diagnosed chronic medical condition; SAE = serious adverse event.

Schedule of Activities for Stage 2
Duration of MnB-Specific Immune Response (Bivalent rLP2086 Subjects Only)

Visit Identifier	11	12	13
Time Period	Month 30	Month 42	Month 54
Visit Description (Time After Vaccination 3)	Immunogenicity 1 (24 Months)	Immunogenicity 2 (36 Months)	Immunogenicity 3 (48 Months)
Visit Window	**±30 Days**	**±30 Days**	**±30 Days**
Confirm continued eligibility	X	X	X
Obtain 5-mL blood sample	X	X	X
Record AEs, and, any research-related injury occurring within 48 hours after blood draw	X	X	X
Record any nonstudy vaccinations	X	X	X
Issue memory aid	X	X	

Abbreviations: AE = adverse event; bivalent rLP2086 = bivalent recombinant lipoprotein 2086 vaccine; MnB = *Neisseria meningitidis* serogroup B.

2.1.4. Assessments

Subjects will receive investigational products at Visit 1, 4, and 6 to conform to a 0-, 2-, 6-month schedule for rLP2086 vaccine. During stage 1, all of the subjects will have blood draws for immunogenicity assessment at visits 1, 5, 7, 8, and 9 which includes baseline and 1 month after each vaccination, as well as 6 and 12 months following the third vaccination dose. For stage 2, all subjects entered for that stage will have blood draws at 24, 36 and 48 months following the last vaccination dose.

Reactogenicity data, including local reactions, systemic events (including fever), and use and type of antipyretics will be collected via an electronic diary (e-Diary) completed by each subject for 7 days following each vaccination.

All Adverse Events (AE) (serious and non-serious) will be collected from the signing of the informed consent document (ICD) to Visit 7. Any SAEs, newly diagnosed chronic medical conditions and medically attended AEs will be collected during the vaccination period and through Visit 8 (6 months after last vaccination dose). AEs/Research-related injuries will be collected at Visit 8 and 9 and at Visit 11, 12, and 13 for the 48-hour period after each vaccination. In addition, acute reactions within 30 minutes after investigational product administration (immediate AEs) will be documented.

Other safety assessments will include medical history, vital signs and physical examination Visit 1 and a brief physical exam and vital signs/temperature assessment at all vaccination visits.

2.1.5. Number of Immunogenicity Assays

Of approximately 396 randomized subjects, up to 264 subjects will be given rLP2086 vaccine and up to 132 subjects will be given the HAV vaccine. It is anticipated that the volume of serum from individual study subjects may not be adequate for performance of hSBA with all 4 primary MnB test strains. Therefore, the study design designates randomization of sera from individual study subjects for initial testing with 2 of the 4 primary hSBA test strains. At a minimum, approximately 50% of subjects from each age group will be randomly selected to test for hSBA on strains CCI (A22) and CCI (B24), and the other 50% of subjects from each age group will be tested for hSBA on strains CCI (A56) and CCI (B44). Serum bactericidal assay (SBA) with all 4 primary MnB test strains may be performed with individual serum specimens when the volume of the sample permits. The maximum number of assay tests per visit in outlined in Table 1 and Table 2.

Table 1.   Number of Immunogenicity Assays in Stage 1

Antigens/Strains	Visit 1	Visit 5	Visit 7	Visit 8	Visit 9
hSBA MnB CCI (A22)	X	X	X	X	X
hSBA MnB CCI (B24)	X	X	X	X	X
hSBA MnB CCI (A56)	X	X	X	X	X
hSBA MnB CCI (B44)	X	X	X	X	X
Maximum # of assays	396*2	396*2	396*2	396*2	396*2
Total assays	792	792	792	792	792

For 50% of the subjects the primary strains are A22 and B24 and for the other 50% the primary strains are A56 and B44. The additional 2 primary strains may be tested if sample volume permits.

Table 2.   Number of Immunogenicity Assays in Stage 2

Antigens/Strains	Visit 11	Visit 12	Visit 13
hSBA MnB CCI (A22)	X	X	X
hSBA MnB CCI (B24)	X	X	X
hSBA MnB CCI (A56)	X	X	X
hSBA MnB CCI (B44)	X	X	X
Maximum # of assays	264*2	264*2	264*2
Total assays	528	528	528

For 50% of the subjects the primary strains are A22 and B24 and for the other 50% the primary strains are A56 and B44. The additional 2 primary strains will be tested if sample permits.

2.2. Study Objectives

2.2.1. Primary Immunogenicity Objectives

- *To describe the immune response as measured by hSBA performed with 4 primary MnB strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <18 months at study entry.*

- *To describe the immune response as measured by hSBA performed with 4 primary MnB strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 18 to <24 months at study entry.*

2.2.2. Primary Safety Objective

- *To evaluate the safety profile of bivalent rLP2086 compared to a control (hepatitis A virus [HAV] vaccine), as measured by local reactions, systemic events, adverse events (AEs), serious adverse events (SAEs), newly diagnosed chronic medical conditions, medically attended events, and immediate AEs in healthy toddlers 12 to <18 months and 18 to <24 months of age at study entry, and in both age strata combined.*

2.2.3. Secondary Immunogenicity Objectives

- *To describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <24 months at study entry (ie, both age strata combined).*

- *To describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination, and 6, 12, 24, 36, and 48 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.*

2.2.4. CCI

- CCI

- CCI

3. INTERIM ANALYSES, FINAL ANALYSES AND UNBLINDING

3.1. Blinding

As the physical appearances of bivalent rLP2086, HAV vaccine, and saline differ, dispensing and administration of the investigational product will be performed by an unblinded dispenser/administrator. The unblinded dispenser/administrator will at all times (other than as described in Section 5.2.2 of the protocol keep the identity of the randomized, allocated vaccination concealed from parent(s)/legal guardian/other family members and blinded study staff. Unblinded study staff will not take part in any subject assessments/interviews/data collection or CRF data entry; however, investigational product dispensing and administration should be noted in the study/medical record (while maintaining the blind) and accountability logs.

So that subject safety and stopping rule criteria can be closely monitored and responded to in a timely fashion, a subset of sponsor representatives will be unblinded (see the IRC charter). These include the medical clinicians, clinical scientists, and statisticians. In addition, an investigational product monitor(s) will be required to monitor the pharmacy dispensing records.

3.1.1. Blinded Study Personnel

All other site personnel, including the investigator, investigator staff, subjects, and subjects' parent(s)/legal guardian, will be blinded to investigational product assignments until randomization allocations are made available.- Pfizer will release unblinding information to the investigator when all subjects have completed Visit 8 and before subjects reach Visit 10. Subjects will receive their randomization allocation at Visit 10. Additional information is included in the SRM (Study Reference Manual).

Stage 2 will be unblinded.

All immunogenicity assays will be performed in a central laboratory by blinded laboratory staff.

3.1.2. DMC Reports

As this study is utilizing E-DMC, the unblinded randomization codes will be released to an Independent Statistical Center (ISC) prior to the DMC meeting. The ISC is not a part of the sponsor's organization and is a statistical team not involved in the conduct of the study. ISC will be producing unblinded data summary reports on safety data and deliver the safety reports to the DMC members through a secure portal independent of the Sponsor.

3.1.3. Assay Testing Allocation

The study design designates randomization of sera from individual study subjects for initial testing with 2 of the 4 primary hSBA test strains. At a minimum, approximately 50% of subjects from each age group will be randomly selected to test for hSBA on strains CCI (A22) and CCI (B24), and the other 50% of subjects from each age group will be tested for hSBA on strains CCI (A56) and CCI (B44). Serum bactericidal assay (SBA) with all 4 primary MnB test strains may be performed with individual serum specimens when the volume of the sample permits. The independent statistical center (ISC, a statistical team not involved in the conduct of the study) will provide the subject list to test hSBA on the 2 strains to the Sponsor's sample management team without the group assignment. The hSBA testing personnel will be blinded to the subject group allocation.

3.2. Primary Analysis

The primary analysis will be performed when all of the immunogenicity data through Visit 7 and all the safety data up to and including Visit 8 are available. The final analysis will include all data not reported in the first CSR and will be performed separately after all of the subjects finish the study when all of the data are available. As this study is not a hypothesis-testing study, no alpha will be adjusted on these analyses.

Interim summaries of immunogenicity and safety data may be generated to summarize key immunogenicity and safety endpoints. As this study is not a hypothesis-testing study, no alpha will be adjusted on these analyses.

