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Statistical Analysis Plan

Protocol number:

rBV A/B-CL-003

Protocol title:

Tolerability and Immunogenicity of a Single 40-µg Dose of Recombinant Botulinum Vaccine A/B (rBV A/B) for the Production of BabyBIG® in Volunteers with Existing Botulinum Immunity

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Revision History

Revision summary since final version 1.0.

Version No.	Effective Date	Description of Changes
1.0	18Feb2025	Initialize

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List of Abbreviations

Abbreviation	
AE	Adverse event
AESI	Adverse Event of Special Interest
BIG-IV	Botulism Immune Globulin Intravenous (Human)
CRF	Case report form
CTMS	Clinical Trial Management System
DSMB	Data and Safety Monitoring Board
eCRF	Electronic case report form
IMM	Immunogenicity Population
MedDRA	Medical Dictionary for Medical Affairs
NAC	Neutralizing antibody concentration
NOCI	New onset chronic illness
PBT	Pentavalent botulinum toxoid
PLD	Plasma-donating Population
PP	Per Protocol Population
PT	Preferred Term
rBV A/B	Recombinant botulinum vaccine A/B
SAE	Serious adverse events
SAF	Safety Population
SAS	Statistical Analysis System
SAP	Statistical Analysis Plan
SOC	System Organ Class

1.0 Introduction

This statistical analysis plan (SAP) details the planned statistical analysis methods required to address the study objectives as described in protocol rBV A/B-CL-003: Tolerability and Immunogenicity of a Single 40-µg Dose of Recombinant Botulinum Vaccine A/B (rBV A/B) for the Production of BabyBIG® in Volunteers with Existing Botulinum Immunity.

This SAP should be read in conjunction with the study protocol, electronic case report form (eCRF), and any other applicable study documents. This version of the SAP is based on the protocol rBV A/B-CL-003 v2.0 02Jul2024 and CRF Version 2.0 02Jul2024. Changes to these documents may result in subsequent changes to the SAP. The final, sponsor-approved version of the SAP must occur prior to database lock.

1.1 Changes to the Planned Analysis

Additional details may have been provided for analyses and data presentations described in the protocol, but no changes were made to analyses specified in the protocol. Any deviations from the approved statistical analysis plan will be described and justified in the final clinical study report.

2.0 Study Objectives and Endpoints

Objectives and Endpoints	
Immunogenicity Objective	**Immunogenicity Endpoints**
To evaluate the effects of a single 40-µg dose of rBV A/B on botulinum toxin type A and type B neutralizing antibody concentration (NAC) over a 12-week period following vaccination in participants previously immunized with Pentavalent botulinum toxoid (PBT) (or PBT and rBV A/B) for occupational protection.	Primary: • The proportion of participants achieving a four times or greater increase (point estimate) in NAC by Week 4 compared with Week 0. A positive response will be defined as achieving this level of increase in at least 50% of the participants for botulinum toxin type A and type B. Secondary: • The proportion of participants achieving two times increase in the area under the plasma concentration-time curve between Week 0 and Week 12 in NAC in comparison to a straight-line extension of the Week 0 NAC to Week 12. A positive response will be defined as achieving this level of increase in at least 50% of participants for botulinum toxin type A and type B; • The proportion of participants achieving a three times or greater increase in NAC by Week 4 compared with Week 0. A positive response will be defined in at least 50% of the participants for botulinum toxin type A and type B.
Safety Objective	**Safety Endpoints**
To obtain safety information during the 12-week study period and at 6 months post-vaccination on the use of rBV A/B in a population of participants previously immunized with PBT (or PBT and rBV A/B) for occupational protection.	Primary safety endpoints collected over the 12-week study period and will be as follows: • Frequency of injection site reactions and systemic reactions that are characterized as severe during the diary reporting period (Days 0-7); • Frequency of injection site reactions and systemic reactions that are characterized as Serious Adverse Events (SAEs) as defined in the protocol; • Participant incidence of treatment-related SAEs as defined in Appendix B; • Participant incidence of serious New onset chronic illnesses (NOCIs); • Clinically significant changes from baseline health resulting in SAEs; • Clinically significant changes in hematology between screening and Week 4 resulting in severe or serious Adverse Events (AEs).

