Clinical Study Protocol IPV-005-ABMG

17 November 2023 (Version 3.0)

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

Product	Novel Oral Poliomyelitis Type-2 Vaccine (live, attenuated) [strain S2/cre5/S15domV/rec1/hifi3]
Protocol Number	IPV-005-ABMG
Clinical Phase	IV
ClinicalTrials.gov Number	NCT05677256
Clinical Indication	Polio vaccine immunization
Issue Date	Version 3.0 (17 November 2023)

Sponsor	FIDEC – Fighting Infectious Diseases in Emerging Countries (with grant support from the Bill and Melinda Gates Foundation) 1390 S Dixie Hwy Suite 1106 Coral Gables, FL 33146 USA
Sponsor Representative	Dr. Ricardo Rüttimann Tel + 1 305 854 0075, Fax + 1 305 856 7847

SIGNATURES

Signature of Sponsor Representative

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

Name: Dr. Ricardo Rüttimann
Tel + 1 305 854 0075, Fax + 1 305 856 7847

This Clinical Study Protocol has been reviewed and approved by the Sponsor in order to ensure compliance with Good Clinical Practice.

Signature:

Date:

Signature of Biostatistician

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

Name: Chris Gast, Ph.D.
Statistical Consultant
Sammamish, WA, USA

This Clinical Study Protocol has been reviewed and approved by the Biostatistician in order to ensure compliance with Good Clinical Practice and the Statistical Analysis Plan.

Signature:

Date:

Signatures of Investigators

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

Name: Dr. K. Zaman, Senior Scientist

Affiliation: International Center for Diarrhoeal Disease Research, Bangladesh (icddr,b)

Address: 68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka- 1212, Bangladesh

I have read this Clinical Study Protocol and agree that it contains all information necessary for proper conduct of the study. I will carry out the study as outlined herein and will complete the study within the designated time.

Signature:

Date:

PROTOCOL HISTORY

Protocol History FIDEC – IPV-005-ABMG			
Document	**Issue Date**	**Amendment Type**	**Comments**
Initial Clinical Study Protocol	Version 1.0 (09 December 2022)	-	-
Protocol Amendment # 1	Version 2.0 (18 June 2023)	Administrative	Change of investigational site and location Adjustment of minor text inconsistencies
Protocol Amendment # 2	Version 3.0 (17 Nov 2023)	Administrative	Local supply and change of IPV brand. Adjustment of minor text inconsistencies.

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PROTOCOL SYNOPSIS

Study Title	**A Phase IV open-label, randomized, parallel-group study to evaluate pharyngeal immunity to poliovirus type-2 in healthy bOPV- versus IPV- vaccinated infants.**		
Product	Trivalent inactivated poliovirus vaccine (manufactured by Bilthoven Biologicals) Novel Oral Poliomyelitis Type-2 Vaccine (live, attenuated) [strainS2/cre5/S15domV/rec1/ hifi3] (manufactured by Bio Farma)	**Clinical Phase**	IV
Protocol Number **ClinicalTrial.gov Number**	IPV-005-ABMG NCT05677256	**Indication**	Polio vaccine immunization

Sponsor	FIDEC
Sponsor Representative	Dr. Ricardo Rüttimann
Clinical Center	International Center for Diarrhoeal Disease Research, Bangladesh (icddr,b)

Objectives:

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 inactivated poliovirus vaccine (IPV), the other group (Group B) with 3 doses of bivalent OPV (bOPV) as a primary schedule at 6, 10, 14 weeks of age.

Secondary objectives 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 serious adverse events (SAEs) and important medical events (IMEs) following administration of IPV, bOPV and nOPV2.

Exploratory objectives 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.

Study Endpoints:

Primary

- 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.

Secondary

Immunogenicity:

- Pharyngeal neutralizing antibody activity, and total and poliovirus type-2–specific concentrations of 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 by causal association.

Exploratory

- 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.

Overview of the Study Design:

This will be 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 will be 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 will 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).

Study Duration:

Each infant will participate for a duration of up to approximately 5 months.

Study Population:

This study will be performed with the participation of approximately 500 infants aged 6 to 8 weeks who have not received prior vaccination against poliomyelitis.

Eligibility Criteria:

Inclusion Criteria:

1. Infants aged 6 to 8 weeks with birth weight > 2,500 g.
2. Healthy infants without obvious medical conditions like immunodeficiency diseases, severe congenital malformations, severe neurological diseases or any other disease that require high doses of corticosteroids or immunotherapies that preclude the subject from participating in the study as established by the medical history and physical examination.
3. Written informed consent obtained from both parents or legal guardian(s) as per country regulations.

Exclusion Criteria:

1. Infants who have received previous vaccination against poliomyelitis.
2. Any confirmed or suspected immunosuppressive or known immunodeficient condition including human immunodeficiency virus infection in the potential participant or any member of the subject's household.
3. Family history of congenital or hereditary immunodeficiency.
4. Major congenital defects or serious uncontrolled chronic illness (neurologic, pulmonary, gastrointestinal, hepatic, renal, or endocrine).
5. Known allergy to any component of the study vaccines or to any antibiotics that share molecular composition with a component of the study vaccines.
6. Uncontrolled coagulopathy or blood disorder contraindicating intramuscular injections (of IPV)
7. Administration of immunoglobulins and/or any blood products since birth or planned administration during the study period.
8. Acute severe febrile illness on the day of vaccination deemed by the Investigator to be a contraindication for vaccination (the child can be included at a later time if within age window and all inclusion criteria are met.).
9. Subject who, in the opinion of the Investigator, is unlikely to comply with the protocol or is inappropriate to be included in the study for the safety or the benefit-risk ratio of the subject.
10. Infants from multiple births or born prematurely (< 37 weeks of gestation).

Test Product, Dose, Mode of Administration:

IPV vaccine (manufactured by Bilthoven Biologicals. The vaccine is a suspension of IPV/0.5mL that will be administered intramuscularly.

nOPV2 vaccine (manufactured by Bio Farma, Indonesia) contains a live attenuated type-2 modified Sabin strain (S2/cre5/S15domV/rec1/hifi3) propagated in Vero cells. Each dose of the nOPV2 vaccine contains no less than $10^{5.0}$ 50% cell culture infective dose ($CCID_{50}$) (0.1 mL, 2 drops). The vaccine will be administered orally.

Statistical Methods:

Sample size

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

A total of 250 infants per group is needed, assuming a reduction of at least 50% in pharyngeal viral shedding rate in IPV recipients with respect to bOPV recipients and taking into account potential loss to follow up.

Viral Shedding

Presence/absence of type-2 virus in pharyngeal and stool samples will be summarized descriptively using counts and proportions among those contributing eligible samples, including exact confidence intervals for the percentage. Between-group inference will be conducted by computing the two-sided 95% confidence interval (CI) for the relative risk (IPV/bOPV) for each time point, and including cross-time point composite variables (e.g., any post-challenge shedding, any post-challenge shedding in the first week, etc.). Supplementary analyses will include a corresponding confidence interval for the risk difference. Quantity of virus in stool, evaluated in a $CCID_{50}$ culture assay, will be summarized descriptively on the $\log_{10}$ scale using the median with percentile-bootstrap based two-sided 95% CIs, with between-group inference performed by the Wilcoxon test, and supplemented with the difference in medians and corresponding two-sided 95% percentile-bootstrap based confidence intervals. Positivity in the culture assay (value > lower limit of quantification [LLOQ]) will be summarized as for positivity in the PCR assay.

Immunogenicity

At each pre-and post-vaccination time point where serum neutralizing antibody titers are obtained:

- Cumulative SC rates (with 95% exact CIs) will be computed from D0 at post-vaccination time points.
- Seroprotection rates (with 95% exact CIs) will be computed
- Geometric mean and median neutralizing antibody titers (with 95% CIs) will be computed

Intestinal mucosal immunity will be evaluated by measuring poliovirus-specific neutralising activity and IgA in stool samples. Poliovirus type-specific neutralising activity will be determined by limiting dilution inhibition of luciferase-expressing wild type-derived polio pseudoviruses in vitro and presented as the reciprocal of the highest sample dilution needed to achieve 60% neutralization as compared with control wells with no added sample. Poliovirus-specific stool neutralization titers of less than four will be considered undetectable and recorded as two. Samples will be serially diluted twofold prior to incubation with virus and then added to target cells. Luciferase expression will be quantified in cell lysates after 24 hours at 37°C, using either the Renilla or NanoGlo Luciferase assay systems. Relative light units will be measured on a BioTek Synergy 2 microplate reader. Antibody titer will be calculated as the reciprocal of the highest sample dilution to yield a 60% reduction in relative light units. Total concentrations of IgA in pharyngeal and stool specimens are quantified, relative to a serum standard, using a multiplex Luminex-based microsphere assay developed by coupling monovalent IPVs to fluorescently coded magnetic microspheres.