3.3. Safety Analysis

The safety data will be reviewed on an ongoing basis, and no alpha will be adjusted for these multiple looks at the safety data.

3.3.1. Internal Review and Data Monitoring Committees

This study will use both an unblinded IRC and an unblinded EDMC. See Section 9.4 of the protocol for detailed responsibilities for both IRC and EDMC.

4. HYPOTHESES AND DECISION RULES

4.1. Statistical Hypotheses

There are no statistical hypotheses specified in the protocol. All safety and immunogenicity analyses will be descriptively summarized.

4.2. Statistical Decision Rules

Not applicable.

5. ANALYSIS SETS

5.1. Full Analysis Set

An intent-to-treat (ITT) population will be defined that includes all subjects who are randomized.

5.2. 'Per Protocol' Analysis Set

The 'Per Protocol' Analysis Set will be used for the immunogenicity analysis. It will be named as Evaluable Immunogenicity Population. The evaluable immunogenicity population will be defined as described below.

This population is the primary population for immunogenicity analyses and includes subjects who:

1. Are eligible for the study (through 1 month post vaccination 3).

2. Have been randomized to a study group.

3. Have received scheduled investigational products as randomized.

4. Have prevaccination blood drawn prior to the first vaccination dose and have post-vaccination 3 blood draw (Visit 7) within 28-42 days from the third vaccination (Visit 6).

5. Have a valid and determinate assay result for the proposed analysis.

6. Have received no prohibited vaccines or treatment.

7. Have no other major protocol violations as determined by the sponsor's global medical monitor.

For above listed items, 1 through 5 will be computerized checks of the data, while items 6 and 7 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, eg, 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 sponsor's global medical monitor will identify those subjects with protocol violations before any immunogenicity analysis is carried out.

5.3. Safety Analysis Set

The safety population will be used for all safety analyses. The safety population will include all subjects who have received at least 1 dose of an investigational product (bivalent rLP2086 or control) and for whom safety data are available. For the safety analysis, subjects will be analyzed according to the investigational product received.

Separate safety populations will be defined for each vaccination visit: vaccination visit 1, vaccination visit 2, vaccination visit 3, and follow-up phase for stage 1.

1. Vaccination 1 Safety Population: This population will include all subjects who received the first dose of investigational product (bivalent rLP2086 or HAV) at visit 1, and for whom safety information from visit 1 to prior to visit 4 is available.

2. Vaccination 2 Safety Population: This population will include all subjects who received the investigational product (bivalent rLP2086 or saline) at visit 4, and for whom safety information from visit 4 to prior to visit 6 is available.

3. Vaccination 3 Safety Population: This population will include all subjects who received the investigational product (bivalent rLP2086 or HAV) at visit 6, and for whom safety information from visit 6 up to visit 7 is available.

4. Follow-up Safety Population for Stage 1: This population will include all subjects who received at least 1 dose of investigational product (rLP2086 or HAV vaccine or saline) and for whom safety information is available from after visit 7 to up to visit 8. Subjects who receive the wrong investigational product and are followed for 6-month safety will not be included in this population.

5.4. Other Analysis Sets

All randomized subjects who have at least 1 valid and determinate assay result related to a proposed analysis will be included in the modified intent-to-treat (mITT) population. This analysis set is for the immunogenicity analysis.

5.5. Treatment Misallocations

- Randomized but not vaccinated: These subjects will be included in the ITT population and excluded from any safety analyses. They might be included in the mITT population if any assay results are available and will be reported under their randomized group for immunogenicity analyses.

- Vaccinated but not randomized: by definition they will be excluded from the immunogenicity analyses since randomized group is missing, but will be reported under the vaccine group they actually received for all safety analyses.

- Randomized but received incorrect vaccine: They will be included in the mITT population if any assay results are available and will be reported under their randomized group for immunogenicity analyses, but may be reported under the group they actually received for some safety analyses (eg, safety data at each dose can be summarized, but safety data at any dose or follow-up can't be summarized as no group can be assigned). All of the safety data will be listed for subjects who did not take the correct vaccine as randomized.

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 Randomization

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

5.6.2. Deviations Assessed Post-Randomization

The full list of protocol deviations (including protocol violations) for the study report(s) will be compiled prior to database release at the visit corresponding to the primary analysis and final analysis. The subjects with major protocol deviations, as determined by Medical Monitor, will be excluded from the evaluable immunogenicity population.

6. ENDPOINTS AND COVARIATES

6.1. Immunogenicity Endpoints

The lower limit of quantitation (LLOQ) for primary test strain CCI (A22) is 1:16. The LLOQ for each of the other 3 primary test strains is 1:8.

6.1.1. Primary Immunogenicity Endpoints

- *Proportions of subjects achieving an hSBA titer ≥ LLOQ 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers aged 12 to <18 months at study entry.*

- *Proportions of subjects achieving an hSBA titer ≥ LLOQ 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers aged 18 to <24 months at study entry.*

6.1.2. Secondary Immunogenicity Endpoints

The following endpoint will be applied to results in healthy subjects aged 12 months to <24 months (ie, both age strata combined) at study entry:

- *Proportion of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086.*

The following endpoints will be applied to results in healthy subjects aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined:

- *Proportions of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains at 1 month after the second vaccination with bivalent rLP2086 and 6, 12, 24, 36, and 48 months after the third vaccination with bivalent rLP2086.*

- *Proportions of subjects with hSBA titers ≥ LLOQ, ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary MnB strains at each applicable blood sampling visit.*

- *hSBA GMTs for each of the 4 primary test strains at each applicable blood sampling visit.*

6.1.3. CCI

CCI

CCI

6.2. Safety Endpoints

6.2.1. Primary Safety Endpoints

The following endpoints will be applied to results in healthy toddlers aged 12 to <18 months and in healthy toddlers aged 18 to <24 months at study entry, and in both age strata combined:

- *Percentages of subjects reporting local reactions (tenderness, redness, and swelling) and by severity after each vaccination visit.*

- *Percentages of subjects reporting systemic events (fever, decreased sleep, increased sleep, decreased appetite, and irritability) by severity after each vaccination visit.*

- *Percentages of subjects reporting the use of antipyretic medication after each vaccination visit.*

- *Percentages of subjects with at least 1 SAE during the following time periods:*

  1. *30 Days after each vaccination.*

  2. *30 Days after any vaccination.*

  3. *During the vaccination phase (from the first study vaccination [Visit 1] through month after the last study vaccination [Visit 7]).*

  4. *During the follow-up phase (from 1 month after the last study vaccination [Visit 7] through 6 months after the third study vaccination [Visit 8]).*

  5. *Throughout the SAE collection period (from the first study vaccination [Visit 1] through 6 months after the third study vaccination [Visit 8]).*

- *Percentages of subjects with at least 1 MAE occurring during the following time periods:*

  1. *30 Days after each vaccination.*

  2. *30 Days after any vaccination.*

  3. *During the vaccination phase (from the first study vaccination [Visit 1] through 1 month after the last study vaccination [Visit 7]).*

  4. *During the follow-up phase (from 1 month after the last study vaccination [Visit 7] through 6 months after the third study vaccination [Visit 8]).*

  5. *Throughout the AE collection period (from the first study vaccination [Visit 1] through 6 months after the third study vaccination [Visit 8]).*

- *Percentages of subjects with at least 1 NDCMC occurring during the following time periods:*

  1. *30 Days after each vaccination.*

  2. *30 Days after any vaccination.*

  3. *During the vaccination phase (from the first study vaccination [Visit 1] through 1 month after the last study vaccination [Visit 7]).*

  4. *During the follow-up phase (from 1 month after the last study vaccination [Visit 7] through 6 months after the third study vaccination [Visit 8]).*

  5. *Throughout the AE collection period (from the first study vaccination [Visit 1] through 6 months after the third study vaccination [Visit 8]).*

- *Percentages of subjects with at least 1 AE occurring during the following time periods:*

  1. *30 Days after each vaccination.*

  2. *30 Days after any vaccination.*

  3. *During the vaccination phase (from the first study vaccination [Visit 1] through 1 month after the last study vaccination [Visit 7]).*

- *Percentages of subjects reporting at least 1 immediate AE after each vaccination.*

These endpoints are detailed in below Section 6.2.2 and Section 6.2.3 based on data sources. The details for each analysis interval are described in these sections.

6.2.2. Reactogenicity Data

Reactogenicity data is solicited adverse events (AEs). The reactogenicity data collected from e-Diary will be: local reactions (redness, swelling, and tenderness at injection site), systemic events (loss of or decreased appetite, drowsiness [increased sleep], and irritability [fussiness]), temperature and use of antipyretic medication.