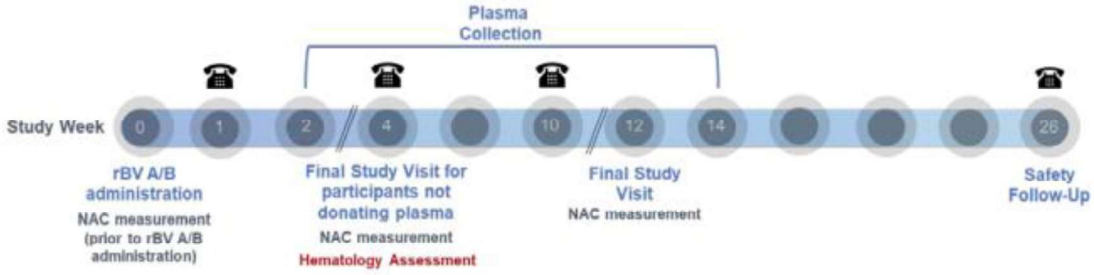
Exploratory Objective	Exploratory Endpoint
To collect source plasma from participants that contain neutralizing antibodies against botulinum toxin type A and type B for the production of BabyBIG (BIG-IV).	The volume of source plasma containing neutralizing antibodies against botulinum toxin type A and type B collected by plasmapheresis for use in BabyBIG manufacture.

3.0 Study Design

3.1 General Description

This protocol has as its ultimate goal the production of additional lots of the approved orphan drug Botulism Immune Globulin Intravenous (Human), (BIG-IV; BabyBIG®) for the treatment of the rare disease infant botulism. To enable production of BabyBIG, a group of high-titer immunized persons are needed who are willing to donate their hyperimmune plasma as a starting material (i.e., Source Plasma) for the manufacture of BabyBIG. This protocol is designed to determine botulinum toxin type A and type B neutralizing antibody levels in healthy participants who were previously immunized with PBT (or PBT and rBV A/B) for occupational protection and who receive rBV A/B (or rBV A/B boost).

This will be a Phase 2, open-label, uncontrolled study to evaluate the safety and immunogenicity of a single 40-µg intramuscular injection of rBV A/B in participants who were previously immunized with PBT (or PBT and rBV A/B) for occupational protection for collection of source plasma for potential use in the production of BabyBIG.



Participants will receive the rBV A/B injection and may elect to undergo plasmapheresis up to twice weekly through Week 14 post-vaccination, so long as they meet donor eligibility requirements. Prior to each donation, a serum sample will be collected for neutralizing antibody concentration (NAC) determination. Participants will be evaluated for safety and immunogenicity at each study visit with longer-term follow-up safety data collected at 6 months.

The study period is defined as the 12-week period that includes the clinic visits and plasmapheresis visits. The study period will end at Week 4 for participants who opt not to participate or who have been determined to be ineligible for donation for the entire study (designated as non-donors). All participants, including donors undergoing plasmapheresis, will also have an additional hematology specimen collected at Week 4. Study participants participating in plasma donations will have a final on-site study visit at Week 12. Non-donors will have a final on-site study visit at Week 4. All participants will have a final phone call for safety follow-up at Week 26.

Up to 30 participants are anticipated to be enrolled. Eligibility will be established by the investigator(s) on the basis of the inclusion and exclusion criteria.

3.2 Randomization and Blinding

This is an open-label study, and no randomization will be performed. Dropouts will not be replaced in this study.

3.3 Sample Size

No formal power calculations will be performed for this study. The sample size will consist of up to 30 participants. On the basis of prior sponsor-investigator experience, this sample size is considered adequate for fulfilling the study objectives.

3.4 Data and Safety Monitoring Board (DSMB)

A data and safety monitoring board (DSMB) will meet quarterly throughout the study and as needed as described in protocol Section **Error! Reference source not found.**. The specific information that will be reviewed will be safety data and neutralizing antibody concentrations. In addition, the DSMB will convene at any time during the study if a stopping rule is met (see protocol Section **Error! Reference source not found.**). Details regarding the displays to be provided for the DSMB meetings will be determined separately from this statistical analysis plan.

3.5 Timing of Analyses

The final analysis will occur once all participants complete their termination visit or their last scheduled assessment (e.g. the Week 26 phone call), or if the sponsor terminates the study for any reason.

4.0 Analysis Populations

4.1 Immunogenicity (IMM) Population

The IMM population will include all participants who have received the vaccine dose, have a baseline NAC, and have at least one post-vaccination immunogenicity assessment up to and including 4 weeks after administration of rBV A/B.

This population will be used for all immunogenicity endpoints.

4.2 Per-protocol (PP) Population

The PP population will include all participants who received the vaccine dose, have a baseline NAC, and have all immunogenicity assessments completed within the protocol-specified timelines (including visit windows) for the time point(s) summarized and do not have major protocol violations expected to affect immunogenicity.