Safety

Safety parameters will be tabulated and analyzed descriptively.

The original terms used in the designated sections of the electronic case report forms (eCRFs) by the study investigators to identify SAEs and IMEs will be fully described and coded according to the Medical Dictionary for Regulatory Activities.

SAEs and IMEs will be summarized by group, type, by severity and occurrence in relation to vaccination, and overall. Determination of causal association of SAEs and IMEs to the study vaccines will be assigned using the individual causality assessment algorithm published by the World Health Organization (WHO).

Summaries, listings, and narratives will be provided, as appropriate.

TIME AND EVENTS SCHEDULE

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[h]** **(+ 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..
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.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

List of Abbreviations

AE	Adverse Event
AEFI	Adverse Event Following Immunization
bOPV	bivalent Oral Poliovirus Vaccine
$CCID_{50}$	50% cell culture infective dose
CI	Confidence Interval
CRO	Contract Research Organization
cVDPV	Circulating Vaccine-Derived Poliovirus
cVDPV2	Circulating Vaccine-Derived Poliovirus type-2
D	Day
DNA	Deoxyribonucleic acid
DTPw-HB-Hib	Diphtheria, Tetanus, Whole-cell Pertussis, Hepatitis B, *Haemophilus influenzae* type b vaccine
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EUL	Emergency Use Listing
GCP	Good Clinical Practice
GPEI	Global Polio Eradication Initiative
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IME	Important Medical Event
IPV	Inactivated Poliovirus Vaccine
IRB	Institutional Review Board
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
NIP	National Immunization Program
nOPV2	Novel Oral Poliovirus Vaccine type-2
OPV	Oral Poliovirus Vaccine
PCV	Pneumococcal Conjugate Vaccine
PP	Per-Protocol
RNA	Ribonucleic acid
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAGE	Strategic Advisory Group of Experts
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction (SAE related to vaccination)
SIE	Shedding Index Endpoint
SUSAR	Suspected Unexpected Serious Adverse Reaction
TVP	Total Vaccinated Population
ULOQ	Upper Limit of Quantification

VAPP	Vaccine-Associated Paralytic Poliomyelitis
VSP	Viral Shedding Population
WHO	World Health Organization

STUDY ADMINISTRATIVE STRUCTURE

SPONSOR	**FIDEC** – Fighting Infectious Diseases in Emerging Countries (with grant support from the Bill and Melinda Gates Foundation) 1390 S Dixie Hwy Suite 1106 Coral Gables, FL 33146 USA
Medical Expert :	Ricardo Rüttimann MD
BIOSTATISTICIAN	**Chris Gast, Ph.D.** Statistical Consultant Sammamish, WA, USA
MONITORING	**DiagnoSearch Life Sciences** Flat # 102, 1st Floor Hiranandani Gardens Powai, Mumbai, Maharashtra 400076 India
MANUFACTURING (nOPV2)	**Bio Farma** JL. Pasteur No.28, Pasteur, Sukajadi, Kota Bandung, Jawa Barat 40161, Indonesia
MANUFACTURING (IPV)	**Bilthoven Biologicals** Antonie van Leeuwenhoeklaan 9 -13, 3721 MA Bilthoven, The Netherlands
CLINICAL ANALYSES	**Centers for Disease Control and Prevention** Polio and Picornavirus Laboratory Branch 1600 Clifton Road, NE MS-G17, Room 6053 Atlanta, GA 30333 USA Contact: Bernardo A. Mainou, Ph.D. Team Lead, Population Immunity Laboratory **Ackerman Lab** Dartmouth College Thayer School of Engineering 15 Thayer Dr Hanover, NH 03755 USA

	Infectious Disease Laboratory, Borwell 314W Dartmouth Hitchcock Health 1 Medical Center Dr Lebanon, NH 03756 USA Contact: Wendy Wieland-Alter
DATA MANAGEMENT, BIOSTATISTICS AND PHARMACOVIGILANCE	**Assign Data Management and Biostatistics (DMB) GmbH** Stadlweg 23, 6020 Innsbruck, Austria

1. INTRODUCTION

1.1 BACKGROUND INFORMATION

In 2013 the Global Polio Eradication Initiative (GPEI) launched the Polio Eradication and Endgame Strategic Plan with the objective to end all polio disease globally. The 4 main objectives of the Polio Eradication and Endgame Strategic Plan are: to detect and interrupt all poliovirus transmission, to strengthen immunization systems and withdraw oral polio vaccine (OPV), to contain poliovirus and certify interruption of transmission and legacy planning [1]. The global effort to eradicate polio has made significant progress, with polio remaining endemic in only two countries — Afghanistan and Pakistan [2].

For a long time, trivalent OPV, containing poliovirus types 1, 2, and 3, was the preferred vaccine for polio control and eradication. Global use of this vaccine enabled the eradication of wild-type poliovirus type 2 and type 3. However, in rare circumstances, use of OPV can result in vaccine-associated paralytic poliomyelitis and in the emergence of circulating vaccine-derived poliovirus (cVDPV) in populations with low immunization coverage. The type-2 strain has been associated with the majority of cases of cVDPV. As part of the Polio Eradication and Endgame Strategic Plan, the Strategic Advisory Group of Experts on immunization (SAGE) called for a globally synchronized switch from trivalent OPV to bivalent OPV (bOPV) in routine immunization programs (i.e., withdrawal of OPV2 to reduce circulating vaccine-derived poliovirus type-2 [cVDPV2]) as the first step towards complete withdrawal of all oral polio vaccines [3]. To mitigate the risks associated with this switch, the SAGE recommended addition of at least one dose of inactivated poliovirus vaccine (IPV) to routine immunization programs. In some countries, the switch from using trivalent OPV to trivalent IPV (i.e. without including a temporary recommendation for bOPV) was accomplished as soon as poliovirus was declared eradicated from the region. IPV use cannot generate cVDPVs but it is not clear if IPV induces mucosal immunity, so IPV is less effective than OPV in interrupting transmission in settings where transmission is predominantly via the fecal–oral route [4, 5].

Indeed, since global cessation of OPV2 use in May 2016, multiple outbreaks of cVDPV2 have been observed, in addition to sporadic cases of disease. In 2022 (up to November 15), 433 cases of cVDPV2 have been reported, the majority of which have occurred in Africa (with the largest number -188 cases- reported in the Democratic Republic of the Congo) [6]. cVDPV2 has also been isolated from environmental samples from many countries and more recently from routinely-acquired wastewater samples from countries which have long used IPV-only schedules for primary infant vaccination (i.e. the United Kingdom [7], the United States [8], and Israel [9] and which have been polio-free for several decades.

It has been shown that IPV-only schedules provide limited primary intestinal immunity and have no impact on the proportion of fecal shedding in first ~2 weeks post OPV challenge, but might reduce the quantity and duration of poliovirus shedding [4]. Pharyngeal immunity induced by IPV-only schedules, as measured by the levels of secretory IgA or IgG in secretions, are reduced relative to the levels of these antibodies induced by OPV-only schedules. Pharyngeal immunity as measured by resistance to a

challenge by OPV viruses appears equivalent to such immunity induced by OPV. It should however be noted that the data are sparse and somewhat contradictory (reviewed in [5]).

1.2 OVERALL RATIONALE FOR THE STUDY

In the light of the switch from OPV to IPV and the continued presence of cVDPV2 in many countries, it is important to understand and quantify the impact of IPV on pharyngeal mucosal immunity, to inform whether and to what extent the mucosal and humoral immune response following IPV could reduce transmission and spread.

This study will assess the effect of vaccination with IPV in parallel with poliovirus type-2 naïve infants (infants having received bOPV) on the pharyngeal and fecal shedding and the induction of immunity following type-2 poliovirus challenge. This understanding would provide critical information on the potential use of IPV in specific settings to interrupt transmission / reduce spread. The results from this study may potentially have important consequences on public health policy in countries which use IPV for infant priming, as they will help to show the extent to which a type-2 mucosal immunity gap remains following a primary series of IPV.