The e-Diary will record reactogenicity data from day 1 to day 7 for each vaccination. The analysis interval for reactogenicity data after each vaccination 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 3.   Analysis Interval for Reactogenicity Data

#	Analysis Interval	Analysis Population	Interval Start date (inclusive)	Interval Stop (inclusive)
1	vaccination 1	Vax 1 Safety	Vax 1 date	Vax 1 date + 6 days (or until resolved day)
2	vaccination 2	Vax 2 Safety	Vax 2 date	Vax 2 date + 6 days (or until resolved day)
3	vaccination 3	Vax 3 Safety	Vax 3 date	Vax 3 date + 6 days (or until resolved day)
4	any vaccination	Safety	Vax 1, Vax 2, or Vax 3	Vax 1, Vax 2, or Vax 3+ 6 days (or until resolved day)

6.2.2.1. Local Reactions Endpoints

The local reactions collected from the e-Diary will be: tenderness 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 variable will be calculated for each vaccination as well as overall reactions for any vaccination. The derivation of this variable is given in Table 4 below.

Table 4.   Derived Variables for Local Reactions

Variable	Yes (1)[a]	No (0)[b]	Missing (.)
Any day 1-7	Parent/legal guardian reports the reaction as 'mild' 'moderate' or 'severe' on any day 1-7	Parent/legal guardian reports the reaction as 'none' on all 7 days or as a combination of 'none' and missing on all 7 days.	Parent/legal guardian 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 e-Diary
b.   For redness and swelling, 'none' means 0 caliper units reported in the e-Diary

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 5 and Table 6.

Table 5.   Grading of Redness and Swelling

Grades	Measurement
None	0 cm (0 caliper units)
Mild	0.5 to 2.0 cm (1 to 4 caliper units)
Moderate	2.5 to 7.0 cm (5 to 14 caliper units)
Severe	>7.0 cm (>14 caliper units)

Table 6.   Grading of Tenderness

Grades	
Mild	Hurts if gently touched (eg, subject whimpers, winces, protests, or withdraws)
Moderate	Hurts if gently touched with crying
Severe	Causes limitation of limb movement

The maximum severity (highest grading) of each local reaction within 7 days of vaccination will be derived for each vaccination as well as any vaccinations. The maximum severity 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 of Each Local Reaction

For subjects experiencing any local reactions (or those with derived reaction presence in Table 4), the maximum duration (last day of reaction −first day of reaction +1) will be derived for each vaccination. 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 reaction:

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

2. Each local reaction on 'any day 1-7' after each vaccination and any vaccination.

3. Maximum severity of each local reaction on 'any day 1-7' after each vaccination and any vaccination.

4. Maximum duration of each local reaction after each vaccination.

5. Any local reaction on 'any day 1-7' after each vaccination and after any vaccination.

6.2.2.2. Systemic Events Endpoints

The systemic events reported via e-Diary will be: fever, loss of or decreased appetite, drowsiness [increased sleep], and irritability [fussiness]. See the table below for the severity scales of each systemic event.

Table 7.   Grading of Other Systemic Events

	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)
Loss of appetite or decreased appetite	Decreased interest in eating	Decreased oral intake	Refusal to feed
Drowsiness (increased sleep)	Increased or prolonged sleeping bouts	Slightly subdued interfering with daily activity	Disabling not interested in usual daily activity
Irritability (fussiness, restless sleep, decreased sleep)	Easily consolable	Requiring increased attention	Inconsolable, crying cannot be comforted

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 each vaccination.

2. Each systemic event on 'any day 1-7' after each vaccination and any vaccination.

3. Maximum severity of each systemic event on 'any day 1-7' after each vaccination and any vaccination.

4. Maximum duration of each systemic event after each vaccination.

5. Any systemic event (including fever) on 'any day 1-7' after each vaccination and after any vaccination.

The derivation of these variables is similar to the derivation of the variables for local reactions (Section 6.2.2.1).

6.2.2.3. Temperature

Oral temperature will be collected in the e-Diary for 7 days (day 1 to day 7) after each vaccination. The highest temperature for each day will be recorded in the e-Diary. The protocol defines fever as an axillary temperature $\geq 38.0°C$. Fever will be categorized according as $38.0°C$ to $\leq 38.4°C$, $38.5°C$ to $\leq 38.9°C$, $39.0°C$ to $39.4°C$, $39.5°C$ to $\leq 40°C$, 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 each vaccination.

2. Fever on 'any day 1-7' after each vaccination and any vaccination.

3. Highest Fever (maximum severity) on 'any day 1-7' after each vaccination and any vaccination.

4. Maximum duration of fever after each vaccination.

Temperatures $<35.0°C$ and $>42.0°C$ will be excluded from the analysis.

6.2.2.4. 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 each vaccination

2. Use of antipyretic medication on 'any day 1-7' after each vaccination and any vaccination

3. Maximum duration of Use of antipyretic medication after each vaccination

4. Onset day of antipyretic use relative to each vaccination

6.2.3. Safety Data Collected Through CRF

The relationship between (S)AEs and the investigational products (bivalent rLP2086 vaccine, saline, and HAV) 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 with an onset prior to first vaccination will be excluded from AE analysis. These will be included in medical history analysis.

Adverse events will be categorized according to MedDRA.

6.2.3.1. Analysis Intervals

There will be up to 7 analysis intervals for most of the safety data collected via CRF.

Table 8.   Analysis Interval for AE, SAE, Medically Attended Events (MAEs), Newly Diagnosed Major Conditions (NDCMC)

#	Analysis Interval	Analysis Population	Interval Start date (inclusive)	Interval Stop (inclusive)	Safety Data
1	Within 30 days of vaccination 1	Vax 1 Safety	Vax 1 date	Vax 1 date + 30 days	AE, SAE, MAE, NDCMC
2	Within 30 days of vaccination 2	Vax 2 Safety	Vax 2 date	Vax 2 date + 30 days	AE, SAE, MAE, NDCMC,
3	Within 30 days of vaccination 3	Vax 3 Safety	Vax 3 date	Vax 3 date + 30 days	AE, SAE, MAE , NDCMC
4	Within 30 days of any vaccination	Safety	Vax 1, Vax 2, or Vax 3 date	Vax 1, Vax 2, or Vax 3 date + 30 days	AE, SAE, MAE, NDCMC,
5	During the vaccination phase	Safety	Visit 1 date	Visit 7 date (or end of vaccination day)	AE, SAE, MAE, NDCMC
6	During the follow-up Phase (Stage 1)	Follow-up Stage 1	Visit 7 date + 1 day, or end of vaccination date + 1 for early withdrawal subjects	Visit 8 date	SAE, MAE, NDCMC,
7	Throughout the AE collection period (Stage 1)	Safety	Visit 1 date	Visit 8 date	SAE, MAE, NDCMC, autoimmune and neuroinflammatory conditions

AE=Adverse events, SAE=serious adverse events, MAE=Medically-attended AE, NDCMC=Newly diagnosed chronic medical conditions

Four (4) analysis intervals will be applied to immediate AEs.

Table 9.   Analysis Interval for Immediate AE

#	Analysis Interval	Analysis Population	Interval Start date (inclusive)	Interval Stop (inclusive)
1	vaccination 1	Vax 1 Safety	Vaccination 1 time	Vaccination 1 time + 30 minutes
2	vaccination 2	Vax 2 Safety	Vaccination 2 time	Vaccination 2 time + 30 minutes
3	vaccination 3	Vax 3 Safety	Vaccination 3 time	Vaccination 3 time + 30 minutes
4	any vaccination	Safety	Vaccination 1, 2, or 3 time	Vaccination 1, 2, or 3 time + 30 minutes

6.2.3.2. 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.

6.3. Other Endpoints

6.3.1. e-Diary Completion

For any given day, an e-diary will be transmitted and considered as complete if all expected data (the 3 local reactions, the 4 systemic reactions (including fever), and the use of antipyretics) are available. If any of the items in the e-Diary are 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 data for the day is complete within the e-diary, the data are transmitted to a centralized database.

The following e-Diary compliance variables will be provided for each vaccination:

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 (in months). Age at time of first vaccination and age at randomization will be derived based on month birthday. For example, if the first vaccination date is one day before the subject's 13th month birthday, the subject is 12 months 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.

6.3.3. Nonstudy Vaccination

Any *Haemophilus influenzae* type b conjugate vaccine (polyribosylribitol phosphate outer membrane protein: PRP-OMP) received prior to the signing of the ICD will be recorded in the case report form (CRF).