Determination of a major protocol violation expected to affect immunogenicity will be done by the sponsor-investigator before reviewing the immunogenicity data for the applicable participant. The precise reasons for excluding participants from the per protocol population will be fully defined and documented. (Note: It is possible for a participant to be included in the primary per protocol analysis for Week 4, even if the subsequent blood draws occur outside of the study windows.)

For all summaries and analyses using the PP population, values collected outside the visit windows and values associated with a major protocol violation expected to affect immunogenicity will be excluded from the specific time point being summarized or analyzed.

This population will be used in the case that any sensitivity analyses are deemed necessary.

4.3 Safety (SAF) Population

The safety population will include all participants enrolled into the study who received the vaccine dose and had at least one post-baseline safety assessment (this assessment can include a response from a participant that no adverse events occurred).

4.4 Plasma-donating (PLD) Population

The PLD population will include all participants who received the vaccine and donated at least one plasma unit.

This population will be used to evaluate the exploratory endpoint.

5.0 General Considerations

5.1 General Data Handling

All analyses will be conducted by using SAS® (SAS Institute, Inc.) version 9.4 or higher.

Study data will be recorded in electronic case report forms (eCRFs) for all screened and enrolled participants.

Summary tables of data will be provided as appropriate, showing the number of participants with non-missing data (n), mean, standard deviation, median, minimum and maximum for continuous variables, and counts and percentages for categorical variables. Appendix listings will also be provided for the clinical study report. Data will be listed by "participant ID."

Relative to the number of digits after the decimal in the raw data, summary statistics will have the following number of digits after the decimal:

- Minimum and Maximum: same number of significant digits as the raw data
- Mean, Median, Q1, and Q3: one additional significant digit
- SD or Standard Error (SE): two additional significant digits
- Percentages <100% will be reported to one decimal place and percentages of 100% will be reported with no decimal place.
- Summary statistics will not exceed four digits after the decimal. Some laboratory parameters or other data may require judicious deviation from this rule.

5.2 General Definitions

Term	Definition
Study Day	Study Day = date of interest – reference date. Day of vaccination = Day 0. Note: if either day is missing, reference date calculations will not be performed. Should imputation be performed, then study day may be computed where appropriate.
Baseline	For all summaries and analyses, including those involving change from baseline endpoints or baseline covariates, a participant's baseline value will be defined as the last non-missing value prior to vaccination.
Post-baseline	Defined as values collected after receipt of vaccination (based on date and time of administration)
Change from Baseline	Defined as: Post-baseline value – Baseline value
Percent Change from Baseline	Defined as: (Post-baseline value – Baseline value)/(Baseline value)*100
Duration on Study (days)	End of study date – enrolled date +1.
Enrolled Date	Date of vaccination.

5.3 Data Imputation Rules

No imputation will be made for missing data, with the exception that partially missing dates will be imputed to the least favorable date. For example, AEs with partially missing dates that occur in the same month as vaccination will be considered treatment-emergent.

5.4 Visit Windows

Immunogenicity values (NAC values) will be assigned to weeks/visits according to the visit windows listed in Section 6.2.3. For displays using the per protocol population, immunogenicity assessment values which are collected outside the visit windows for the relevant time points will be excluded from per protocol analyses. Safety values will be evaluated and displayed as belonging to the intended visit, regardless of the actual study day on which they were collected.

6.0 Analysis Methods

6.1 Study Participant Data

6.1.1 Participant Enrollment and Disposition

This is an open-label study, and no randomization will be performed. Dropouts will not be replaced in this study. Participants who meet certain exclusion criteria may be re-screened for study enrollment under certain conditions as described in the protocol.

Participant enrollment, inclusion in analysis populations, number of participants discontinuing before study completion, and reasons for premature discontinuation will be summarized with frequencies and, where appropriate, percentages of enrolled participants. Data will also be provided in an appendix listing of the clinical study report.

6.1.2 Protocol Violations

Determination of major protocol violations expected to affect immunogenicity for each participant (and hence affecting the PP population) will be performed by the Sponsor-Investigator before reviewing the immunogenicity data for the applicable participant. For all summaries and analyses using the PP population, values associated with a major protocol violation expected to affect immunogenicity will be excluded from the specific time point being summarized or analyzed.

All protocol violations will be documented in the clinical trial management system (CTMS). The CTMS protocol violation data will be listed in an appendix listing of the clinical study report.

6.1.3 Demographic and Baseline Characteristics

Demographic and baseline characteristic data will be collected during screening prior to vaccination. All demographic and baseline characteristic data will be summarized and listed.