1.3 RISK BENEFIT ANALYSIS

1.3.1 *Potential Risks*

The IPV and bOPV used for primary vaccination are commercially available and have been approved by the Ministry of Health of Bangladesh for the immunization of infants and children.

The novel oral poliovirus vaccine type-2 (nOPV2) received an Emergency Use Listing (EUL) from the World Health Organization (WHO) in November 2020 [10] and was initially used to combat outbreaks of cVDPVs in a number of African and East Mediterranean countries. As of October 2022, 500 million doses of nOPV2 had been administered in 23 countries [11].

Experience in the clinical trial setting has been obtained in several trials [12, 13]. In the M5 study [14], solicited adverse events (AEs) in children and infants following administration of the nOPV2 vaccine included abnormal crying, drowsiness, fever, irritability, loss of appetite, and vomiting. No serious adverse events (SAEs) or important medical events (IMEs) considered related to vaccination were reported for nOPV2. Severe AEs considered related to vaccination were reported, the most frequent of which was abnormal crying. All were transient, usually resolving within 3 days. No clinically relevant differences were observed in SAEs, AEs or IMEs related to vaccination between nOPV2 and mOPV2 groups. SAEs that entailed hospitalization (mild bronchitis and moderate pneumonia) were not considered related to study vaccine. There were no AEs that led to death, to withdrawal, or to permanent study discontinuation. Preliminary assessment of currently ongoing trials have not evidenced any vaccine-related safety issues [15].

The attenuated strains of poliovirus multiply in the gut. The fecal excretion of the vaccine virus may persist for several weeks, especially in unprotected populations, and may also be transmissible to the contacts of the vaccinees contributing to the herd immunity against

polio. In populations with low immunity, a close contact with a recently vaccinated subject may very rarely be at risk of developing paralytic poliomyelitis caused by the vaccine (VAPP). Clinical data from the Phase I and Phase II adult studies (M4a and M4) and the Phase II pediatric study (M5) support the improvements in genetic and phenotypic stability of the nOPV2 vaccine, with no meaningful increase in neurovirulence observed [12, 13, 14, 16, 17]. Genetic stability analyses do not suggest any anticipated increase in the likelihood of VAPP with the vaccine which will be used in this study. In addition, as part of the nOPV2 roll-out under EUL, samples are collected from cases of acute flaccid paralysis and from environmental systems in countries where nOPV2 is being used. All isolates have been determined to have no consequence on public health [15], with the exception of some cVDPVs associated with nOPV2, through recombination, but at a much lower rate than would have been expected for the Sabin 2 vaccine [23].

1.3.2 *Potential Benefits*

Infants who will be vaccinated with nOPV2 in this study will potentially develop high levels of immunity against poliovirus type-2. Indeed, a key benefit of nOPV2 is the intestinal immunity (in addition to humoral immunity) against type-2 poliovirus disease which is conferred through vaccination by the oral route.

If the data are conclusive, the results will assist in the design of a new polio vaccination strategies that may enhance the effectiveness of the vaccination campaigns to prevent emergence and further spread of cVDPV2.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of the study is to compare the poliovirus type-2 pharyngeal mucosal excretion 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.

2.2 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 of IPV, bOPV and nOPV2.

2.3 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.

3. STUDY ENDPOINTS

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.

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.

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.

4. STUDY DESIGN

4.1 OVERVIEW OF THE STUDY DESIGN

This will be a multicenter, randomized, controlled, 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 will be included in the study 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 will 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).

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, 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.

4.2 DISCUSSION OF STUDY DESIGN

Subjects will be randomized in a 1:1 ratio to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across groups.

The study will be conducted in an open-label manner. The evaluation of immunogenicity endpoints is not expected to be biased given their objective nature. 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.

The safety profile of vaccines used under the NIP is known and the safety profile of nOPV2 has also been shown to be good (see Section 1.3.1), so that the assessment of vaccine safety in this study will be limited to SAEs and IMEs. Due to the low frequency of these events and their nature, which allows an objective reporting, blinding of the investigator/study team to treatment attribution is considered inconsequential.

In addition to the vaccination with IPV or bOPV, infants will receive the DTPw-HB-Hib and PCV vaccine as recommended by the local NIP.

5. SELECTION OF STUDY POPULATION

For details on the sample size calculation, please refer to Section 10.2.

5.1 INCLUSION CRITERIA

1. Infants aged 6 to 8 weeks with birth weight >2,500 g.
2. Healthy infants without obvious medical conditions like immunodeficiency diseases, severe congenital malformations, severe neurological diseases or any other disease that require high doses of corticosteroids or immunotherapies that preclude the subject from participating in the study as established by the medical history and physical examination.
3. Written informed consent obtained from both parents or legal guardian(s) as per country regulations.

5.2 EXCLUSION CRITERIA

Subjects meeting any of the following criteria are excluded from participation in this study:

1. Infants who have received previous vaccination against poliomyelitis.
2. Any confirmed or suspected immunosuppressive or known immunodeficient condition including human immunodeficiency virus infection in the potential participant or any member of the subject's household.
3. Family history of congenital or hereditary immunodeficiency.
4. Major congenital defects or serious uncontrolled chronic illness (neurologic, pulmonary, gastrointestinal, hepatic, renal, or endocrine).
5. Known allergy to any component of the study vaccines or to any antibiotics that share molecular composition with a component of the study vaccines.
6. Uncontrolled coagulopathy or blood disorder contraindicating intramuscular injections (of IPV)
7. Administration of immunoglobulins and/or any blood products since birth or planned administration during the study period.
8. Acute severe febrile illness on the day of vaccination deemed by the Investigator to be a contraindication for vaccination (the child can be included at a later time if within age window and all inclusion criteria are met.).
9. Subject who, in the opinion of the Investigator, is unlikely to comply with the protocol or is inappropriate to be included in the study for the safety or the benefit-risk ratio of the subject.

10. Infants from multiple births or born prematurely (< 37 weeks of gestation).

5.3 CRITERIA FOR ELIMINATION FROM THE PER-PROTOCOL POPULATION

A review of protocol deviations, including, among others, vaccinations occurring out of window, administration of other polio vaccines, and OPV administration to household members, will be conducted prior to database lock based on predefined criteria. Subjects with major protocol deviations detected via monitoring or automated database search which are deemed likely to interfere with the assessment of endpoints will be removed from the Per-Protocol Population at the time of occurrence of the first such deviation. 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.

5.4 ADDITIONAL CONSTRAINTS

Information on prohibited therapies can be found in Section 7.

6. VACCINES

Study vaccines:

The IPV vaccine is a suspension of IPV/0.5mL containing inactivated poliovirus type 1 (Mahoney strain), type 2 (MEF-1 strain) and type 3 (Saukett strain). Each of the three strains of poliovirus is individually grown in Vero cells. The vaccine is manufactured by Bilthoven Biologicals.

The nOPV2 vaccine contains a live attenuated type-2 modified Sabin strain (S2/cre5/S15domV/rec1/hifi3) propagated in Vero cells and is produced by Bio Farma, Indonesia.

Other vaccines:

DTPw-HB-Hib, PCV, and bOPV vaccines will be provided by the NIP.

6.1 PHYSICAL DESCRIPTION OF THE STUDY VACCINES

The IPV vaccine is a suspension for injection presented in vials for intramuscularly administration. The vaccine color varies from orange-yellow to orange-red.

The nOPV2 vaccine will be provided to the sites in vials presented as an aqueous yellow-red solution for oral use. The vaccine will be administered orally.

6.2 CHARACTERIZATON OF VACCINE LOTS

Each dose of the IPV vaccine will contain no less than 40 D-antigen units of poliovirus type 1 (Mahoney strain), 8 D-antigen units of type 2 (MEF-1 strain) and 32 D-antigen units of type 3 (Saukett strain).

Each dose of the nOPV2 vaccine will contain no less than $10^{5.0}$ 50% cell culture infective dose ($CCID_{50}$) of poliovirus type-2 (0.1 mL, 2 drops).

6.3 OTHER MEDICATION ADMINISTERED IN THE STUDY

The following concomitant vaccines DTPw-HB-Hib and PCV-10 will be administered in the opposite thigh to which IPV will be applied, according to the time and events schedule. bOPV vaccine will be administered orally.

These will be administered according to the manufacturer's instructions.

