Statistical Analysis Plan

Final Analysis

A Phase IV open-label, randomized, parallel-group study to evaluate pharyngeal immunity to poliovirus type-2 in healthy bOPV- versus IPV-vaccinated infants

Protocol: IPV-005-ABMG

Confidential

Sponsor: FIDEC – Fighting Infectious Diseases in Emerging Countries (with grant support from the Bill and Melinda Gates Foundation)
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List of Abbreviations

Abbreviation	Definition
ATC	Anatomical Therapeutic Chemical
bOPV	Bivalent OPV
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CSP	Clinical Study Protocol
CSR	Clinical Study Report
DML	Dartmouth Lab
DMP	Data Management Plan
DRM	Data Review Meeting
DTS	Data Transfer Specifications
EP	Enrolled Population
IPV	Inactivated poliovirus vaccine
LLOQ	Lower limit of quantification
NIP	National Immunization Program
nOPV2	Novel OPV2
PCV-10	Pneumococcal conjugate vaccine
PD	Protocol Deviation
PP	Per-Protocol
PPP	Per-Protocol Population
PT	Preferred Term
PV	Polio Virus
RCD	Reverse cumulative distribution
RT-PCR	reverse-transcription polymerase chain reaction
SAP	Statistical Analysis Plan
SC	Seroconversion
SIE	Shedding Index Endpoint
SOC	System Organ Class
SP	Seroprotection
TLF	Tables, listings figures
TVP	Total Vaccinated Population
ULOQ	Upper limit of quantification
VSPP	Viral Shedding Population – Pharyngeal
VSPS	Viral Shedding Population – Stool

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1. OVERVIEW

1.1 Study Objectives

1.1.1 Primary Objective

The primary objective of the study is to compare the poliovirus type-2 pharyngeal mucosal excretion in the first week, and at 2 and 4 weeks following the administration of a challenge novel OPV2 (nOPV2) dose at 18 weeks of age in 2 parallel groups of infants. One group (Group A) will be primed with 3 doses of IPV, the other group (Group B) with 3 doses of bOPV as a primary schedule at 6, 10, 14 weeks of age.

1.1.2 Secondary Objectives

The secondary objectives of the study are:

- To assess and compare the pharyngeal mucosal neutralizing and immunoglobulin class-specific immune response to poliovirus type-2 at Day (D)0, 2 and 4 weeks following administration of a challenge dose of nOPV2 in both groups.
- To assess the humoral immunogenicity to poliovirus type-2 at D0, 4 and 8 weeks following administration of a challenge dose of nOPV2 in both groups.
- To assess the safety expressed as incidence, severity and causality of SAEs and IMEs following administration of IPV, bOPV and nOPV2.

1.1.3 Exploratory Objectives

The exploratory objectives of the study are:

- To assess poliovirus type-2 intestinal mucosal excretion in the first week, and at 2 and 4 weeks following administration of a challenge dose of nOPV2 in both groups.
- To assess poliovirus type-2 intestinal mucosal neutralizing and immunoglobulin class-specific immune response at 2 and 4 weeks following administration of a challenge dose of nOPV2 in both groups.

1.2 Study Design

This is a multicenter, randomized, open-label, parallel-group, phase IV study in healthy infants from Bangladesh aged 6 to 8 weeks who have never been vaccinated against poliomyelitis.

Approximately 500 infants are included and randomized with a 1:1 ratio to the following treatment groups:

- Group A: approximately 250 subjects to receive 3 doses of IPV administered at 6, 10, 14 weeks of age
- Group B: approximately 250 subjects to receive 3 doses of bOPV administered at 6, 10, 14 weeks of age

A total of 10 on-site visits are planned.

All infants receive concomitant routine vaccinations included in the local National Immunization Program (NIP), i.e. pentavalent vaccine (diphtheria, tetanus, whole-cell pertussis, hepatitis B, *Haemophilus influenzae* type b, [DTPw-HB-Hib]), and pneumococcal conjugate vaccine (PCV-10).

The total study duration will be approximately 5 months. Each infant will participate for a duration of approximately 5 months, which includes a pre-challenge phase of 3 months when they will receive 3 doses of either IPV or bOPV,

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and the challenge and follow-up phase when they will receive a single challenge dose of nOPV2 and have immunogenicity and safety assessments up to 2 months following the challenge.

