STATISTICAL ANALYSIS PLAN
for
PATH Protocol CVIA 065 (CC-ID6)

Study Title:

A Phase 1 Randomized Study to Examine the Safety, Tolerability, and Immunogenicity of Inactivated Poliovirus Vaccine (IPV) with or without *E.coli* Double Mutant Heat Labile Toxin (dmLT) and Impact on Poliovirus Shedding Post-bOPV Challenge in Healthy IPV-Primed Adult Subjects

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18 JUN 2020

Protocol Number Code:	CVIA 065
Development Phase:	Phase 1
Products:	• Licensed trivalent IPV (IMOVAX®-Polio) for intramuscular (IM) administration (Sanofi Pasteur, France) • dmLT, also known as LT (R192G/L211A), (IDT Biologika, Germany) • Licensed Polio Sabin™ One and Three (oral), Bivalent Oral Poliomyelitis vaccine Types 1 and 3 (bOPV) (GSK)
Form/Route:	Intramuscular, oral
Indication Studied:	Polio
Sponsor:	PATH Center for Vaccine Innovation and Access (CVIA) 455 Massachusetts Ave, Suite 1000, Washington, DC 20001, USA
Date of the Analysis Plan:	18 JUN 2020
Version Number:	2.0

This study was performed in compliance with Good Clinical Practice.

CVIA 065 STATISTICAL ANALYSIS PLAN REVISION HISTORY

Version Number	Version Date	Summary of Changes
2.0	18 JUN 2020	Updated for changes in V3.0 of the protocol: endpoints moved from Secondary to Exploratory; homing marker CCR9 was removed; revised exclusion criteria; increased allowable window for Day 50 to ±2 day; clarified topline analysis composition; and clarified unblinding of homing marker assay.
1.0	26 MAR 2020	Original

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LIST OF ABBREVIATIONS

µg	microgram
AE	Adverse Event
ALS	Antibodies in Lymphocyte Supernatant
ALT	Alanine Aminotransferase
AML	Algemeen Medisch Laboratorium, Antwerp, Belgium
ASC	Antibody-Secreting cell
AST	Aspartate Aminotransferase
ATC	Anatomic Therapeutic Chemical
AUC	Area under the curve
BMI	Body Mass Index
bOPV	Bivalent Oral Polio Vaccine
CDC	(U.S.) Centers for Disease Control and Prevention
cVDPV	circulating Vaccine Derived Poliovirus
cGMP	Current Good Manufacturing Practice
CI	Confidence Interval
CRP	C-reactive protein
CSR	Clinical Study Report
CVIA	Center for Vaccine Innovation and Access
dmLT	*E.coli* Double Mutant Heat Labile Toxin
DRM	Data Review Meeting
EC	Ethics Committee
eCRF	Electronic Case Report Form
ETEC	Enterotoxigenic *Escherichia coli*
FAMHP	Federal Agency for Medicines and Health Products
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GM	Geometric Mean count
GMFR	Geometric Mean Fold Rise
GMT	Geometric Mean Titer
GPEI	Global Polio Eradication Initiative
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICH	International Council on Harmonisation
ICF	Informed Consent Form
ID	Intradermal
Ig	Immunoglobulin
IM	Intramuscular
IPV	Inactivated Poliovirus Vaccine
IRB	Institutional Review Board
KM	Kaplan Meier

LIST OF ABBREVIATIONS (CONTINUED)

LLN	Lower Limit of Normal
LLOQ	Lower Limit of Quantitation
LT	Enterotoxigenic *E. coli* (ETEC) Labile Toxin
MedDRA	Medical Dictionary for Regulatory Activities
mcg	microgram (also µg)
mg	milligram
mL	milliliter
mLT	*E.coli* Double Mutant Heat Labile Toxin
mOPV	Monovalent Oral Polio Vaccine
NAb	Neutralizing Antibody
OPV	Oral Polio Vaccine
PATH REC	PATH's Research Ethics Committee
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase chain reaction
PEFs	Poliovirus Essential Facilities
PI	Principal Investigator
PO	By mouth
PP	Per Protocol
PT	Preferred Terms
RCD	Reverse Cumulative Distribution
RI	Routine Immunization
SAE	Serious Adverse Event
SAGE	Strategic Advisory Group of Experts
SAP	Statistical Analysis Plan
SDCC	Statistical Data Coordinating Center
SIE	Shedding Index Endpoint
SOC	System Organ Class
SRC	Safety Review Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULB	Université Libre de Bruxelles, Belgium.
ULN	Upper Limit of Normal
ULOQ	Upper Limit of Quantitation
UMB CVD	University of Maryland, Baltimore. Center for Vaccine Development.
UZA	Antwerp University Hospital
VAPP	Vaccine Associated Paralytic Polio
VDPV	Vaccine Derived Poliovirus
WBC	White Blood Cells
WHO	World Health Organization
WPV	Wild Poliovirus