6.4 PACKAGING AND LABELING

nOPV2 and IPV vaccines will be labelled and packed according to local law and regulatory requirements.

Detailed information on the packaging and labeling is specified in the Investigational Product Handling Instructions for the study.

6.5 STORAGE AND VACCINE ACCOUNTABILITY

The Investigator (or his/her designee) is responsible for the safe storage of all study vaccines assigned to the clinical site, in a locked, secure storage facility with access limited to those individuals authorized to dispense the study vaccine, and maintained within the appropriate ranges of temperature. All study vaccines must be stored as specified at delivery and in the original packaging.

The IPV vaccine should be stored at +2 to + 8 °C and is not to be frozen. Once opened, the vaccine can be used up to 28 days provided it is stored at +2 to + 8 °C.

The nOPV2 vaccine should be stored in a freezer at approximately –20°C (±5 °C). After thawing, the vaccine can be stored at +2 to +8°C for up to 2 weeks. Once opened, a vial must be used within 48 hours, when it expires.

Regular temperature logging of the study vaccine storage room at the clinical site should be performed. In case a deviation in storage conditions should occur, the clinical site must not further dispense the affected study vaccines and notify the Sponsor.

The Investigator is responsible for ensuring that all study vaccines received at the clinical site are inventoried and accounted for throughout the study.

Study vaccines should be dispensed under the supervision of the Investigator, a qualified member of the clinical staff, or by a hospital/clinic pharmacist. The Investigator must maintain accurate records demonstrating date and amount of vaccine administered to and by whom. Study vaccines will be supplied only to subjects participating in the study.

The Sponsor's designated site monitor will periodically check the supplies of study vaccines held by the Investigator or pharmacist to ensure accountability and appropriate storage conditions of all study vaccines used.

Unused study vaccine must be available for verification by the site monitor during on-site monitoring visits. After the last visit of the last subject in the study, any unused vaccine will be returned to the manufacturer, and any used vaccine will be destroyed at the clinical site with the Sponsor's written permission (in this case a certificate of destruction will be provided and filed in the Trial Master File [TMF]).

6.6 RANDOMIZATION AND BLINDING

This is an 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.

6.7 DOSE AND ADMINISTRATION

The dose of IPV vaccine should be 0.5 mL administered strictly intramuscularly in the anterolateral aspect of the thigh, using a standard needle and syringe.
One dose of nOPV2 vaccine (0.1 mL) is contained in two drops, which are delivered using the dropper supplied with the vaccine.

Other study vaccines provided under the NIP should be administered as per the manufacturer's instructions.

The vaccinees will remain under medical supervision for at least 30 min following the administration of study vaccine.

6.8 COMPLIANCE

All vaccine administrations in the study will be supervised by the Investigator or his/her designee.

7. PRIOR AND CONCOMITANT THERAPY

All therapies (prescriptions and over-the-counter medications) other than the study vaccines administered from informed consent until the last study visit must be recorded in the source documents and in the concomitant therapy section of the electronic case report form (eCRF) (name of the drug, dosage, route and dates of administration).

The Sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

There will be no restrictions in using concomitant therapies except for any medication that has a potential effect on the immune system in the opinion of the Investigator.

Infants will receive routine vaccinations (DTPw-HB-Hib and PCV vaccine) according to the local NIP, which will be provided by the NIP.

8. ASSESSMENTS

8.1 TIMING OF ASSESSMENTS

An overview of the timing of vaccine administration and assessments is given in the Time and Events Schedule.

Parents/guardian(s) will be given a full explanation of the nature of the study and written informed consent (approved by the local ethics committee) will be obtained from parents/guardian(s) according to local requirements before any study-related assessment will be carried out.

SAEs, IMEs and the administration of concomitant medication will be monitored continuously from informed consent until the last study-related activity at Day 56.

After informed consent process has been completed, the assessments will proceed as outlined in the Time and Events Schedule. The subjects will be kept under medical supervision for at least 30 minutes after administration of the study vaccines. The subjects will also receive routine vaccinations according to the NIP, as outlined in the Time and Events Schedule.

Unscheduled visits can be planned, for instance to obtain additional information to ensure the safety of the subject. Additional blood and urine samples may be taken at the discretion of the Investigator if deemed necessary for safety observation.

Findings made during unscheduled visits should be reported in the source documents and in the designated sections of the eCRF.

8.2 VIRAL SHEDDING

Pharyngeal and stool samples for the determination of the presence/absence of poliovirus type 2, viral titers (stool only), and mucosal immune response will be taken at the time points specified in the Time and Events Schedule.

Detailed descriptions of the collection, handling, transport and processing of the biological samples will be included in the laboratory manual.

8.3 IMMUNOGENICITY

8.3.1 *Immunogenicity Variables*

Blood, pharyngeal and stool samples for the determination of neutralizing type-2 poliovirus antibodies will be taken at the time points specified in the Time and Events Schedule.

Up to 4 mL of blood will be taken at each of the 3 pre-/post-vaccination time points. The total amount of blood to be taken for this study is thus approximately 12 mL. Neutralizing antibodies against poliovirus in blood samples will be determined using a sero-neutralization assay [19].

Total and poliovirus type-2 neutralizing antibody activity and IgA concentrations in pharyngeal samples will be measured at Dartmouth College and Dartmouth Hickcock

Health by limiting dilution inhibition of a luciferase-expressing type-2–specific wild-type–derived polio pseudovirus in vitro, expressed as the titer needed to achieve 60% neutralization. Titers <4 will be considered undetectable. Total IgA and poliovirus type-2–specific concentrations in pharyngeal specimens will be assessed relative to a serum standard, using a multiplex Luminex-based microsphere assay developed by coupling monovalent IPVs to fluorescently-coded magnetic microspheres, with relative concentration units below the limit of detection (≤10).

Samples that remain after protocol-specific assessments have been performed may be used for further exploratory work on polio, including cryofreezing for future immunological studies. No human deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) analysis will be performed.

8.3.2 *Immunogenicity Criteria*

The following endpoints will based on neutralizing type-2 poliovirus antibody titers measured in serum:

- Seroconversion: 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.
- Seroprotection: defined as poliovirus type-2-specific titers ≥1:8.
- Geometric mean and median titers.

The neutralizing type-2 poliovirus antibody titers in pharyngeal samples (for all samples for which virus titers can be estimated) will be expressed as geometric mean and median titers.

8.4 SAFETY EVALUATIONS

The safety assessment in this study will be based on SAEs and IMEs.

SAEs and IMEs will be monitored continuously from the time of informed consent signature until D56. At regular intervals during the study, parents will be asked non-leading questions to determine the occurrence of any SAEs/IMEs.

For detailed definitions and reporting procedures of SAEs/IMEs, see Sections 10.3.1.

8.5 EXPLORATORY EVALUATIONS

Summaries of the detection of poliovirus type 2 via RT-PCR and viral titer ($CCID_{50}$/g of stool) among those positive for virus will be computed by group and time point, for stool samples. An SIE will be calculated as the average of log_{10}-transformed values of viral concentration in stool samples as determined using RT-PCR (viral identity) and $CCID_{50}$ (titer) from stool samples taken following nOPV2 challenge, and this index will be summarized by group.

Descriptive analysis and plots of the reverse cumulative distribution of the SIE will be generated.

Total antibody and IgA concentrations in stool samples will be assessed as described in Section 8.3.1.

8.6 APPROPRIATENESS OF MEASUREMENTS

The assessments which will be made in this study are either standard or are scientifically justified.

Each biological assay contains a reference material used as a control. Additional re-test of some samples may be conducted to ascertain the presence of temporal variability in assay results.

9. STUDY TERMINATION/COMPLETION

9.1 STUDY COMPLETION

9.1.1 *Subject Completion*

A subject will be considered to have completed the study if all study-related procedures have been completed for them 56 days after administration of the nOPV2 challenge dose and they are not withdrawn.

9.1.2 *Study Completion Date*

The study completion date is considered to be the date on which the final serology analysis is available.

9.2 REMOVAL OF SUBJECTS FROM THE STUDY

Parents(s)/guardian(s) have the right to withdraw subjects from the study at any time for any reason, including personal reasons. A subject can be withdrawn without giving a reason. The Investigator should however try to find out why a subject is withdrawn from the study and document the reason for withdrawal in the source documents and on the eCRF.