1.3 Endpoints

1.3.1 Primary Endpoint

The primary endpoint is:

- The presence of poliovirus type-2 in pharyngeal samples detected by reverse-transcription polymerase chain reaction (RT-PCR) in the first week, and at D14 and D28 in both groups.

1.3.2 Secondary Endpoints

Immunogenicity:

- Pharyngeal neutralizing antibody activity, and total and poliovirus type-2-specific concentrations of pharyngeal mucosal immunoglobulin A (IgA) on D0, D14 and D28 in both groups.
- Cumulative seroconversion (SC) rate of poliovirus type-2 neutralizing antibodies on D28 and D56 in both groups.
- Geometric mean and median titers of poliovirus type-2 neutralizing antibodies on D0, D28 and D56 in both groups.
- Seroprotection (SP) rate to poliovirus type-2 on D0, D28 and D56 in both groups.

Safety:

- Incidence of SAEs and IMEs from the date of informed consent throughout the study period in both groups by severity and causal association.

1.3.3 Exploratory Endpoints

- The presence of poliovirus type-2 in stool samples detected by RT-PCR in the first week, and at D14 and D28 in both groups.
- Shedding Index Endpoint (SIE) of poliovirus type-2 expressed as the average poliovirus type-2 viral titer from stool samples collected in the first week, and at D14 and D28 in both groups.
- The presence of poliovirus type-2 neutralizing antibodies in stool samples at D14 and D28 in both groups.
- Total and poliovirus type-2–specific concentrations of IgA from stool specimens collected on D0, D14 and D28 in both groups.

1.4 Sample Size Calculation

This study is designed to evaluate poliovirus type-2 pharyngeal mucosal immunity following an IPV (vs bOPV) series in infants in a setting of no homotypic (to challenge vaccine type) OPV exposure.

Data to inform the anticipated rates of type 2 excretion, detectable via PCR, are sparse. Data suggest pharyngeal shedding rates range from 38% to 75% for individuals with lower serum neutralizing antibody following exposure to wild-type poliovirus type 1. Data from Faden, Modlin, Thoms et al show high secretory IgA antibody presence among those receiving three doses of IPV, suggesting an IPV-related impact on pharyngeal shedding may be observed upon

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OPV challenge. Data from a study by Onorato, Modlin, McBean et al suggest low pharyngeal excretion following three doses of IPV.

In order for study results to be impactful on vaccination policy as it relates to IPV usage to stem pharyngeal-associated spread of poliovirus, it is recognized that the impact will likely need to be of a large magnitude. It is unknown if pharyngeal shedding following nOPV2 challenge will be substantial in magnitude, or perhaps very limited. In acknowledgment of the sparsity of relevant data, it was further desired to approach determination of sample size from a conservative perspective, allowing for lower rates of pharyngeal shedding among bOPV participants than those suggested by the low-titer shedding results in the study by Marine, Chin and Gravelle, and in acknowledgment of the lack of relevant historical data with nOPV2, which may be expected to yield lower viral shedding than observed in historical studies following exposure to wild type-1 virus. With these data and considerations in mind, it was assumed that pharyngeal viral shedding rates at 1 week post-nOPV2 vaccination would be approximately 15% for IPV vaccinees and 50% for bOPV vaccinees (relative risk [RR] 0.3, a 70% reduction in shedding rate). A significant impact on viral shedding was considered to be an RR which is less than 50% (50% reduction associated with IPV vaccination). Therefore, the hypothesis to be tested is H0: RR ≥50% vs H1: RR < 50%. With 225 evaluable participants per group, there is 90% power to confirm a 50% reduction in the pharyngeal shedding rate, under these assumptions, using the upper bound of the two-sided score-based 95% CI for the relative risk (IPV/bOPV). To account for 10% loss to follow-up and loss from the Per-Protocol Population prior to collection of the first post-challenge pharyngeal sample, the sample size is inflated to 250 per group.