Any nonstudy vaccine given from the signing of the ICD to study completion will be recorded in the CRF.

Nonstudy vaccines will be categorized according to the WHO Drug Dictionary.

6.4. Covariates

The control group and each dose-level group from different cohorts within the same age stratum will be pooled for analysis. All of the immunogenicity analyses will be summarized for each age stratum separately, as well as for the combined group. Similar to the immunogenicity analysis, the control group and each dose-level group from different cohorts within the same age stratum will be pooled for analysis. All of the safety analyses will be summarized for each age stratum separately as well as for the overall populations. Note that for both the immunogenicity and the safety summaries, the summaries combining age cohorts will only be provided if the same dose-level is chosen for the expanded enrolment for each cohort. For example, if the low dose is chosen in the 12-18 months old age cohort and the high dose is chosen for the 18-24 months old age cohort, then the safety and immunogenicity results for the 12-24 months old group combined will not be presented. If there are no events for a specific safety analysis for the combined age strata, summaries by each age strata may not be performed.

The following endpoints will further be summarized by race (White, Black, Asian and Other), by gender (Male, Female), and by country.

- Proportion of subjects with hSBA titer $\geq$ LLOQ for each of the 4 primary MnB test strains at each time point

- GMTs for each of the 4 primary MnB test strains at each time point

- Primary safety endpoints related to reactogenicity, AE, SAE, and MAE

Composite responses and 4-fold responses may additionally be summarized by age, race, gender, and country. Summaries by a sub-group (e.g. race, gender, country) may be performed in cases where 5% or more of subjects in two or more of the sub-groups have identical values. Sub-group analyses for safety analyses will not be done for rare events (where the percentage of subjects with events is less than 1% in all the vaccine groups for the summary).

7. HANDLING OF MISSING VALUES

7.1. Immunogenicity Data

As assay data are expected to be missing completely at random (MCAR), the primary analysis for the primary objectives will be based upon the observed, determinate observations. If all subjects have hSBA tested for all of the 4 primary MnB test strains, descriptive summaries will be provided to describe the reason the hSBA data are missing and the relationship between the missing data indicator and other design variables or covariates (age, race, sex, center, etc.) and the observed hSBA data. Additionally, a sensitivity analysis using a mixed-effects model with repeated measurement (MMRM) will be applied to the primary endpoints. The MMRM uses the maximum likelihood estimation, and it is under the assumption that the data are missing at random (MAR). If only 50% of the subjects will have 2 strains tested and the remaining 50% have the other 2 strains tested, no sensitivity analyses will be planned because the missing assumption is MCAR.

7.1.1. Missing Data Investigation

For the hSBA assay results, the following values will be set to missing: QNS (insufficient sera), indeterminate results, and Not Done. Subjects without blood draw (ie, dropout) will also have missing data for immunogenicity.

The proportion of subjects with missing immunogenicity data will be summarized at each blood sampling visit for each strain. The denominator will be the ITT population (all randomized). The category of missing reasons (QNS, indeterminate, Not Done, dropout) will also be summarized.

7.1.2. Missing Data Analysis

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 & Hsu, 2011)).[1]

Descriptive summaries will be provided to describe the relationship between the missing data indicator and other design variables, covariates, (age, race, gender, etc.) and observed hSBA data[2] (Refer to Section 8.2.4.1 for more details).

The Panel on Handling Missing Data in Clinical Trials suggested that sensitivity analysis should relate inferences to one or more parameters that capture departures from the primary missing data assumption. Since the primary analysis is based on MCAR, the sensitivity

analysis will be based on Missing At Random (MAR). Thus, a sensitivity analysis using mixed effects model with repeated measurement (MMRM) will be applied to the hSBA related endpoints (GMT and proportion of subjects with titers greater or equal to LLOQs for each of the primary strains). The MMRM uses maximum likelihood estimation and it is under the assumption that the missingness is at random (MAR). Refer to Section 8.2.4.2 and Section 8.2.4.3 for details.

7.1.3. Immunogenicity Data Below LLOQ

The LLOQ are listed in below table:

Table 10.   LLOQ for hSBA

Strain	LLOQ
CCI (A22)	1:16
CCI (B24)	1:8
CCI (A56)	1:8
CCI (B44).	1:8

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

7.2. Safety Data

7.2.1. Reactogenicity Data

For derived variables based on reactogenicity data, if any day of the 7-day e-Diary is available, the 'any day 1-7' data will be considered as non-missing. Subjects are excluded from the analysis if they don't receive the particular dose or the safety data is missing on all days within the interval.

The reactogenicity data are collected through e-Diary, which does not allow subjects to skip the question. Therefore, for a specific day, as long as the e-Diary data is transferred for that day, all of the reactogenicity data for the subject on that day is non-missing. The e-Diary transmission and completion status will be summarized per Section 6.3.1. The e-Diary completion summary will provide the missing data information on the reactogenicity data.

Based on data from available studies, the missing data on reactogenicity is minimal, which is consistent with Li, et al. (2011).[3] No sensitivity analysis is planned for reactogenicity data.

7.2.2. Safety Data Collected Through CRF

Subjects are excluded from the analysis if they do not receive the vaccine dose or if safety data are missing for a specified interval.

The sponsor is making every effort to follow up with subjects for safety data (6-month after the last vaccination).

The missing data rate on the safety data in each analysis interval will be summarized for each group. The numerator will be the # of subjects with missing CRF safety data information. The denominators vary with the analysis interval.

#	Analysis Interval	Denominator
1	Within 30 days of vaccination 1	# of subjects received vax 1
2	Within 30 days of vaccination 2	# of subjects received vax 2
3	Within 30 days of vaccination 3	# of subjects received vax 3
4	Within 30 days of any vaccination	# of subjects received any vax
5	During the vaccination phase	# of subjects received any vax
6	During the follow-up Phase for Stage 1	# of subjects received any vax
7	During AE collection period (Visit 1 to Visit 8).	# of subjects received any vax

Further details about the data handling approach are available in the appendix.

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

8.1.1.1. GMTs

For each primary strain, hSBA titers measured at each blood sampling time point (Visit 1, Visit 5, Visit 7, Visit 8, Visit 9, Visit 11, Visit 12, and Visit 13) will be logarithmically transformed for analysis and geometric mean hSBA titers will be computed for each tested primary strain for each group at each blood sampling time point along with 2-sided 95% 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.

8.1.2. Analyses for Binary Endpoints

8.1.2.1. Immunogenicity Data

The number and proportions of subjects achieving hSBA titers $\geq$LLOQ, $\geq$1:4, $\geq$1:8, $\geq$1:16, $\geq$1:32, $\geq$1:64, and $\geq$1:128 at each blood sampling time point, and 4-fold rise on hSBA from baseline to 1 month post-vaccination 2 and 1 month post-vaccination 3, will be descriptively summarized along with the exact 2-sided 95% CI (or Clopper-Pearson confidence limit) for the primary MnB test strains CCI (A22) , CCI (B24), CCI (A56), and CCI (B44).

The exact confidence interval for a 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,

$$ \text{use: } p_L = \frac{rF_L}{(rF_L + (n - r + 1))} \quad \text{and for the upper limit } p_U, \text{ use } \quad p_U = \frac{(r+1)F_U}{(n-r) + (r+1)F_U} $$

where F_L is the quantile from the F distribution for $\alpha=0.025$, 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 $\alpha=0.975$, 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 (1991).[4]

8.1.2.2. Safety Data

All of the safety endpoints (including reactogenicity data and (S)AE) will be summarized with percentages and 95% exact CI for each group.

The safety data will be compared between rLP2086 (by dose) and control group as below:

- The percentages of subjects reporting immediate AEs, reporting reactogenicity events (including use of antipyretic medication) will be compared with unconditional exact test (Chan & Zhang, 1999)[5] for the pair-wise comparisons. The 95% exact CIs on the difference will be also provided. The exact CIs will be computed using the noninferiority procedure, using the standardized test statistic and gamma=0.000001.

- Fisher's exact test will be used to compare the percentage of subjects reporting (S)AE, MAEs, and NDCMCs. The difference in the percentages between rLP2086 (by dose) and control group and 2 sided 95% CIs for the difference will be provided. The CIs of the difference will be computed using the Miettinen-Nurminen[6] method.

The confidence intervals and statistical tests presented for the safety data will not be used to test hypotheses but will be used to determine which events may need further clinical investigation. No adjustment for multiplicity is needed.