Subjects **may** be withdrawn from the study in the event of:

- A severe AE or an SAE;
- Difficulties in obtaining blood or other samples;
- Failure of the subject and/or subject's parents/guardian(s) to comply with the protocol requirements or to cooperate with the Investigator.

Subjects **must** be withdrawn from the study in the event of:

- Withdrawal of consent by parents/guardian(s);
- For safety reasons, if, in the Investigator's opinion, in the best interest of the subject.

In the event of a subject being withdrawn from the study, the monitor and Sponsor should be informed: in the event of withdrawal due to an SAE (for details on AE reporting see Section 10.3), the Sponsor should be notified within 24 hours; in the event of withdrawal for other reasons, the Sponsor should be notified within 2 days from the event.

If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned.

Subjects who are withdrawn from the study prior to completion of the scheduled study procedures for any reason (e.g. SAE, withdrawal of consent) should be invited to complete the assessments as much as possible: as long as the subjects' parents/guardian(s) consents, all relevant assessments of the day on which the subject withdrew from the study should be completed, at least those related to safety. In case of an SAE/IME, the appropriate follow-up will be done.

Subjects who are withdrawn from the study will not be replaced.

10. STATISTICAL METHODS

10.1 STATISTICAL ANALYSIS

All statistical analyses will be performed by Assign Data Management and Biostatistics GmbH, Stadlweg 23, 6020 Innsbruck using SAS® software (SAS Institute Inc., Cary, NC, USA) under the supervision and responsibility of the Sponsor.

This document describes the basic elements of the statistical analysis to be performed. All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalized before database lock.

Unless otherwise specified, descriptive statistics include mean, standard deviation, median, maximum, minimum, and range for continuous variables and the number and percentage in each group for categorical variables.

Unless specified otherwise in the SAP, 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, and Miettinen-Nurminen score-based CIs will be used for rate differences.

10.1.1 *Study Populations*

The following populations will be considered for analysis:

- The Enrolled Population is defined as all subjects for whom informed consent is provided, regardless of the subject's randomization and vaccination status.
- The Total Vaccination Population (TVP) is defined as all subjects who received nOPV2.
- The Per-Protocol (PP) Population consists of all eligible study participants who are in the TVP, who received all three doses of bOPV or IPV according to the randomized schedule, and who provided an evaluable baseline sample and at least one post-baseline sample and excludes those subjects who received any therapy that could significantly affect the subject's immune status and those subjects who met criteria outlined in Section 5.3. The PP Population will be adapted by time point to allow subjects to contribute data to PP 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.
- The Viral Shedding Population (VSP) consists of all PP Population members who do not contribute a type-2 positive pharyngeal or stool sample prior to receipt of the nOPV2 challenge dose.

Unless specified otherwise, the Enrolled Population will be used for safety analysis and analysis of demographics. Immunogenicity will be evaluated primarily in the PP Population. Virologic analyses will be primarily conducted in the VSP.

10.1.2 *Initial Characteristics of Participants*

Descriptive statistics will be provided per group for demographic (e.g., age, weight, race, sex) and other initial subject characteristics (e.g., medical and surgical history, concomitant diseases). Demographics will be compared using Chi-squared and Fisher's exact test for categorical variables, and the Wilcoxon test for continuous variables.

Prior and ongoing medical history will be coded with the Medical Dictionary for Regulatory Activities (MedDRA), summarized and listed.

Prior and concomitant medications will be coded using the WHO_DRUG Dictionary, summarized, and listed.

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.

10.1.3 *Virologic Data (viral shedding)*

The presence of type 2 poliovirus in pharyngeal (primary endpoint) and stool (exploratory endpoint) samples will be summarized by time point and overall (post-baseline) as a categorical variable. 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 confidence interval. This analysis will be repeated for PCR detection of type 2 in stool samples, and for culture-positivity rates in the $CCID_{50}$ assay, for eligible samples. Such analyses will be further expanded for composite endpoints involving these assays and time point to be defined in the SAP, including any post-challenge shedding, any post-challenge shedding in the first week (D3, D5, and/or D7), etc.

Additionally, for stool samples, those positive for type 2 (only) 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. 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, with assay upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) used to impute values achieving those limits 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 will be included in the primary SIE computation. Comparisons of $\log_{10}$ $CCID_{50}$/g between groups at each time point will be conducted by computing the difference in medians and its corresponding

two-sided 95% CI computed via the percentile bootstrap, with primary inference arising from the Wilcoxon test p-value.

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.

10.1.4 *Mucosal immunity*

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. The patterns of response of enteric antibody are well described with that to OPV being many fold that to IPV. This OPV enteric response is associated with protection against shedding on a variety of OPV challenges. Less is known about pharyngeal responses. The approach to analysis is to look for correlations between the vaccination history and the height of preexisting immunity, the vaccination history and the shedding seen in the pharynx and stool, and the nature of the class specific luminex binding response in the pharynx. The current study will not enable us to compare mucosal responses between OPV and IPV as one arm, bOPV, will have received no prior type 2 vaccine. The response to nOPV2 in infants in Panama is currently being examined with Dr. Elizabeth Brickley and these results will help to develop the statistical model for this analysis.

10.1.5 *Serum neutralizing antibody*

Serum neutralizing antibody analyses will be descriptive in nature, and no comparisons between groups are deemed relevant, given the large anticipated difference in 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).
- Seroprotection rates (with 95% exact CIs) will be computed.
- Geometric mean and median neutralizing antibody titers (with 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/ULOQ, then reverse-transformed to obtain the geometric mean and corresponding CI. The median $\log_2$ titer will be paired with bootstrap-based CIs. Median titers will be analyzed on the $\log_2$ scale, and paired with two-sided 95% percentile bootstrap-based CIs.
- 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. Subgroup analyses, if performed, will be detailed in the SAP.

10.1.6 *Safety Data*

Safety parameters will generally be tabulated and analyzed descriptively.

All SAEs/IMEs will be summarized by group and overall, and by type, severity and occurrence in relation to vaccination. Determination of causal association will be assigned using the individual causality assessment algorithm published by WHO in 2013 [18]. After applying the Adverse Event Following Immunization (AEFI) causality algorithm, the SAE or IME will be classified as consistent or inconsistent with causal association to immunization. Events with insufficient or conflicting evidence to make a determination of association will be deemed indeterminate according to the algorithm.

Summaries, listings, and narratives will be provided, as appropriate.

10.1.7 *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.

10.2 DETERMINATION OF SAMPLE SIZE

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 [20]. 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 OPV challenge [21]. Data from a study by Onorato, Modlin, McBean *et al* suggest low pharyngeal excretion following three doses of IPV [22].

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 [20], 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 H_0: RR ≥50% vs H_1: 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.

10.3 ADVERSE EVENT REPORTING

10.3.1 *Definitions*

Adverse Events

An AEFI has been defined by the working group on vaccine pharmacovigilance of the Council for International Organizations of Medical Sciences and the WHO as "any untoward medical occurrence which follows immunization and which does not necessarily have a causal relationship with the usage of the vaccine. The AE may be any unfavorable or unintended sign, abnormal laboratory finding, symptom or disease"

An AEFI can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a vaccine product.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after vaccine administration even though they may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either vaccine or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with vaccine administration.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

- Pre-existing conditions or signs and/or symptoms present in a subject prior to the study vaccination. These events will be recorded in the medical history section of the eCRF.

Serious Adverse Events

The Investigator will be responsible for recording and reporting within 24 hours and according to regulatory timelines all SAEs observed during the study (pre-challenge phase and challenge and follow-up phase) period.

An SAE, experience or reaction, is any untoward medical occurrence (whether considered to be related to study drug or not) that at any dose:

- Results in death.
- Is life-threatening (the subject is at a risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization: Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.
- Results in persistent or significant disability/incapacity.
- Is a congenital abnormality/birth defect detected only after study inclusion.

Important Medical Event

The Investigator will be responsible for recording and reporting within 24 hours and according to regulatory timelines all IMEs observed during the study (pre-challenge phase and challenge and follow-up phase) period.

IMEs are medically significant events that do not meet any of the SAE criteria above, but require medical or surgical consultation or intervention to prevent this event to become one of the serious outcomes listed in the SAE definition above. Examples of IMEs include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias (e.g., neutropenia or anemia requiring blood transfusion, etc.) or convulsions that do not result in hospitalization. Although IMEs are not SAEs, they are processed in the same way as SAEs. Every aspect described for SAEs (including study objectives and endpoints) also applies to an IME.