1.5 Schedule of Assessments

Assessments	Pre-challenge phase			Challenge and follow-up						
Visit	1	2	3	4	5	6	7	8	9	10
Time of Visit (days) Visit Window	D –84 (+14,-7 days)	D –56 (+7 days)	D –28 (+7 days)	D 0 (+ 14 days)	D 3 ± 1 day)	D 5 (± 1 day)	D 7 (± 1 day)	D 14 (± 2 days)	D 28 (+ 7 days)	D 56/EoS[f] (+ 7 days)
Weeks of age	5-8	10-12	14-16	18-20						
Informed consent[a]	X									
In-/exclusion criteria	X			X						
Medical history/concomitant diseases	X			X						
Medication/Vaccination history[b]	X			X						
Demographic data	X			X						
Physical examination	X	X	X	X					X	X
Randomization	X									
Administration of bOPV/IPV[d]	X	X	X							
Administration of nOPV2				X						
Vaccination according to the NIP[g]	X	X	X							X
Serum sample for polio antibodies[c]				X[c]					X	X
Pharyngeal sample[c]				X[c]	X	X	X	X	X	
Nasal strip[c]				X				X	X	
Stool sample[e]				X[c]	X	X	X	X	X	
Adverse events[f]	X	X	X	X	X	X	X	X	X	X

D= day

a. No study-related assessment will be carried out before signature of the informed consent form has been obtained.
b. Including polio vaccination history.
c. Prior to administration of nOPV2.
d. Participants will concomitantly receive DTPw-HB-Hib and pneumococcal conjugate vaccines at V1, V2 and V3..

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e. Parents/guardians will be asked to collect if available the first stool in the provided receptacle and to bring the samples with them to the site.
f. Only SAEs/IMEs and intake of concomitant medication(s) will be monitored continuously from informed consent signature date until D56.
g. Infants participating in the study should receive vaccines as recommended by the NIP. They will receive:
    - At 6-8 weeks of age (V1/D -84): pentavalent (diphtheria, tetanus, whole-cell pertussis, hepatitis B, *Haemophilus influenzae* type b, [DTPw-HB-Hib]), and 10 valent pneumococcal conjugate (PCV-10) vaccines;
    - At 10-12 weeks of age (V2/D -56): pentavalent DTPw-HB-Hib vaccines and PCV-10 vaccines;
    - At 14 – 16 weeks of age (V3/D-28): pentavalent DTPw-HB-Hib and PCV-10 vaccines;
    - At D56, IPV, for participants randomized to the bOPV group.
    - These vaccines are provided by the local NIP.
h. Should the EoS visit take place earlier than D56, serum samples for polio antibodies will not be taken.

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2. GENERAL CONSIDERATIONS

2.1 Conduct of Analysis

After clinical database lock, a final analysis is to be conducted, and a Clinical Study Report is to be written. Since not all external data are available immediately after database closure, the final analysis will initially be conducted using all data available at that time. At a later time point, when all external data are available, the analysis will be repeated, containing all the data.

2.2 Statistical Software and Quality Control

All statistical analyses will be performed using SAS® version 9.4 Tables, figures and data listings will be generated in Microsoft® Word® as well as PDF® format.

Quality control of SAS® programs will include a review of the whole process of result generation:

- Review of all analysis SAS® programs
- Review of SAS® log for errors, warnings and other notes that could indicate mistakes in the programs
- Review of all tables, listings and figures for completeness and correctness

An independent re-programming will be done at least for the primary endpoint.

2.3 Blinding and Randomization

This is a randomized, open-label study.

Allocation of each subject to the 2 treatment groups will be described in a computer-generated randomization schedule prepared prior to start of the study by Assign DMB using SAS® software (SAS Institute Inc., Cary, NC, USA).

The randomization will apply a 1:1 ratio and will be balanced using randomly permuted blocks across the groups.

Blinding of subjects and assessors will not be possible due to the different methods of administration (injected versus oral) employed in this study. Laboratory personnel will be blinded to group until such time as the neutralizing antibody assays are complete, and samples from all groups will be randomized for concurrent testing.

For further details please refer to the Randomization Plan (Final 2.0, 04-Sep-2023).

2.4 Descriptive Analyses

Unless otherwise specified below, descriptive analyses of continuous variables (summary statistics) will be described with the number of non-missing observations, arithmetic mean, standard deviation (±SD), median, quartiles (Q1 and Q3), minimum and maximum.