8.2. Statistical Analyses

8.2.1. Analysis of Primary Endpoints

The primary analysis for the primary objectives is the proportion of subjects with hSBA titer ≥ LLOQ 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers aged 12 to <18 months, and 18 to <24 months, at study entry respectively. The evaluable immunogenicity population will be used for this summary and both percentages and CIs will be displayed.

The control group and each dose-level group from different cohorts within the same age stratum will be pooled for analysis. All of the immunogenicity analyses will be summarized for each age stratum separately, as well as for the combined group. Note that for both the immunogenicity and the safety summaries, the summaries combining age cohorts will only be provided if the same dose-level is chosen for the expanded enrolment for each cohort. For example, if the low dose is chosen in the 12-18 months old age cohort and the high dose is chosen for the 18-24 months old age cohort, then the safety and immunogenicity results for the 12-24 months old group combined will not be presented. For any non-selected dose level from the sentinel cohort where the vaccination series is not completed, no immunogenicity assay testing will be performed on those subjects.

8.2.2. Analysis of Secondary Endpoints

All of the analyses mentioned in Section 8.2.1 to be performed on the mITT population are considered as secondary analyses.

Secondary analyses also will include percent of subjects with hSBA titers≥ LLOQ for each of the primary 4 MnB test strains 1 month following the third vaccination for both the evaluable immunogenicity population and for the mITT population.

The percentage of subjects with hSBA titers ≥ LLOQ for each of the primary 4 MnB test strains 1 month following the second vaccination , and at 6, 12, 24, 36, and 48 months after the third vaccination will be analyzed using both the evaluable immunogenicity population and the mITT population.

The percentage of subjects with hSBA titers≥ LLOQ, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 for each of the 4 primary MnB test strains at each applicable blood sampling visit will be analyzed using both the evaluable immunogenicity population and the mITT population.

The GMTs for each of the primary MnB test strains at each applicable blood sampling visit will be summarized for the evaluable immunogenicity population and the mITT population.

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

8.2.3. CCI

CCI

CCI

8.2.4. Sensitivity Analyses

8.2.4.1. Assessing Missing hSBA with Other Variables

All of the subjects, for the combined age strata, will be dichotomized to 2 categories – with missing hSBA or non-missing hSBA. If a subject has missing data at any blood sampling visit up until Month 7 for any of the 2 selected strains, the subject will be categorized as 'Missing (1)'; if the subjects have hSBA for all blood sampling visit up until Month 7 for the 2 selected primary strains, the subjects will be categorized as 'Non-missing (0)'. Then the summary statistics will be provided for the following variables by the missing indicator:

Age (mean, std), Race, Gender, Center, Vaccine Group, GMT on each strain for each group at each visit.

Additional summaries may be provided based on each strain, where the Missing (1) will be assigned to subject with missing data at any blood sampling visit up until Month 7 for that strain and the non-Missing (0) will be assigned to subject with all time points up until Month 7 for that strain. The same variables will be summarized for each indicator.

8.2.4.2. Assessing Factors of Race, Age and Gender on hSBA GMT

A mixed-effect model with repeated measurement[7] will be utilized to assess the effect of race, center and gender, in which both baseline and the post-vaccination titers up until Month 7 (in logarithmic scale) are modeled as dependent variables for each primary 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 (ie, First-order autoregressive, compound symmetry). If only 50% of the subjects will have 2 strains tested and the remaining 50% have the other 2 strains tested, no sensitivity analyses will be planned because the missing assumption is MCAR.

Log (hSBA) = Group + race + gender + age at randomization +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 subjects in the combined age strata in the mITT population, using ½ LLOQ to impute the hSBA values below LLOQ, for the primary strains only.

If only 50% of the subjects will have 2 strains tested and the remaining 50% have the other 2 strains tested, no sensitivity analyses will be planned because the missing assumption is MCAR.

8.2.4.3. hSBA Titer Greater Than or Equal to LLOQ Response Endpoints

Similar to GMT, a generalized linear model with mixed-effect on repeated measurement will be utilized (GLIMMIX). The response variables are binary variables and a Logit link will be used. The model will be similar to above.

The model-estimated response rates at post-dose 2 and post-dose 3 and the 95% CI will be summarized for each of the 2 strains for each group.

If only 50% of the subjects will have 2 strains tested and the remaining 50% have the other 2 strains tested, no sensitivity analyses will be planned because the missing assumption is MCAR.

8.2.5. Analysis of Safety Data

Similar to the immunogenicity analysis, the control group and each dose-level group from different cohorts within the same age stratum will be pooled for analysis. All of the safety analyses will be summarized for each age stratum separately as well as for the overall populations. The summaries combining age cohorts will only be provided if the same dose-level is chosen for the expanded enrolment for each cohort. If there are no events for a specific safety analysis for the combined age strata, summaries by each age strata may not be performed.

8.2.5.1. 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, for each vaccination. The presence of each local reaction, each systemic event, fever, use of antipyretic medication, any local reaction, any systemic events will also be summarized for 'any day 1-7' after any vaccination similarly. 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, by each vaccination as well as 'after any vaccination'. 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 for each vaccination. The number of unknown durations will also be summarized. The onset day for each local reaction and systemic event will also be summarized.

All of these summaries will be summarized according to the vaccine received. The safety population at each corresponding dose will be used for the analyses for each vaccination.

The data summaries will be descriptively presented by group.

Proportion differences (between rLP2086 dose groups and HAV/saline control group), associated 95% CIs and p-values will only be provided, by each vaccination and after any vaccination for the following endpoints:

- any local reactions (overall and by type of event) and any severe local reactions (only by type of event)

- any systemic events (overall and by type of event) and severe systemic events (only by type of event)

- any use of anti-pyretics

- any fever (≥38.0°C) and also any temperature above or equal to 39.0-39.9, and 40 degrees centigrade.

P-values from the comparison and 95% exact CIs on the difference will be computed using the noninferiority procedure, using the standardized test statistic and gamma=0.000001.

Also, the increasing severity and potentiation of local and systemic reactions across all 3 vaccinations will be summarized with number and proportion of subjects with such characteristics, along with 95% exact CI.

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

8.2.5.1.1. Unscheduled Visits (Unplanned Visits)

A listing will be generated for all of the subjects with unscheduled visits (or unplanned visits) for severe reactions.

8.2.5.2. Safety Data Collected Through CRF

8.2.5.2.1. SAE

The percentage of subjects reporting at least one SAE, and the number of episodes will be summarized for each group for each of the first 7 analysis intervals (Section 6.2.3.1) with 95% exact confidence intervals for the percentages. Proportion differences (between rLP2086 dose groups and HAV/saline control group), associated 95% CIs and p-values from Fisher's exact test will be provided for analysis intervals 'During the vaccination phase' and 'Throughout the AE collection period (Visit 1 to Visit 8)'.

Similarly, the percentage of subjects reporting at least one related SAE, total # of related SAEs reported by each group. No between-group proportion differences, associated CIs or p-values for comparisons between groups will be generated for related SAEs.

In addition, for analysis interval throughout the AE collection period (Visit 1 to Visit 8), the SAEs will be summarized by each preferred term (MedDRA) with percentage and number of events for each group. Listings of related SAEs will be provided.

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

8.2.5.2.2. Newly Diagnosed Chronic Medical Condition (NDCMC)

The percentage of subjects reporting at least one newly diagnosed chronic medical conditions, and the number of episodes will be summarized, by severity and overall, for each group for each of the first 7 analysis intervals with 95% Clopper-Pearson CI for the percentages. Proportion differences (between rLP2086 dose groups and HAV/saline control group), associated 95% CIs and p-values from Fisher's exact test will be provided for analysis intervals 'During the vaccination phase' and 'Throughout the AE collection period (Visit 1 to Visit 8)' for any severity.

Similarly, the percentage of subjects reporting at least one related newly diagnosed chronic medical condition, and total # of related newly diagnosed chronic medical conditions will be reported by each group. No between-group proportion differences, associated CIs or p-values will be generated for related newly diagnosed chronic medical conditions.

In addition, for the analysis interval throughout the AE collection period (Visit 1 to Visit 8), the NDCMCs will be summarized by each preferred term (MedDRA) with percentage and number of events for each group.

8.2.5.2.3. 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 database lock. These events can be an SAE or AE.

A listing of neuroinflammatory and autoimmune conditions matching this preferred terms list will be provided.

8.2.5.2.4. Medically Attended Events (MAE)

The Medically attended AE will be analyzed as per Section 8.2.5.2.1 in addition there will be:

- An analysis to include the summary of medically attended AE by severity for each analysis interval.