10.3.2 *Intensity of Adverse Events*

The investigator will assess the incidence and maximum intensity that occurred over the duration of the event for all SAEs/IMEs recorded during the study. The assessment will be based on the investigator's clinical judgment.

The intensity should be assigned to one of the following categories:

1 (mild) = An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

2 (moderate)	=	An AE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An AE which prevents normal, everyday activities (in adults, such an AE would, for example, prevent attendance at work and would necessitate the administration of corrective therapy.)
4 (life-threatening)	=	An AE that puts the subject at risk of death
5 (fatal)	=	An AE that results in death

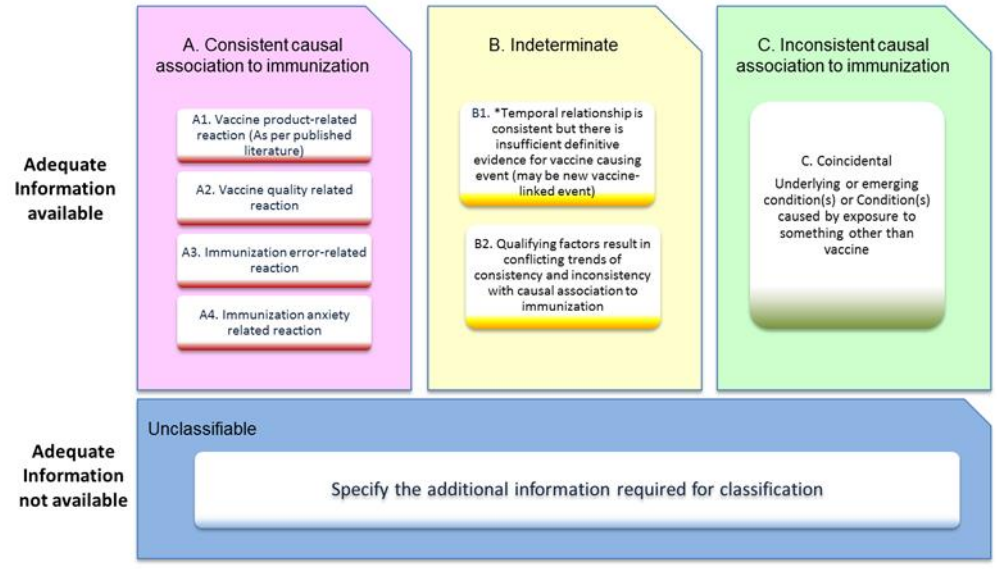
An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 0, or when it is Grade 4 or 5.

10.3.3 *Causality Assessment*

The investigator is obligated to assess the relationship between investigational vaccine and the occurrence of each SAE/IME. The investigator will use clinical judgement to determine the relationship. Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational vaccine will be considered and investigated. The investigator will also consult the Investigator's Brochure to determine his/her assessment.

Causality should be assessed by the investigator using AEFI causality algorithm developed by WHO for individual AEFI evaluation [18]. When appropriate information is available the investigator should arrive to the following possible conclusions:

Figure 1. Adverse Event Following Immunization Causality Algorithm



*B1 : Potential signal and maybe considered for investigation

SAEs considered to be causally associated to the vaccination will be termed serious adverse reactions (SARs).

If an event meets the criteria to be determined as 'serious' (see Section 0), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine, if applicable.
- Erroneous administration.
- Other cause (specify).

10.3.4 *Outcome*

The outcome of each SAE/IME must be rated as follows:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

10.3.5 *Recording of Adverse Events*

All SAEs/IMEs occurring during the clinical investigation must be documented in the source documents and on the AE forms of the eCRF. The Investigator will inquire about the occurrence of SAEs/IMEs at every visit during the study.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the SAE/IME to the study vaccine in the source documents and on the eCRF. All measures required for SAE/IME management must be recorded in the source documents and reported according to Sponsor's instructions.

All SAE/IME occurring at any time during the study will be followed by the Investigator until satisfactory resolution (e.g., value back to baseline value) or stabilization or until final database lock. If necessary, in order to obtain additional information to ensure safety to the subject, additional blood and urine samples may be taken at the discretion of the Investigator. Certain long-term SAE/IME related to therapy cannot be followed until resolution within the setting of this study. In these cases follow-up will be the responsibility of the treating physician.

10.3.6 *Reporting of Serious Adverse Events to the sponsor*

All SAEs independently from the circumstances or suspected cause must be reported on a SAE Form by the Investigator to the Sponsor, the Contract Research Organization (CRO), and to Assign DMB Safety Desk (contractor for pharmacovigilance) within 24 h of their knowledge of the event, via e-mail.

The SAE form should include a clearly written narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

Follow-up and outcomes should be reported for all subjects who experience an SAE.

It is critical that the information provided on the SAE Form matches the information recorded in the source documents and on the eCRF for the same event.

Copies of additional laboratory tests, consultation reports, postmortem reports, hospital case reports, autopsy reports, and other documents should be sent when requested and applicable. Follow-up reports relative to the subject's subsequent course must be submitted to the Sponsor and Assign DMB (contractor for pharmacovigilance) until the event has subsided or, in the event of permanent impairment, until the condition stabilizes.

10.3.7 *Reporting of Serious Adverse Events to Competent Authorities/Ethics Committees*

FIDEC assumes responsibility for appropriate reporting of AEs to the regulatory authorities. FIDEC will also report to the Investigator all SAEs that are unlisted (unexpected) and associated with the use of the nOPV2 vaccine. The Investigator must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

AE reporting, including suspected unexpected serious adverse reactions (SUSARs), will be carried out in accordance with applicable local regulations.

After termination of the clinical study (see Section 9.1.2), any unexpected safety issue that changes the risk-benefit analysis and is likely to have an impact on the subjects who have participated in the study, together with proposed actions, will be reported by the Sponsor to the competent authority(ies) concerned as soon as possible.

10.4 DATA AND SAFETY MONITORING BOARD

Not applicable. Investigators will be aware of group attribution during collection and analysis of AEs, as the study is open-label.

11. ETHICAL ASPECTS

11.1 STUDY-SPECIFIC DESIGN CONSIDERATIONS

Potential subjects' parents/guardian(s) will be fully informed of the nature of the study and of the risks and requirements of the study before any study-related assessment will be carried out. During the study, subjects' parents/guardian(s) will be given any new information that may affect their child's decision to continue participation. They will be informed that their child's participation in the study is voluntary and that they may withdraw their child from the study at any time with no reason given and without penalty or loss of benefits to which they or their child would otherwise be entitled. Only subjects whose parents/guardian(s) are fully able to understand the risks, benefits, and potential AEs of the study and who provide their consent voluntarily will be enrolled in the study.

11.2 REGULATORY ETHICS COMPLIANCE

11.2.1 *Investigator Responsibilities*

The Investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the study, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB/IEC, or the regulatory authority(ies).

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles originating from the Declaration of Helsinki (1964 and revisions), and that the clinical study data are credible.

11.2.2 *Independent Ethics Committee and Institutional Review Board (IEC/IRB)*

An IRB/IEC should safeguard the rights, safety, and well-being of all study subjects. Special attention should be paid to studies that may include vulnerable subjects.

Before the start of the study, the Investigator (or Sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- Final protocol and, if applicable, amendments.
- Sponsor-approved informed consent form (ICF) (and any updates or any other written materials to be provided to the subjects).
- Sponsor-approved subject recruiting materials.

- Prescribing information of the licensed vaccines.
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable.
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by the IEC/IRB).
- Information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects.
- Any other documents that the IEC/IRB may require to fulfill its obligation.

This study will be undertaken only after the IEC/IRB has given full written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials, and any other written information to be provided to the subjects, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study, the Investigator (or Sponsor where required) will send the following documents and updates to the IEC/IRB for its review and approval, where appropriate:

- Protocol amendments.
- Revision(s) to the ICF and any other written materials to be provided to the subjects' parents.
- New or revised subject recruiting materials approved by the Sponsor.
- Revisions to compensation for study-related injuries or payment to subjects or their parents/guardian(s) for participation in the study.
- Prescribing information of the licensed vaccines.
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually).
- Reports of SUSARs.
- New information that may adversely affect the safety of the subjects or the conduct of the study.
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects.
- Report of death of any subjects under the Investigator's care.
- Notification if a new Investigator is responsible for the study at the clinical site.
- Development Safety Update Report, Short-Term Study Specific Safety Summary and Line Listings, where applicable.
- Any other requirements of the IEC/IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to

the study subjects. If a deviation from or a change to the protocol was implemented to eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IEC/IRB as soon as possible.