Categorical variables (frequency statistics) will be described with the number of non-missing observations and percentages (%). Percentages will be calculated within each stratum on the total number of non-missing observations, if not stated otherwise.

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2.5 Inferential Analyses

Unless specified otherwise, statistical tests and CIs will be computed using a two-sided 5% significance level. Exact (Clopper-Pearson) CIs will be used for univariate summaries of dichotomous variables. If other CIs are used, this will be described in the respective sections.

2.6 Center and Country Effect and Stratification Variables

Specific analyses described below will include subgroup analysis according to clinical site.

2.7 Handling Missing Data

The reasons for any missing data will be ascertained and appropriate statistical methods will be used to accommodate these absences in the analyses of the study data that minimize potential biases and maximize efficiency conditional on the causes for data being missing. Data values that are identified by quality control procedures to be spurious will not be used in final analyses of the study data. Generally, values will not be imputed, and the analysis will be limited to observed values. However, if observations during or after analysis suggest extensive missing data or a systemic pattern of missingness, exploratory analyses may be conducted to evaluate sensitivity of the overall conclusions to the missing data.

2.8 Analysis Sets

2.8.1 Enrolled Population

The Enrolled Population (EP) is defined as all subjects for whom informed consent is provided, regardless of the subject's randomization and vaccination status. With the exception of safety endpoints, EP subjects are analyzed according to the randomized schedule, regardless of any wrong or missing pre-challenge dose administration. For the safety endpoints the following bullets will be considered:

- Subjects are analyzed according to the vaccine actually received, regardless of any missing pre-challenge doses, i.e.
- If any dose administration are mixed (e.g., one dose of IPV and two doses of bOPV), it will be discussed during the data review meeting to determine if an additional column is needed for safety endpoints.

2.8.2 Total Vaccinated Population

The Total Vaccinated Population (TVP) is defined as all subjects who received nOPV2 after receiving all three doses of the assigned pre-nOPV2 challenge dose.

2.8.3 Per-Protocol Population

The Per-Protocol Population (PPP) consists of all eligible study participants who received nOPV2 (i.e., are in the TVP), who received all three doses of bOPV or IPV according to the randomized schedule, and who provided an evaluable and not per-protocol disqualified baseline sample (Visit 4) and at least one post-baseline sample for any sample type, e.g. for analysis of serum neutralizing antibody, the PPP will be used, limited to those with an evaluable and PP qualified pre-nOPV2 and any post-nOPV2 sample.

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Subjects who received any therapy that could significantly affect the subject's immune status and those subjects who have major protocol deviations (PDs) (see Section 2.9) will be excluded. The PPP will be adapted by time point to allow subjects to contribute data to per-protocol analyses until such time as they become disqualified. All deviations and violations occurring in the study will be reviewed prior to database lock and classified as either minor or major.

For tables using the PPP on subject level (e.g., demographics, disposition) one of the following evaluable and PP qualified sample pairs (baseline + post baseline) needs to be available:

- Pharyngeal sample evaluated via PCR
- Pharyngeal sample evaluated for neutralizing antibody, or total or type-2-specific concentrations of pharyngeal IgA
- Serum sample evaluated for neutralizing antibodies

2.8.4 Viral Shedding Population – Pharyngeal/Stool/Nasal

The Viral Shedding Population – Pharyngeal/Stool/Nasal (VSPP/VSPS/VSPN) is a subset of the PP Population. The condition from above (PP qualified baseline + post baseline sample) refers to the respective sample type (Pharyngeal/Stool/Nasal).
Furthermore, the respective baseline sample, when tested, needs to be negative for type 2 poliovirus (via PCR).

If >10% of samples are missing or non-evaluable at the laboratory, an additional analysis will be performed among the PPP who do not contribute a respective type 2 positive sample at baseline; this will be termed the VSPP2/VSPS2/VSPN2.

2.9 Protocol Deviations and Data Review Meeting

Potential PDs and violations occurring in the study which may warrant exclusion from per-protocol analysis will be reviewed prior to database closure in a Data Review Meeting (DRM). In the DRM the potential PDs are classified as follows:

- Major, if there is a reasonable possibility of a meaningful and relevant impact of the deviation on assessment of primary endpoint or secondary immunogenicity endpoints. Classifications of major protocol deviations will be conducted on a time point level (if possible) to allow subjects to contribute data to per-protocol analyses until such time as they become disqualified.
- Minor, if no or no relevant or possible impact on immunogenicity is expected.