- An analysis to include 'moderate and severe' medically attended AE summary by preferred term for the analysis intervals 5 to 7.

- Proportion differences (between rLP2086 dose groups and HAV/saline control group) , associated 95% CIs and p-values from Fisher's exact test will be provided for analysis intervals 'During the vaccination phase' and 'Throughout the AE collection period (Visit 1 to Visit 8)' for any severe conditions and any severity.

8.2.5.2.5. Immediate AE

The number and percentage of subjects reporting adverse events during the protocol specified first 30-minute observation period will be summarized by randomization group for each vaccination.

This immediate AE report will be summarized for each vaccination and any vaccination. These summaries will include 95% Clopper-Pearson's CI. Unconditional exact 95% CI on the proportion differences and p-value will also be provided for the analysis interval 'Within 30 days of any vaccination'.

The immediate AE categorized as SAE (defined as immediate AE and also meet the definition of SAE) will be listed.

8.2.5.2.6. AE

The AEs will be analyzed similar to SAE, except that only the first 5 intervals are applicable as non-serious AEs (except MAEs and non-serious NDCMCs) are only collected during the vaccination phase.

Non-serious AEs are not required to be reported during the follow-up phase, however, if non-serious AEs during the follow-up phase were recorded in the database (excluding MAEs and non-serious NDCMCs), these data will be listed separately.

Note that for the analysis interval of vaccination phase the AEs will be summarized by each preferred term (MedDRA) with percentage and number of events for each group. Percentages and 95% CIs on the proportion differences will also be provided when at least 4 subjects have an adverse event for a specific preferred term in any of the displayed vaccine groups.

In addition, related AEs will also be summarized by each preferred term with percentage and number of events for each group.

8.2.5.2.7. 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 vaccination phase.

2. Any SAE throughout the AE collection period (Visit 1 to Visit 8),

3. Any medically attended AE throughout the AE collection period (Visit 1 to Visit 8).

4. Any newly diagnosed chronic medical conditions throughout the AE collection period (Visit 1 to Visit 8).

5. Any newly diagnosed chronic medical conditions categorized as throughout the AE collection period (Visit 1 to Visit 8).

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 group.

8.2.5.3. AEs and any RRIs Occurring Within 48 Hours After Blood Draw

From Visit 8 to Visit 13 AEs and research-related injuries (RRI) occurring within 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. AEs and RRIs occurring within 48 hours of a blood draw will be listed in the CSR. Events with an onset equal to the date of Visit 8 will be reviewed by the study lead clinician; unless otherwise documented before the database release, any events with an onset equal to the date of Visit 8 will be included on the appropriate listing(s) of AEs and RRIs.

8.2.5.4. Death

Any death data will be listed.

8.2.6. Analyses of Study Conduct

8.2.6.1. Subject Disposition, Vaccination Administration, Blood Samples

The number and percentage of subjects who are randomized, take each dose of investigational product, withdrew during the vaccination phase, completed the vaccination phase, completed and withdrew in the follow-up phase in stage 1, entered stage 2 and completed stage 2, and are major protocol violators and are major protocol violators (excluded from evaluable immunogenicity population at 1 month following vaccination 3), will be summarized. The reasons for withdrawal will also be tabulated.

In addition, the relationship of randomized vaccine group to actual vaccine received will be presented as a cross tabulation of the actual sequence received versus the randomized vaccine administration regimen.

For each blood draw, the number and percentage of subjects randomized, vaccinated (visits 1, 4, 6), and providing blood samples within the protocol-specified time frame, as well as before and after the specified time frame, will be tabulated.

Similarly, for each vaccination visit, the number and percentage of subjects who are enrolled, vaccinated (visits 1, 4, 6), and vaccinated within the protocol-specified time frame, before and after the specified time frame will be tabulated.

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 each randomized group and total population.

A listing of subjects' non-compliant vaccine administration will be provided. The protocol violators will also be listed. Subjects receive the vaccine not as randomized will be listed as well. 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.

8.2.6.2. 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.

8.2.6.3. 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.

8.2.6.4. Nonstudy Vaccination

Nonstudy vaccination summaries will be provided for the following analysis intervals: prior to the vaccination and during the vaccination phase. The denominator for the percentages will be the safety population.

8.2.7. Subgroup Analysis

Some immunogenicity and safety endpoints will be descriptively summarized by race, by gender, and by country, and within age strata as described in Section 6.4.

All of the safety and immunogenicity analyses will be summarized for each age stratum separately as well as for the overall populations. Note that for both the immunogenicity and the safety summaries, the summaries combining age cohorts will only be provided if the same dose-level is chosen for the expanded enrolment for each cohort. For example, if the low dose is chosen in the 12-18 months old age cohort and the high dose is chosen for the 18-24 months old age cohort, then the safety and immunogenicity results for the 12-24 months old group combined will not be presented. If there are no events for a specific safety analysis for the combined age strata, summaries by each age strata may not be performed.

For the safety endpoints (AE, SAE collected, medically attended AE and newly diagnosed chronic medical conditions) collected, a separate summary table including analysis interval of 'Vaccination period', 'Follow-up Phase (Stage 1)' (excluding AEs) and 'Throughout the AE collection period' (excluding AEs), will be provided for the subjects who completed the full series (3 doses) and those who only completed partial series (1 or 2 doses). Similar subgroup analysis will also be provided for those safety endpoints with relatedness of vaccination phase.

All of the 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.

9. REFERENCES

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10. APPENDICES

10.1. Data Handling

10.1.1. AE Start Date

10.1.1.1. Data Cleaning Process

Data entered through remote data capture (RDC) is under review at an ongoing basis. The data manager and clinical team send queries to the site to resolve inconsistency or missing data issues. The start date may be recorded as complete YYYY-MM, YYYY only, or completely missing.

10.1.1.2. Endpoints Impacted

The following variables will be impacted because the endpoints require AE start date in order to allocate each AE to a corresponding analysis interval:

- Proportion of subjects who develop at least one adverse event within 30 days after each vaccination visit.

- Proportion of subjects who develop at least one adverse event within 30 days after each vaccination visit after any vaccination visit.

    Proportion of subjects who develop at least one adverse event during the vaccination phase.

Table 11. Analysis Interval

#	Analysis Interval	Interval Start date (inclusive)	Interval Stop (inclusive)
1	Within 30 days of vaccination 1	Vax 1 date	Vax 1 date +30 days
2	Within 30 days of vaccination 2	Vax 2 date	Vax 2 date +30 days
3	Within 30 days of vaccination 3	Vax 3 date	Vax 3 date + 30 days
4	Within 30 days of any vaccination	Each of above 3	Each of above 3
5	During the vaccination phase	Vax 1 date	1-month post-vaccination 3 blood sampling date (or the day the subject withdraw from the study for early withdrawal subjects)

It will also impact the medically attended AE, SAE, or newly diagnosed medical conditions, in which situations, similar data handling rule will be used.

10.1.1.3. Details on Data Handling

The general approach for data handling of the missing AE start date is to be conservative with regards to treatment emergence (or assume the worst for the sponsor).

10.1.1.3.1. Completely Missing

The AE start date will be imputed as the date of the first dose of study vaccination as long as the AE stop date is not prior to first vaccination date.

10.1.1.3.2. Partial Missing

Partial missing date with only month and year available:

1. If AE partial date indicates AE start date is prior to first dosing date, no imputation is needed since it can be slotted as 'prior to vaccination phase'.

The protocols require to record all of the AE occurred after signing the informed consent document until the end of vaccination phase. For below example, this AE occurred prior to first vaccination based on year and month and can be slotted as an AE occurred prior to first injection with month and year. The date will not be imputed.

AE	AE Start Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Depression	2011-12	2012-01-05	2012-02-24	2012-06-14

If AE partial date indicates AE start date is after the vaccination phase, no imputation is needed either.

AE	AE Start Date	Date of Dose 1	Date of Dose 2	End of Vaccination Phase
Depression	2012-04	2012-01-05	2012-02-24	2012-03-14

2. If AE partial date could not determine if AE start date is prior to first dosing date, impute day to the first day of the month first and compare the imputed date with the first vaccination date and AE stop date.

   2.1   If the imputed date is <u>prior to</u> the date of first dosing date and stop date is after first dosing date, **impute the date to the date of first dosing date**

AE	AE Start Date	AE Stop Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Common cold	2012-01	2012-02	2012-01-05	2012-03-01	2012-06-13

The partial date in above example will be imputed as '2012-01-05', the date of the first vaccination date.