The Investigator (or Sponsor where required) will notify the IEC/IRB about the study completion within 90 days after the end of the study (see Section 9.1.2).

11.2.3 *Informed Consent*

The parents/guardian(s) of each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and the reviewing IEC/IRB. The informed consent should be in accordance with the principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before enrollment in the study, the Investigator or an authorized member of the clinical staff must explain to the parents/guardian(s) of potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects' parents/guardian(s) will be informed that the subject's participation is voluntary and that they may refuse to allow the subject to participate or withdraw consent for the subject to participate at any time, without penalty or loss of benefits to which the parents/guardian(s) and/or subject was entitled. Finally, they will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that the subject's records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject's parents/guardian(s) is authorizing such access, and agrees to allow the subject's study physician to recontact the subject's parents/guardian(s) for the purpose of obtaining consent for additional safety evaluations, if needed.

The ICF will include a paragraph whereby the subject's parents/guardian(s) allow or not the use of the subject's biological samples for additional polio related research, if needed.

The language about the study used in the oral and written information, including the ICF, should be non-technical and practical and should be understandable to the subjects' parents/guardian(s). The subjects' parents/guardian(s) will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry of the subject into the study, consent should be appropriately recorded by means of the subject's parents/guardian(s) personally dated signature. After having obtained consent, a copy of the ICF must be given to the subject's parents/guardian(s).

If a subject's parents/guardian(s) is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the ICF after the oral consent of the subject's parents/guardian(s) is obtained, if permitted by local law.

11.2.4 *Privacy of Personal Data*

The collection and processing of personal data from subjects enrolled in the study will be limited to those data that are necessary to investigate the safety, quality, and immunogenicity of the study vaccines used in the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data need to agree to keep the identity of the study subjects confidential.

The informed consent obtained from the subjects' parents/guardian(s) includes explicit consent for the processing of personal data and for the Investigator to allow direct access to subjects' original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

12. ADMINISTRATIVE REQUIREMENTS

12.1 PROTOCOL AMENDMENTS/NOTIFICATIONS

Neither the Investigator nor the Sponsor will modify this protocol without a formal amendment (except modifications that do not alter the benefit/risk-see next paragraph). All protocol amendments must be issued by the Sponsor and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IEC/IRB approval nor when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazard to the subjects, in which case an amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IEC/IRB must be provided to the Sponsor or its designee.

When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

12.2 SUBJECT IDENTIFICATION AND ENROLLMENT LOGS

The Investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure subject confidentiality, no copies will be made. All reports and communications related to the study will identify subjects by initials and/or assigned number only.

12.3 SOURCE DOCUMENTATION

At a minimum, source documentation must be available for the following to confirm data collected in the eCRF: subject identification, eligibility, and study identification; study discussion and date of informed consent, dates of visits, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow-up of AEs, concomitant medication, study vaccine receipt/dispensing/return records, study vaccine administration information, laboratory printouts, date of study completion, and reason for early discontinuation of study vaccine or withdrawal from the study, if applicable.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded on the eCRF are consistent with the original source data.

It is recommended that the author of an entry in the source documents be identifiable.

At a minimum, the type and level of detail of source data available for a study subject should be consistent with that commonly recorded at the clinical site as a basis for standard medical care. Specific details required as source data for the study will be reviewed with the Investigator before the study and will be described in the monitoring guidelines (or other equivalent document). The nature and location of all source documents will be identified in the Source Document Identification Form. Data that will be recorded directly into the eCRF are specified in the Source Document Identification Form.

12.4 CASE REPORT FORM COMPLETION

Electronic Data Capture (EDC) will be used for this study. The study data will be transcribed by study personnel from the source documents onto an eCRF, and transmitted in a secure manner to the Sponsor. The electronic file will be considered to be the eCRF.

All eCRF entries, corrections, and alterations must be made by the Investigator or other authorized study-site personnel.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheet will become part of the subject's source documentation. Such worksheet should not resemble an eCRF. All data related to the study must be recorded on the eCRFs prepared by the Sponsor. Data must be entered into the eCRFs in English. Designated site personnel must complete the eCRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

The Investigator must verify that all data entries on the eCRFs are accurate and correct.

12.5 MONITORING

The monitoring of the study will be done under the responsibility of the Sponsor by the designated CRO.

The monitor will perform on-site monitoring visits as frequently as necessary. The monitor will record the dates of the visits in a study site visit log that will be kept at the clinical site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the eCRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the Sponsor and clinical staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the clinical staff.

Direct access to source documentation (medical records) must be allowed at all times for the purpose of verifying that the data recorded in the eCRF are consistent with the original source data. Findings from this review of eCRFs and source documents will be discussed with the clinical staff. During on-site monitoring visits (notified and agreed upfront with the clinical staff), the relevant clinical staff will be available, the source documentation will be accessible, and a suitable environment for review of study-related documents will be provided. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

12.6 DATA MANAGEMENT

Data management of the study will be performed under the responsibility of the Sponsor by Assign DMB.

After the monitor has reviewed the data entered into the eCRFs for completeness and accuracy and the data are released by the Investigator, data will be uploaded into the clinical database to perform cleaning activities. Computerized data cleaning checks will

be used in addition to manual review, including listings review, to check for discrepancies and to ensure consistency and completeness of the data.

If necessary, queries will be generated in the EDC tool. The Investigator or an authorized member of the clinical staff must adjust the eCRF (if applicable) and complete the query. If corrections to an eCRF are needed after the initial entry into the eCRF, this can be done in 3 different ways: 1- site personnel can make corrections in the EDC tool at their own initiative or as a response to an auto query (generated by the EDC tool), 2- the site manager can generate a query (field data correction form) for resolution by the clinical staff, and 3- the clinical data manager can generate a query for resolution by the clinical staff.

Laboratory data will be reconciled against records in the clinical database.

The clinical database will be locked as soon as it is considered clean. Only authorized and well-documented updates to the study data are possible after database lock. The locked database is used in the final statistical analysis for study reporting. Measures will be undertaken to protect subject data handed over by the Investigator to the data management department and during inspections against disclosure to unauthorized third parties. Subject confidentiality will be maintained at all times.

12.7 DATA QUALITY ASSURANCE

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor or designate.

The Sponsor or his designee will review the eCRF system for accuracy and completeness during (on-site) monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. After upload of the data into the clinical study database, their accuracy will be verified using appropriate validation programs.

In accordance with ICH/GCP guidelines, the Sponsor will be entitled to audit the facilities used in the clinical and laboratory parts of the study, as well as to access all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.8 ON-SITE AUDITS

Representatives of the Sponsor's clinical quality assurance department or any other qualified auditor appointed by the Sponsor may visit the clinical site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eCRFs. Subject privacy must, however, be respected. The Investigator and clinical staff are to be present

and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or its designees.

Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.9 STUDY TERMINATION

The Sponsor has the right to terminate the study at any time. In the event of early termination of the study or temporary halt by the Sponsor, the IEC/IRB and the regulatory authorities should be notified within 15 calendar days and should be provided with a detailed written explanation of the reasons for the termination/halt.

An end-of-study declaration will be submitted to the regulatory authorities and IEC/IRB after the complete study has ended. This notification will be submitted within 90 days after the end of the study.

12.10 RECORD RETENTION

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eCRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 15 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 5 years have elapsed since the formal discontinuation of clinical development of the study vaccine. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

12.11 USE OF INFORMATION AND PUBLICATION

All information, including but not limited to, information regarding the study vaccines or the Sponsor's operations (e.g., patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the Sponsor to the Investigator and not previously published, and any data generated as a result of this study are considered confidential and remain the sole property of the Sponsor. The Investigator agrees to maintain this information in confidence, to use this information only to accomplish this study, and not to use it for other purposes without the Sponsor's prior written consent.

The Investigator understands that the information generated in this clinical study will be used by the Sponsor in connection with the continued development of the study vaccine, and thus may be disclosed as required to other clinical Investigators or regulatory agencies. To permit information derived from the clinical studies to be used, the Investigator is obliged to provide the Sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated under the responsibility of the Sponsor and will contain eCRF data from all clinical sites that participated in the study. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating Investigator.