Here, the designations of "major" and "minor" are distinct from any other classification of protocol deviations, e.g. arising from clinical monitoring.

2.10 Subject Data Listings

Listings will be sorted by treatment group / cohort, subject ID, and/or parameter and/or visit (in this order), where

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applicable. Treatment group / cohort will be shown in all listings.

2.11 Columns in Tables

Every group has its own column in the analysis. Group A will be labeled as “IPV”, and Group B will be labeled as “bOPV”. For Overall Study Information, Baseline tables and Safety tables an additional total column will be added that presents both treatment groups pooled. Furthermore, for the disposition table there is an additional column for subjects who were not randomized.

2.12 Medical Coding

Medical history, concomitant procedures and SAEs/IMEs are coded using Medical Dictionary for Regulatory Activities (MedDRA). Vaccination history and prior/concomitant medication is coded using WHO Drug Reference List and Anatomical Therapeutic Chemical (ATC) Classification System. Further details on Coding are provided in the Coding Guideline, attachment A04 of the Data Management Plan (DMP).

2.13 Changes in the Conduct of the Study or Planned Analysis

As the PCR multiplex assay automatically produces data for the three PV serotypes, the presence for PV1 and PV3 will also be summarized post hoc, in case they are of value to the polio research community. All the rest of the analyses described within this document follow the descriptions and planned analyses in the CSP. However, additions as compared to analyses described in the CSP may be contained in this document (e.g. additional subsets defined for immunogenicity analysis). Any changes or deviations from this SAP in the statistical analysis will be justified and explained in the CSR.

2.14 Data From External Laboratories

Some data, which are analyzed as part of the Final Analysis, will be provided by external laboratories. Below is a summary containing the data received from Centers for Disease Control and Prevention (CDC) and Dartmouth Lab (DML). For more details see the according Data Transfer Specifications (DTS).

Laboratory	Result	Content	Data used in following section of the SAP
CDC	Serum sample results	Neutralizing antibody titers	Section 6
	Pharyngeal sample results	PCR results	Section 4
	Stool sample results	PCR results	Section 4
		Log10 CCID50	Section 4
DML	Nasal strip luminex results	Mean fluorescence intensity results	Section 5
		total IgA ug/ml	Section 5
	Nasal strip neutralization assay results	PV2 60% neutralizing titer	Section 5
	Stool luminex results	Mean fluorescence intensity results	Section 5
		total IgA ug/ml	Section 5
	Stool neutralization assay results	PV2 60% neutralizing titer	Section 5

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3. OVERALL STUDY INFORMATION AND BASELINE EVALUATION

Analyses will be performed for TVP and PPP with the following exceptions: Disposition table and Screening failure table will be created using the EP, Demographics will be presented for the EP and PPP and eligibility criteria violated table will only be shown for the TVP.

- Disposition, including receipt of doses, inclusion in study populations and reasons for dropout will be summarized and listed, and a CONSORT diagram will be prepared.
- Screening failures will be summarized and listed (including not randomized subjects).
- Major and minor protocol deviations will be summarized including by category, and listed.
- Violated eligibility criteria will be summarized and listed.
- Descriptive statistics will be provided per group for demographics (e.g., age, weight, race, sex). Demographics will be compared using Fisher's exact test for categorical variables, and the Wilcoxon test for continuous variables.
- Prior and ongoing medical history will be summarized by System Organ Class and Preferred Term and listed.
- Medical history will be considered "prior" if eCRF field "Ongoing at Visit 1" is answered with "no" and considered "ongoing" if the eCRF field "Ongoing at Visit 1" is answered with "yes" or is missing.
- Prior and concomitant medications (including vaccinations) will be summarized by ATC Level 2 and ATC Level 3 and listed.
    - Medications stopped prior (<) to first pre-challenge dose will be considered prior medications, all other medications are considered to be ongoing/concomitant. Medications with a missing or incomplete end date where it cannot clearly be decided if the end date was before or after first pre-challenge dose will be considered ongoing/concomitant.
- Vaccination history (for non-study and non-NIP vaccinations) will be listed.
- Concomitant NIP Vaccinations will be summarized by vaccination and listed.
- Concomitant procedures will be summarized by System Organ Class and Preferred Term and listed.
- Visit attendance will be summarized and listed.
- Physical examination results including the number of physical examinations performed as well as number of abnormal findings will be summarized, and all details will be listed.