2.2 If the imputed date is after the first dosing date; **check the subsequent dosing date and AE stop date**:

A. If AE stop date is prior to the subsequent dosing date: stop here and use the first day of the month for imputation

AE	AE Start Date	AE Stop Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Common cold	2011-12	2011-12-03	2011-10-10	2011-12-05	2012-04-12

The partial date in above example will be imputed as '2011-12-01', the first day of the month.

B. If AE stop date is after the subsequent dosing date: Compare the month of subsequent dose and the month of the AE

B1. If the AE start month is the same as the subsequent dose month: use the **subsequent dose date to impute the AE start day**

AE	AE Start Date	AE Stop Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Common cold	2012-01	2012-02-17	2011-11-22	2012-01-17	2012-05-09

The partial date in above example will be imputed as '2012-01-17', the day of the second vaccination date.

B2. If the AE start month is not the same as the subsequent dose month: Stop here and keep the original **1st day of the month as imputed date**.

AE	AE Start Date	AE Stop Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Common cold	2012-03	2012-04	2011-10-10	2011-12-05	2012-04-12

The partial date in above example will be imputed as '2012-03-01', the first day of the month.

Partial missing date with only year available:

The imputation approach will be the same as the missing day.

1. If AE partial date indicates AE start date is prior to first dosing date, **no imputation** is needed since it can be slotted as 'prior to vaccination phase'.

AE	AE Start Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Depression	2011	2012-01-05	2012-02-24	2012-06-14

2. If AE partial date could not determine if AE start date is prior to first dosing date, impute day to January 1st of the year and compare the imputed AE start date with the earliest dosing date in the same calendar year and AE stop date.2.1.   Compare the stop date with the earliest dosing date in that calendar year, if the stop date is earlier than the earliest dosing date in the same calendar year, the AE start date will remain the **first day of the calendar year**

AE	AE Start Date	AE Stop Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Common cold	2012	2012-01-12	2011-11-23	2012-02-08	2012-06-13

The partial date in above example will be imputed as '2012-01-01', the first day of the year.

    2.2. Compare the stop date with the earliest dosing date in that calendar year, if the stop date is later than the earliest dosing date in the same calendar year, the AE start date will be imputed as the **earliest dosing date in that calendar year**

AE	AE Start Date	AE Stop Date	Date of Dose 1	Date of Dose 2	Date of Dose 3
Common cold	2012	2012-06-12	2011-11-23	2012-02-08	2012-06-13

The partial date in above example will be imputed as '2012-02-08', the earliest dosing date in calendar year 2012.

10.1.1.4. Summary

With above imputation method, the most conservative approach was used. Therefore, if no apparent indication from the stop date and the part start date, the AE can be slotted to any of the primary endpoint analysis interval (within 30 days of vaccination). In addition, these events will be slotted to the vaccination phase, given the event is either imputes to the date of a vaccination day of the 1st day of the month (or year).

To be specific, 2.2.B1 (missing day) and case 2.2 (missing month and day) in Section 10.1.1.3.2 will be allocated to **AE within 30 days after dose 2**.

10.1.1.5. Other Consideration

With imputed AE start date, the other variables might be impacted would be the AE duration. For MnB studies, the only duration to be summarized is the Reactogenicity data collected through e-Diary (Section 10.1.4). The duration of regular AE will not be summarized. However, each AE will be provided in the listing with a duration. If any AE with a missing or partial start date, the listing will not display the AE start day relative to the vaccination, nor the duration as the date is missing and the 2 variables cannot be derived with a missing date (ie, the raw data on the dates will be displayed).

10.1.2. AE Start Time

10.1.2.1. General Notes on Data Entry and Cleaning Process

The start time is to be recorded if the AE started on the same date of the vaccination. In addition, subjects are required to be observed for 30 minutes post-vaccination.

If any AE occurred on the vaccination day has a missing start time, a query will be sent to the site for confirmation of the time. If the site has confirmed that no additional data is available the start time will be recorded as unknown.

10.1.2.2. General Rule for Data Handling

In general, the AE start time will not be imputed.

10.1.2.2.1. AE Start Time Earlier Than Vaccination Time

If the AE start time is earlier than the first vaccination time, that AE will not be considered as an AE occurred during the vaccination phase, nor included as an AE within 30 days after the first vaccination.

If the AE start time is earlier than the second (or third) vaccination time, that AE will be included as an AE occurred during the vaccination phase. However, it will not be considered as an AE within 30 days after the second (or third) vaccination.

If AE start time is earlier than the vaccination time, the AE will not be considered as immediate because the immediate AE is defined as an AE occurred within 30 minutes **after** the vaccination.

10.1.2.2.2. AE Start Time Missing Due to Imputation of Partial AE Start Date

As mentioned in Section 10.1.1.4, the AEs will be included in various AE analysis interval. The AEs imputed on the day of vaccination with the missing time will not be allocated to an interval that is prior to the vaccination.

However, these AEs will not be included as immediate AE because actual start time in the CRF would either include a specific time or 'unknown', if it is truly occurred on the same day of the vaccination based on protocol requirement. The imputation date in Section 10.1.1 allows conservative calculation of AE in different analysis interval.

10.1.2.2.3. Unknown AE Start Time

Any AE with unknown AE start time will not be included in the immediate AE counting. As mentioned above, the site is required to observe the subjects for 30 minutes and record start time of any AE during the observation time. For any AE occurred on the same day of the vaccination day with an unknown start time, queries will be sent to site asking if the AE occurred within the observation period. If site confirmed the AE started during the observation period, the vaccination time will be entered so that this AE can be included as immediate AE. Otherwise, an estimated time is entered in the database.

Therefore, for any AEs with unknown time in the clean database, they are not included as immediate AEs. However, these AEs will be counted as within 30 days after that vaccination.

10.1.3. AE Events Collapsing

The AE log page is designed to collect one adverse event per record, therefore, no multiple AEs should be collapsed to one AE.

10.1.4. e-Diary Symptom Resolved Date

10.1.4.1. General Notes on Data Entry and Cleaning Process

According to protocol, investigators will be required to review the e-diary data online at frequent intervals to evaluate subject compliance and as part of the ongoing safety review. In addition, the investigator or designee must contact the parent/legally authorized representative in order to obtain stop dates for any reactions ongoing on the last day that the e-diary was completed.

The data manager and sponsor review the symptom resolved data at an ongoing basis to determine if any query is needed. Site is queried if the symptom resolve date is missing.

10.1.4.2. General Rule for Data Handling

10.1.4.2.1. Missing / Partial Symptom Resolved Date

If the symptom resolved date is completely missing or partially missing after the query, the dates will not be imputed. Therefore, in the summary table of 'Duration of Local Reaction' or 'Duration of Systemic Events', these events will be summarized as 'Unknown' duration.

In addition, the proportion of subjects with unknown duration among the subjects who reported such event will be summarized.

10.1.4.2.2. Calculation of Duration with Nonmissing Symptom Resolved Date

The symptom resolved date page can be filled in a scheduled visit which can have the symptom resolved page filled at unscheduled visit. In the programming handling, all of the CRF pages will be pooled together regardless the page origin. If the symptom resolved page indicated the symptom is continuing, the duration will be counted as 'Unknown'.

First, the program logic will find all of the e-Diary events with the symptom present on the last day of the e-diary entry. It may not necessarily be day 7. For example, if subject's e-diary is only transmitted until day 6, as long as the event is present on day 6, that event is picked for further consideration on the duration.

Second, the symptom resolved date from both data sources (planned page and unscheduled page) will be slotted to different interval based on the relative day to each vaccination as illustrated below:
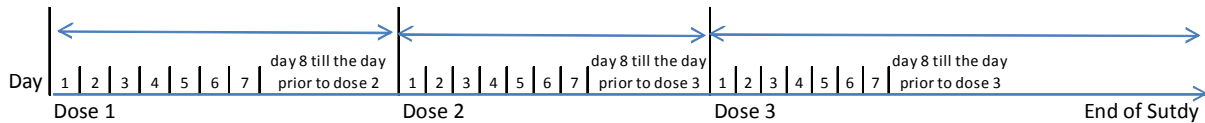
Third, if the event occurred on the last day of the e-Diary entry after dose 1 has a resolved date in the first period (after dose 1 but prior to dose 2), the ending date for that event will be used to calculate the symptom duration. If the event occurred after the specific dose with a symptom resolved date not in the same period (ie, e-diary is after dose 1 but symptom resolved date is after dose 2 or after dose 3), the duration for that event will be assigned as 'Unknown'. At the same time, a query is sent to the site for confirmation if the duration indeed lasted more than one period.