6.1.4 Medical History

Medical history will be collected during screening (Days -7 to -1). Data will be presented in a by-participant listing.

6.1.5 Prior and Concomitant Medications

All prior and current medications taken within 60 days prior to Screening will be collected and recorded in the eCRF. All concomitant medications used to treat a severe localized injection site reaction or severe systemic reaction from Week 0-1 as well as any serious adverse events (SAEs) (including serious new onset chronic illnesses [NOCIs]) throughout the study (through Week 26) will be recorded in the eCRF. Concomitant medications will be queried at each visit. Concomitant medication data will be listed.

6.1.6 Vaccine Exposure and Compliance

A single dose of the rBV A/B vaccine will be administered to each participant at the Vaccination Visit (Day 0). The vaccine formulation is 40 µg total antigen (20 µg Antigen A and 20 µg Antigen B) with 100 mM sodium chloride, 15 mM sodium phosphate, and 50 mM sodium acetate with Alhydrogel™ (0.2% weight per volume) balanced to a pH of 5.5 for a total dose volume of 0.5 mL. Vaccine administration and exposure data, including whether or not each

participant received the injection, will be provided in an appendix listing of the clinical study report.

6.2 Immunogenicity

The IMM population will be used for all immunogenicity endpoints. The PP population will be used in the case that any sensitivity analyses are deemed necessary.

All immunogenicity endpoints will be performed for both botulinum toxin type A and type B NACs.

6.2.1 Primary Endpoint Analysis

The primary immunogenicity endpoint is the proportion of participants achieving a four times or greater increase in NAC by Week 4 compared to Week 0. The cut-off value for determining whether the increase is achieved for each participant will be calculated as [(Week 0 value) x 4] + (Week 0 value). Equivalently, a percent change from baseline greater than or equal to 400% can also be used to determine whether the increase is achieved. A positive response will be defined as achieving this level of increase in NAC in at least 50% of participants in the IMM population. The proportion of IMM participants who achieve this level of increase in NAC will be provided along with a 95% confidence interval. Graphs may be used to show corresponding participant-level data.

This primary analysis will be performed for both botulinum toxin type A and type B NACs. In the event that the primary endpoint is not met for neutralizing antibodies against botulinum toxin type A or type B, the Sponsor-Investigator will determine if the plasma collected during the study contains sufficient antibody titers for meeting specifications for BabyBIG production. If so, the trial will still be considered successful.

Details regarding the collection schedule for blood samples for NAC can be found in Section 6.2.3.

6.2.2 Secondary Endpoints and Analyses

The first secondary immunogenicity endpoint is the proportion of participants achieving a two times increase in the estimated area under the plasma concentration-time curve in the period between Week 0 to Week 12 in comparison to straight-line extension of the Week 0 NAC to Week 12. The cut-off value for determining whether the increase is achieved for each participant will be calculated as (Week 0 value) x (total number of days from Week 0 to Week 12 collection) x 2. A positive response will be defined as achieving this level of increase in at least 50% of the participants in the IMM population. If a participant does not have NAC collections through Week 12, the date of the last NAC collection will be used in place of the Week 12 date for both the concentration-time curve and the straight-line extension. In the cases where there are multiple NAC values on the same date for a particular participant, the mean of those values will be used in calculating the estimated area under the plasma concentration-time curve.

The second secondary immunogenicity endpoint is the proportion of participants achieving a three times or greater increase in NAC above baseline by Week 4 compared to Week 0. The cut-off value for determining whether the increase is achieved for each participant will be calculated as [(Week 0 value) x 3] + (Week 0 value). A positive response will be defined as achieving this level of increase in at least 50% of the participants in the IMM population.

Proportions will be provided for both secondary endpoints along with 95% confidence intervals.

Further details regarding the collection schedule for blood samples for NAC can be found in Section 6.2.3.

6.2.3 Neutralizing Antibody Concentration (NAC) Values

Blood collection for determination of NAC values will be performed prior to vaccination at the Vaccination Visit (Day 0), for non-plasma donating participants at the Week 4 visit, and for plasma-donating participants at plasmapheresis visits and the Week 12 final study.

For summary tables involving NAC values, the week numbers will be assigned as described below. Day values will be assigned based on the derivation provided in Section 5.2. Week 4 and Week 12 have larger windows than other weeks in order to facilitate collection of NAC values during the time periods of primary interest and align with protocol-defined windows for those respective study weeks.