More details about table content will be defined in the mock tables.

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4. ANALYSIS OF VIRAL SHEDDING

Virologic analyses of each sample type will be primarily conducted in the VSP corresponding to that sample type. Tables will be repeated in the TVP only showing the baseline visits.

- The presence of type 2 poliovirus in pharyngeal (primary endpoint) and stool (exploratory endpoint) samples will be summarized by time point and overall (post-challenge) as a categorical variable.
- Furthermore, type 2 coinfection (defined as detection of type 2 along with types 1 and/or 3 in a given sample) will be analyzed.
- Generally, samples collected within-window according to the schedule of events will be eligible for analysis, but minor deviations will be considered for eligibility in the blinded data review meeting. Samples will generally not be excluded from the evaluations conducted with RT-PCR due to presence of types 1 and/or 3.
- The primary endpoint comparison will be based on the computation of relative risk of type 2 positivity at 1, 2, and 4 weeks following nOPV2 challenge vaccination (IPV/bOPV), with the corresponding score-based two-sided 95% confidence interval (CI) for the relative risk. If the upper bound of this CI is less than 0.5, this will be considered to have demonstrated a significant impact on challenge virus shedding of IPV vaccination.
- This will be supplemented with the computation of the corresponding risk difference and associated Miettinen-Nurminen score-based confidence interval.
- For the CIs for the risk difference and relative risk the following rules apply for special cases:
  - When there is a 0% response or a 100% response in both groups, the Wald-Modified method will be used to calculate the CI for relative risk, and the Agresti-Caffo method will be used to calculate the CI for risk difference, as score-based CIs are otherwise not computable in such settings.
  - For relative risk, when division by zero occurs, the relative risk will be set to "Not Calculable" in the output, but the corresponding Wald-Modified CI will still be shown.
- This analysis will be repeated for PCR detection of type 2 in stool samples
- Such analyses will be further expanded for composite endpoints involving these assays and time points. Relative risk and risk difference computations and corresponding 95% CIs will be computed for composite endpoints as well. The assays and time points used for the composite endpoints are:
  1. Any post-challenge shedding
  2. Any post-challenge shedding <7 days after challenge (after receiving nOPV2) (including samples up to Visit 6 (D5))
  3. Any post-challenge shedding >=7 days after challenge (after receiving nOPV2) (including all samples from Visit 7 (D7) onwards)
- For stool samples, those positive for type 2 will be evaluated for viral titer in a $CCID_{50}$ assay, with results summarized on the $\log_{10}$ scale with the median and corresponding two-sided 95% CIs computed via the percentile bootstrap method, separately for each group and time point. The difference in medians will be analyzed similarly with the difference in medians presented, along with its bootstrap-based 95% CI.

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- The summaries of $CCID_{50}$ will also be presented among those PCR-positive for type 2 (only) virus ("among shedders" analysis)
- A Shedding Index Endpoint (SIE) will be computed as the mean $\log_{10}$ $CCID_{50}$ per gram of stool across all post-challenge stool sampling time points. The mean will be computed by imputing the assay upper (3.05 $\log_{10}$ $CCID_{50}$ per gram) and lower (8.30 $\log_{10}$ $CCID_{50}$ per gram) limits of quantification (ULOQ), respectively where necessary, and with type 2 PCR-negative samples contributing a value of zero to the computation.
- Only participants contributing all samples, with each producing an assay result (either a $CCID_{50}$ result when PCR-positive, or a PCR-negative result) will be included in the primary SIE computation.
- Plots will be produced to display positivity across time point, separately for each sample type and group, and including RCDs by time point and group for viral titer.
- As a post hoc analysis, frequency results for type 1 and type 3 PCR-positivity will be summarized in case they are of value to the polio research community, similar to type 2 positivity as described above.