If the site confirmed that the event did span to more than one period, the duration will be calculated as segment. For example, if the first day of the e-diary is day 2 of dose 1 and the ending date is day 40 after dose 3, and each day of the e-diary entry is presented with the symptom on dose 2 and dose 3, the duration for that subject at each dose period will be calculated as:

After dose 1: (Dose 2 date -1) – (Dose 1 date) +1

After dose 2: (Dose 3 date -1) – (Dose 2 date) +1

After dose 3: (Symptom resolved date) – (Dose 3 date) +1

In addition, the subjects with duration spanned to multiple dosing period will be footnoted with overall duration longitudinally.

10.1.5. Additional Data Handling

10.1.5.1. Nonstudy Vaccine Date

This will impact the summary table of 'Nonstudy Vaccine'. For all MnB studies, all previous use of PRP-OMP , and last dose of MCV4, Tdap, or HPV prior to study start will be entered in the nonstudy vaccine page labeled as 'Prior xxx Use'. The concomitant vaccine administered after study start is required to be entered in the CRF page labeled as 'Concomitant Vaccine' (or label with similar meaning). All of the study only required the concomitant vaccine to be recorded after informed consent is signed till the one month after last study vaccine administration (or end of vaccination visit for early withdrawals).

- If the CRF page label indicates the nonstudy vaccine is collected in the designated 'Prior xx Use' page, this nonstudy vaccine will be summarized in the analysis interval of 'prior to vaccination' when the nonstudy vaccine administration is missing.

- If the CRF page label indicates the nonstudy vaccine is collected in the designated 'Concomitant Vaccine Use' page, this nonstudy vaccine will be summarized in the analysis interval of 'Concomitant Vaccine' when the nonstudy vaccine administration is missing.

- If a nonstudy vaccine is entered in the 'Concomitant Vaccine Use' page but the date indicates the nonstudy vaccine is administered prior to first dose of study vaccination, this nonstudy vaccine will not be summarized in the analysis interval of 'Concomitant Vaccine'. Since 'Prior xx Use' page only collect certain prespecified vaccines, it will not be included in the summary of analysis interval of 'prior to vaccination'. This nonstudy vaccine will be included in the nonstudy vaccine listing.

- If a nonstudy vaccine is entered in the 'Concomitant Vaccine Use' page but the date indicated the nonstudy vaccine is administered after the end of vaccination phase, this nonstudy vaccine will not be summarized in the analysis interval of 'Concomitant Vaccine'. This nonstudy vaccine will be included in the nonstudy vaccine listing.

10.1.5.2. Nonstudy Medication Date

Pfizer general rule of imputing missing date of nonstudy medication may be used. However, summary table to include nonstudy meds used during the vaccination will not be provided, while a table to summarize all nonstudy medication use will be provided. Additionally, all original date (or partial date) in the database will be displayed in the listing. Therefore, the Pfizer imputation rule will not have any impact of the tables or listings.

10.1.5.3. All Subjects

Our safety population should include any vaccinated subjects, the 'All Subjects' should include subjects who were randomized or vaccinated; any subjects who were screen failure but not randomized will not be included as 'All Subjects'.

10.1.5.4. Missing AE CRF / Incomplete AE CRF Information

Proportion of subjects with missing AE CRF will be calculated.

SAP Section 7.2.2 describes the denominators and numerators. However, the main SAP section did not differentiate the 'missing AE CRF for the **whole perio**d' with 'missing AE CRF for **some time** within analysis interval'. The latter is referred as 'any incomplete AE CRF information'. Below paragraph describes the detailed data handling on calculating the numerators on 'incomplete AE CRF information'.

This variable will be based on the 'follow-up CRF' page – as long as subject checked 'yes' on 'Was AE discussed' for a visit, this subject is not considered as with incomplete AE CRF information for any analysis interval prior to that visit or inclusive of this visit.

Table 12. Algorithm of Incomplete AE Safety Data Information

#	Analysis Interval	Analysis Population	Denominator	Numerator
1	Within 30 days after vaccination 1	Vaccination 1 Safety Population	# of subjects received vax 1	# of subjects with 'follow-up CRF' page checked as 'No' for all visits after vaccination 1
2	Within 30 days after vaccination 2	Vaccination 2 Safety Population	# of subjects received vax 2	# of subjects with 'follow-up CRF' page checked as 'No' for all visits after vaccination 2
3	Within 30 days after vaccination 3	Vaccination 3 Safety Population	# of subjects received vax 3	# of subjects with 'follow-up CRF' page checked as 'No' for all visits after vaccination 3
4	Within 30 days after any vaccination	Safety Population	# of subjects received any vax	# of subjects with 'follow-up CRF' page checked as 'No' for all visits after vaccination 1
5	During the vaccination phase	Safety Population	# of subjects received any vax	Same as above
6	During the follow-up Phase for Stage 1	Follow-up Safety Population for stage 1	# of subjects received any vax	# of subjects with 'follow-up CRF' page checked as 'No' for all visits after Visit 7 and up until Visit 8
7	During AE collection period	Safety Population	# of subjects received any vax	Total # of unique subjects from row #5 and row #6

10.1.5.4.1. Analyses Impacted

There are 5 safety populations defined: Safety Population; Vaccination 1 Safety Population; Vaccination 2 Safety Population; Vaccination 3 Safety Population, and Follow-up Safety Population for Stage 1. The analysis on the CRF related safety summary will use the corresponding safety population listed in Table 12 as the population definition is the same as the denominator minus the numerator, except for the analysis interval of 'During the AE collection period'.

The primary analysis on SAE, medically attended AE, newly diagnosed medical condition 'During the AE collection period' will use the overall 'safety population' because this will capture any events that were reported during the study stay regardless study/vaccination phase completion status.

Additional analysis will be performed on the follow-up safety population for stage 1 for the endpoints of SAE, medically attended AE, newly diagnosed medical condition during the AE collection period, so that the estimation are based on subjects with complete safety information.

Supplementary analyses may be performed on 'SAEs during the AE collection period' among subjects who completed the study; '(S)AE during the vaccination phase' among subjects who completed the vaccination phase.

10.1.5.5. Telephone Contact Attempt

A telephone contact attempt is to be made 12-month after last study vaccination administration for all subjects who have been vaccinated. In the case that 12-month telephone contact was made to unvaccinated subjects, this information will not be summarized in the disposition table; however, it will be listed in the subject demographic information.

10.1.5.6. End of Study and End of Vaccination Phase

10.1.5.6.1. Completion Status

A query will be sent where a subject marked the study / vaccination phase is completed when some important procedures were not performed (eg, marked 'Vaccination Phase Completed' but did not have post-vaccination 3 blood draw; marked 'Study Completed' but without a successful telephone contact, etc.).

If site confirmed that it was hard to draw blood for the subject or due to other reasons but all other procedures at that visits were performed, this subject may be included as completing 'vaccination phase'. In order to reconcile the inconsistency between the number of subjects who had 1-month after vaccination 3 blood draw and the total number of subjects who completed the vaccination phase, the subjects will be footnoted in the disposition table indicating 'unable to take blood draw' or other pertinent reasons.

If the site replaced the 12-month telephone call with an office visit, the subject will be counted as 'Study completed' and additional clarification will be added in the table footnote.

10.1.5.6.2. Dates

The 2 dates will be used to derive total subject-duration for the calculation of incidence rates.

2 subject summary pages will be provided (one for end of vaccination phase and one for end of study); therefore the 2 dates can be derived from the 2 pages.

Table 13.   End of Study Date and End of Vaccination Phase Date

Study	# of Subject Summary Page	End of Vaccination Date	End of Study Date
B1971035	2	• Visit Date for 'Subject summary Page- Vaccination Phase' (This date should be on the same day of last visit during vaccination phase or later)	• Visit Date for 'Subject summary Page- End of Study' (This date should either be the Visit 10 or Visit 13 date, whichever is later)

10.1.5.6.3. Reason for Discontinuation

The 2 CRF pages included identical questions. If subjects withdraw during the vaccination phase, the subjects should be marked as 'Study Not Completed' even there was a successful telephone contact made at 6-month after last vaccination. Therefore, there are edit checks to query the site when the withdraw reason or withdraw phase do NOT match between the 2 pages. Most of the time, the site would follow the query instruction, and it will not cause any inconsistency for programming. However, if the site is not willing to make the change, the programming will only use the withdraw phase and withdraw reason from the 'End of Vaccination Phase' to produce listings and tables.