Clinical narratives will be written for the following events (for example):

- All deaths (irrespective of vaccine relationship).
- All other SAEs and IMEs after vaccination.
- All discontinuations of the study vaccine due to AEs (irrespective of vaccine relationship).
- At the discretion of the team and after statistical analysis of the data, certain discontinuations not related to AEs or treatment failure, i.e., related to lost to follow-up or withdrawal of consent (irrespective of treatment group).
- Any events of special interest explicitly requested by the regulatory agencies.

The Principal Investigator will sign off the final version of the Clinical Study Report. A summary of this final version will be provided to the Investigators, the applicable regulatory authorities, and the IECs/IRBs, if required by the applicable regulatory requirements, within 1 year after the end of the study (see Section 9.1.2)

The Sponsor shall have the right to publish study data and information without approval from the Investigator. If an Investigator wishes to publish information from the study, a copy of the manuscript must be provided to the Sponsor for review at least 30 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the Sponsor in writing, the Investigator will withhold such publication for up to an additional 30 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or

regulatory compliance, the Sponsor will review these issues with the Investigator. The Sponsor will not mandate modifications to scientific content and does not have the right to suppress information. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

12.12 REGISTRATION OF CLINICAL STUDIES AND DISCLOSURE OF RESULTS

The Sponsor will register the existence and disclose the results of this clinical study as required by law, on Clinicaltrials.gov and the WHO International Clinical Trials Registry Platform.

For the public disclosure of clinical study documentation or data, e.g., the study protocol or clinical study report, appropriate measures will be taken to redact such material so as to protect the privacy and confidentiality of the data as applicable to the study subjects in agreement with the legislative authority requiring such disclosure.

12.13 INVESTIGATOR INDEMNITY

The Sponsor holds and will maintain an adequate insurance policy covering damages arising out of FIDEC-sponsored clinical research studies.

The Sponsor will indemnify the Investigator and hold him/her harmless for claims related to damages arising from the investigation, provided that the study vaccine was administered under the Investigator or deputy's supervision and in strict accordance with accepted medical practice and the study protocol.

The Investigator must notify the Sponsor immediately upon notice of any claims or lawsuits.

12.14 CONFIDENTIALITY

All study documents are provided by the Sponsor to the Investigator and appointed clinical staff in confidence. None of this material may be disclosed to any party not directly involved in the study without the Sponsor's written permission.

The Investigator must assure that subjects' anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the subjects' study numbers, names, addresses, and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from the Sponsor.

13. REFERENCES

1. World Health Organization. Polio Eradication and Endgame Sratregic Plan 2013-2018. Available at: https://polioeradication.org/who-we-are/strategic-plan-2013-2018/#:~:text=The%20strategy%20has%20three%20goals,re%2Demergence%20of%20any%20poliovirus.
2. World Health Organization Global Polio Eradication Initiative (GPEI). Where we work. 2022 Available at: https://polioeradication.org/where-we-work/. Accessed 30 November 2022.
3. World Health Organization. Meeting of the Strategic Advisory Group of Experts on immunization, April 2012 - conclusions and recommendations. Wkly Epidemiol Rec. 2012; 87:201-216.
4. Hird TR, Grassly NC. Systemic review of mucosal immunity induced by oral and inactivated poliovirus vaccines against virus shedding following oral poliovirus challenge. Plos Pathog. 2012 8(4): e1002599.
5. Emmanuel Vidor. Poliovirus—inactivated. In: SA Plotkin, WA Orenstein, PA Offit (Eds.), Vaccines (7th ed.), Elsevier, Philadelphia, 2013, pps. 854-865.
6. World Health Organization Circulating vaccine-derived polioviruses. Available at: https://polioeradication.org/polio-today/polio-now/this-week/circulating-vaccine-derived-poliovirus/. Accessed 30 November 2022.
7. Klapsa D, Wilton T, Zealand A, et al. Sustained detection of type 2 poliovirus in London sewage between February and July, 2022, by enhanced environmental surveillance. Lancet 2022; 400: 1531–38.
8. Ryerson AB, Lang D, Alazawi MA, et al. Wastewater Testing and Detection of Poliovirus Type 2 Genetically Linked to Virus Isolated from a Paralytic Polio Case —New York, March 9–October 11, 2022. Morbid Mort Wkly Rep 2022; 71(44):1418-24.
9. Zuckerman NS, Bar-Or I, Sofer D, et al. Emergence of genetically linked vaccine-originated poliovirus type 2 in the absence of oral polio vaccine, Jerusalem, April to July 2022. Eurosurveillance 2022; 27(37):pii=2200694.
10. World Health Organization, First ever vaccine listed under WHO emergency use. 2020. Available at: https://www.who.int/news/item/13-11-2020-first-ever-vaccine-listed-under-who-emergency-use. Accessed 30 November 2022.
11. World Health Organization Global Polio Eradication Initiative (GPEI). cVDPV2 outbreaks and the type 2 noval oral polio vaccine (nOPV2) - Fact Sheet. Available at: https://polioeradication.org/wp-content/uploads/2022/10/GPEI-nOPV2-Factsheet-EN-20221011.pdf. Accessed 30 November 2022.
12. Van Damme P, De Coster I, Bandyopadhyay AS, et al. The safety and immunogenicity of two novel live attenuated monovalent (serotype-2) oral poliovirus vaccines in healthy adults: a double-blind, single-centre phase 1 study. Lancet. 2019 Jul 13; 394(10193):148-158.
13. De Coster I, Leroux-Roels I, Bandyopadhyay AS, et al. Safety and immunogenicity of two novel type-2 oral poliovirus vaccine candidates compared with a

monovalent type-2 oral poliovirus vaccine in healthy adults: two clinical trials. Lancet. 2021; 397(10268):39-50.
14. Sáez-Llorens X, Bandyopadhyay AS, Gast C, et al. Safety and immunogenicity of two novel type-2 oral poliovirus vaccine candidates compared with a monovalent type-2 oral poliovirus vaccine in children and infants: two clinical trials. Lancet. 2021; 397(10268):27-38.
15. World Health Orgainization. Global Advisory Committee on Vaccine Safety (GACVS). 17 September 2021. Wkly Epidemiol Rec 2022: 97:17-24.
16. Wahid R, Mercer L, Macadam A, et al. Assessment of genetic changes and neurovirulence of shed Sabin and novel type 2 oral polio vaccine viruses. npj Vaccines. 2021; 6: 94 https://doi.org/10.1038/s41541-021-00355-y.
17. Wahid R, Mercer LD, De Leon T, et al. Genetic and phenotypic stability of poliovirus shed from infants who received novel type 2 or Sabin type 2 oral poliovirus vaccines in Panama: an analysis of two clinical trials. Lancet Microbe 2022 Nov 1;S2666-5247(22)00254-3 https://doi.org/10.1016/S2666-5247(22)00254-3.
18. Tozzi AE, Asturias EJ, Balakrishnan MR, Halsey NA, Law B, Zuber PL. Assessment of causality of individual adverse events following immunization (AEFI): a WHO tool for global use. Vaccine. 2013; 31(44):5041-6.
19. Weldon, W.C., Oberste, M.S., Pallansch, M.A. Standardized Methods for Detection of Poliovirus Antibodies. In: Martín, J. (eds) Poliovirus. Methods in Molecular Biology, 2016 vol 1387. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-3292-4_8.
20. Marine WM, Chin TDY, Gravelle CR. Limitation of fecal and pharyngeal poliovirus excretion in Salk-vaccinated children. Am J Hyg. 1962;76:173–195.
21. Faden H, Modlin JF, Thoms ML, et al. Comparative evaluation of immunization with live attenuated and enhanced-potency inactivated trivalent poliovirus vaccines in childhood: systemic and local immune responses. J Infect Dis. 1990;162:1291–1297.
22. Onorato IM, Modlin JF, McBean AM, et al. Mucosal immunity induced by enhanced-potency inactivated and oral polio vaccines. J Infect Dis. 1991;163:1–6.
23. Davlantes E, Jorba J, Henderson E., et al. Circulating Vaccine-Derived Poliovirus Type 2 Emergences Linked to Novel Oral Poliovirus Vaccine Type 2 Use — Six African Countries, 2021–2023. https://www.cdc.gov/mmwr/volumes/72/wr/mm7238a4.htm?s_cid=mm7238a4_w.