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5. MUCOSAL IMMUNITY ANALYSIS

Mucosal immunity will be evaluated primarily in the appropriate VSP(s), which requires PP population membership.

- Mucosal immunity will be analyzed in stool and nasal strips
- Mucosal immunity will be measured by two assays:
    - Neutralization, reflecting functional antibody and
    - Class-specific binding antibody that demonstrates the role of IgA in mucosal immunity. This will be measured as both total (as µg/ml) and type-2-specific IgA (as mean fluorescence intensity).
    - For stool, only D14 and D28 will be analyzed for neutralizing activity; for IgA from stool, this will include D0, D14, and D28.
    - For nasal strips, this will include D0, D14, and D28.
- If frequency of breastfeeding ("non-breastfeeding" in eCRF) drops more than 10% below the baseline rate for any time point, additional tables will be presented showing IgA-related mucosal immunity results stratified by breastfeeding vs non-breastfeeding status for this and all subsequent time points. E.g. if breastfeeding percentage is >10% lower at Visit 8 as compared to baseline (Visit 4), then Visits 8 and following visits will be presented stratified by Visit 8 breastfeeding status.
- Data will be summarized at each collection time point with the GMT/GMC as a continuous variable ($\log_2$ scale for neutralization titer, $\log_{10}$ scale for IgA concentration), then back-transformed. Positivity in each assay will also be summarized as a categorical variable. Positivity is defined as:
    - Neutralization titer ≥16 (as primary analysis)
    - Neutralization titer ≥4 (as sensitivity analysis)
    - For IgA, positivity is defined as any nonzero value. Values recorded as "zero" will be assigned one-half of the lowest nonzero value for each type separately, for analysis purposes.
- For the neutralizing titer the LLOQ and ULOQ on the log2 scale are 2 and 9, respectively.
- For the total and type-2 specific IgA the LLOQ on the log10 scale is one-half of the lowest nonzero value. No ULOQ is available for the total and type-2 specific IgA.
- Comparisons between groups will be performed with the GMT/GMC ratio computed via a linear model of the log results which incorporates left/right censoring at LLOQ/ULOQ as well as the relative risk and risk difference for positivity, including corresponding two-sided 95% confidence intervals, computed with the methodology defined above for positivity in the viral shedding assay.
- Computation of the GMT/GMC and GMT/GMC ratios will come from SAS PROC LIFEREG incorporating censoring at assay-specific LLOQ and/or ULOQ on the log scale, with back-transformation as appropriate.
- Scatterplots with linear regression lines and overlaid Spearman correlations and corresponding p-values for tests of correlation=0 will be created for each of the following, employing the relevant log scale identified above for each assay:
    - Pre-nOPV2 immunity (type 2 fecal neutralizing activity and type-2-specific IgA; pharyngeal and nasal samples) and stool viral shedding (viral titer) at each post-nOPV2 sample collection/testing time point, and stool SIE)

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- Type 2 IgA vs type 2 fecal neutralizing titer for each post-nOPV2 collection/testing time point; pharyngeal, stool, and nasal samples; within sample type and between sample type
- Type 2 stool viral shedding (viral titer) at D7 post-challenge and all D14 measures of mucosal immunity
- Pre-nOPV2 type 2 serum neutralizing antibody and post-nOPV2 measures of mucosal immunity (type 2 neutralizing activity and type-2-specific and total IgA)
- Correlations will be computed for all participants combined, and separately for each group, displayed on one figure with all relevant points, but color and shape distinguishing the groups.
- Correlation figures will be recomputed using the peak of the post-nOPV2 value across time point, where relevant

- A logistic regression will examine, for each post-challenge time point independently, the relationship between positivity in the mucosal neutralizing assay relative to pre-nOPV2 log2 serum neutralizing antibody.

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6. SERUM NEUTRALIZING ANTIBODY ANALYSIS

Immunogenicity will be evaluated primarily in the PPP. Serum neutralizing antibody analysis will be repeated for the TVP.