- Week 1 - values collected from Day 4 through Day 10
- Week 2 - values collected from Day 11 through Day 17
- Week 3 - values collected from Day 18 through Day 24
- Week 4 - values collected from Day 25 through Day 31 [Day 28 (±3 days)]
- Week 5 - values collected from Day 32 through Day 38
- Week 6 - values collected from Day 39 through Day 45
- Week 7 - values collected from Day 46 through Day 52
- Week 8 - values collected from Day 53 through Day 59
- Week 9 - values collected from Day 60 through Day 66
- Week 10 - values collected from Day 67 through Day 73
- Week 11 - values collected from Day 74 through Day 76
- Week 12 - values collected from Day 77 through Day 91 [Day 84 (±7 days)]
- Week 13 - values collected from Day 92 through Day 94
- Week 14 - values collected from Day 95 through Day 101

6.3 Interim Analysis

There are no planned interim analyses for this study.

6.4 Safety

All safety analysis reporting will be based on the Safety Population.

6.4.1 Adverse Events

Only SAEs and AEs of special interest (AESIs) will be evaluated for this study and only from time of vaccination and onward (i.e., all AEs will be treatment-emergent). AESIs will include a) severe localized injection site reactions and severe systemic reactions that occur from Day 0 to Day 7 and b) severe clinically significant changes in hematology between screening and Week 4. SAEs will be recorded from administration of the investigational product at the vaccination visit through the follow-up phone call at Week 26. AE data will be coded using Medical Dictionary for Medical Affairs (MedDRA) v26.0.

Severe localized injection site reactions and severe systemic reactions that occur from Day 0 to Day 7 (from the Week 1 telephone call) will be summarized by category (localized or

systemic) and maximum severity. An overall summary with point estimates of incidence and 95% confidence intervals will be provided for severe localized injection site reactions and severe systemic reactions, along with average durations for each. The time period covering the study as a whole will also be summarized.

A summary of all SAEs by system organ class (SOC) and preferred term (PT) will be produced. A similar summary will be produced for SAEs that are categorized as serious NOCIs. The SAEs qualifying as serious NOCIs will be identified by CDPH, and the list will be used for selection of applicable SAEs in the data. Summaries by maximum severity and strongest relationship to vaccine will be produced for all SAEs. SAEs, serious NOCIs, and AESIs (severe localized injection site reactions and severe systemic reactions, clinically significant changes in hematology between screening and Week 4), SAEs or AESIs resulting in death, and SAEs or AESIs leading to discontinuation from the study will be provided in appendix listings of the clinical study report.

When calculating the incidence of SAEs and AESIs, each SAE and AESI will be counted only once for a given participant within a MedDRA category (e.g., overall, system organ class, or preferred term). When AEs are summarized within levels of another AE assessment (e.g., causality or severity), AEs will be counted once per participant at the worst level of the assessment (e.g., strongest relationship to vaccine or greatest severity).

6.4.2 Clinical Laboratory Evaluations

Samples for hematology, serum chemistry, and urinalysis will be collected at screening. Hematology samples will also be collected at Week 4. Treatment-emergent abnormal hematology values at Week 4 will be summarized. Hematology laboratory abnormalities will be assessed for relatedness and may be presented by relatedness in tables based on the investigator's assessment. A summary of clinically significant changes in hematology (i.e. treatment-emergent clinically significant values) between screening and week 4 will be produced.

6.4.3 Vital Signs and Other Physical Examinations

Vital sign measurements will include temperature, blood pressure, heart rate, and respiratory rate. These measurements will be provided in an appendix listing of the clinical study report.

Physical examinations will be performed during screening and at the initial plasmapheresis visit. Physical examination findings will be recorded with medical history or AEs.

6.4.4 Participant Diaries

Participants will complete a home diary on a daily basis for 7 days post-vaccination, as well as during the study period to report any adverse events and concomitant medications. Significant data from the diaries will be recorded with adverse event data or concomitant medication data by study staff on study source documents during study phone call follow up and/or on-site visits, so there will be no separate summaries or listings of diary data.

6.5 Exploratory Endpoint

The exploratory endpoint will be the volume of source plasma containing neutralizing antibodies against botulinum toxin type A and type B collected by plasmapheresis. This exploratory endpoint will use the plasma-donating population.

Plasmapheresis data will be summarized and provided in an appendix listing of the clinical study report. Prior to final analysis, CDPH will provide a line listing of plasma units to be excluded from the plasmapheresis data summary, i.e., units that are unusable because they were destroyed or should be excluded for some other reason. Plasma units may be destroyed for multiple reasons including low volume, contamination, or incomplete testing. Further details regarding the collection schedule for serum samples for NAC can be found in Section 6.2.3.