- Serum neutralizing antibody analyses will be descriptive in nature, and no comparisons between groups are deemed relevant for secondary and exploratory endpoints, given the large anticipated difference in pre-challenge type 2 immunity expected to be conferred by IPV relative to bOPV. For descriptive analyses, at each pre-and post-vaccination time point where neutralizing antibody titers are obtained:
    - Cumulative seroconversion rates (with 95% exact CIs) will be computed (post-baseline time points only).
        - Seroconversion is defined as seropositive (titer ≥1:8) in those initially seronegative, or among those initially seropositive, a minimum 4-fold rise in titer. Seroconversion will be computed from D0 for all subjects.
        - Seroconversion will be computed only among those with seroconversion possible to observe (i.e. restricted to those with D0 antibody titer ≤8.5 $\log_2$)
    - Seroprotection rates (with 95% exact CIs) will be computed.
        - Seroprotection is defined as poliovirus type-2-specific titers ≥1:8.
    - Geometric mean and median neutralizing antibody titers (with two-sided 95% CIs) will be computed. Mean titers will be estimated on the $\log_2$ scale using likelihood-based estimation, using a normal error assumption, to accommodate censoring at assay LLOQ (2.5 $\log_2$) / ULOQ (10.5 $\log_2$), then reverse-transformed to obtain the geometric mean and corresponding CI. Median titers will be analyzed on the $\log_2$ scale and paired with two-sided 95% percentile bootstrap-based CIs.
    - Geometric mean fold rises and corresponding two-sided 95% confidence intervals will be computed by using the antilog (base 2) transformation on the mean and CI derived on the $\log_2$ scale of the differences between post-baseline and baseline nAb titers, using the *t* distribution method.
        - A supplementary analysis of fold-rises will be performed among those with a value >LLOQ and <ULOQ for both the pre-vaccination and post-vaccination samples.
    - Plots of the reverse cumulative distribution of antibody titers will be generated for each group.
- Generally, samples collected within-window according to the schedule of events will be eligible for analysis, but minor deviations will be considered for eligibility in the blinded data review meeting.
- Type 1 and type 3 serum neutralizing antibody will be summarized for type 2, as a post hoc analysis.

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7. SAFETY ANALYSIS

The EP and TVP will be used for safety analysis. Only treatment-emergent adverse events will be summarized (those occurring after any study vaccination), with all events in the EP identified in listings. The listing will include a TVP and a post-challenge yes/no column. The vital signs table will be created only for EP.

- All SAEs/IMEs will be summarized by group and overall
- SAEs/IMEs prior to and after nOPV2 will be summarized by group and overall
    - If it is not possible to determine if SAE/IME was prior to/after nOPV2 (e.g. in case of missing dates) it will be counted as “after”
- SAE/IME will be presented by type (seriousness criteria)
- SAE/IME will be presented by SOC and PT
- SAE/IME will be presented by severity and by causal association
- SAEs considered to be causally associated to the vaccination will be termed serious adverse reactions (SARs).
- Medically attended SAEs/IMEs will be presented by SOC and PT.
- Clinically relevant vital signs will be summarized over time, and all vital signs will be listed.
- Subjects receiving other vaccinations not per protocol (non-study polio vaccinations, or non-NIP vaccinations) will contribute safety data prior to administration of the non-study/non-NIP vaccination, although they will still be followed for safety for the remaining study duration, with data presented in separate summaries.
- Subjects receiving other vaccinations not per protocol will be flagged as such if “Any new vaccination history (excluding any vaccinations administered as part of the study) since Visit 1” from the eCRF page “Vaccination History” is answered with “yes”. The date of the first respective vaccination will be used as a cut-off for the safety data. All safety data prior or on the day of the respective vaccination will be flagged as “Prior or on vaccination not per protocol”. All safety data after the day of the respective vaccination will be flagged as “After vaccination not per protocol”. This only applies to vaccinations recorded after the first study vaccination.

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8. SHELLS OF TABLES, DATA LISTINGS AND FIGURES

For this analysis, shells of tables, data listings and figures (mock TLF) will be generated. The mock TLFs will serve as a basis for programming. The following differences between the mock TLFs and the final analysis will be allowed:

- Capital versus non-capital letters
- Empty rows and Blanks
- Layout: column width and size
- Order of items within a list, new categories, minor changes of labels
- Amendment of explaining footnotes

No Table of Contents will be provided for mock TLFs as not all tables, data listings and figures may be produced. The mock TLFs will also be finalized.

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