1. PREFACE

This Statistical Analysis Plan (SAP) for "A Phase 1 Randomized Study to Examine the Safety, Tolerability, and Immunogenicity of Inactivated Poliovirus Vaccine (IPV) with or without *E. coli* Double Mutant Heat Labile Toxin (dmLT) and Impact on Poliovirus Shedding Post-bOPV Challenge in Healthy IPV-Primed Adult Participants" (PATH protocol CVIA 065) describes and expands upon the statistical information presented in the protocol.

This document describes all planned analyses and provides reasons and justifications for these analyses. It also includes sample tables, figures, and listings planned for the final analyses (see Appendix 1, Appendix 2, and Appendix 3). Regarding the final analyses and Clinical Study Report (CSR), this SAP follows the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines, as indicated in Topic E3 (Structure and Content of Clinical Study Reports), and more generally is consistent with Topic E8 (General Considerations for Clinical Trials) and Topic E9 (Statistical Principles for Clinical Trials). The structure and content of the SAP provide sufficient detail to meet the requirements identified by the US Food and Drug Administration (FDA) and ICH, while all work planned and reported for this SAP will follow internationally accepted guidelines published by the American Statistical Association and the Royal Statistical Society for statistical practice.

This document contains a review of the study design, general statistical considerations, comprehensive statistical analysis methods for efficacy and safety outcomes, and a list of proposed tables and figures. Any deviation from this SAP will be described and justified in protocol amendments and/or in the Clinical Study Report (CSR), as appropriate. The reader of this SAP is encouraged to also review the study protocol for details on conduct of the study and the operational aspects of clinical assessments.

2. INTRODUCTION

Attenuated strains of poliovirus (Sabin type 1, 2, and 3) have historically been used for oral polio vaccine (OPV). Despite its well-established safety record, OPV use can be associated with rare emergence of genetically divergent vaccine-derived polioviruses (VDPVs) whose genetic drift from the parental OPV strains indicates prolonged replication or circulation [1]. For this reason, the global eradication of poliomyelitis (polio) requires the cessation of all OPV in routine immunization (RI), as soon as possible after the eradication of wild poliovirus (WPV) transmission [2]. To minimize the risk of continued circulating vaccine-derived poliovirus type 2 (cVDPV2) cases and vaccine associated paralytic polio (VAPP), the type 2 component of OPV (OPV2) was phased out from all RI activities in a globally coordinated manner in April 2016 [1]. Additionally, the plan for polio eradication of the World Health Organization (WHO)/Global Polio Eradication Initiative (GPEI) called for introduction of a single dose of inactivated polio vaccine (IPV) in RI along with a worldwide withdrawal of OPV2. The introduction of IPV will help facilitate interruption of transmission with the use of monovalent OPV2 in the case of outbreaks. Following

certification of eradication of wild polio, the GPEI Strategic Plan envisions a transition to an all-IPV schedule, at which time OPV would be completely withdrawn from RI [3]. IPV would then be the sole means of immunization against polio, although monovalent type 1, 2 or 3 OPV (mOPV) may be used locally in the event of polio outbreaks occurring after global OPV cessation. WHO's Strategic Advisory Group of Experts (SAGE) on Immunization recommended in April 2017 that after global OPV withdrawal, (i) countries should include at least two doses of IPV in their routine immunization schedule; (ii) countries without Poliovirus Essential Facilities (PEFs) should maintain IPV in their routine immunization schedule for at least 10 years to address immediate (VDPVs), intermediate (immunodeficiency-associated VDPVs), and longer-term (e.g., containment failure) risks; and (iii) countries with PEFs should continue to use IPV as long as mandated by the Global Action Plan to minimize poliovirus facility-associated risk [4].

The comparative ability of OPV and IPV to induce mucosal immunity has been explored since the early days of polio research. It has been known for many years that OPV, by being more effective than IPV in primary induction of gut immunity, can limit the fecal shedding of polio virus. Recent trials with IPV using bivalent OPV (bOPV) schedules have explored this phenomenon and confirmed a marginal impact of IPV in inducing type-specific intestinal mucosal immunity, particularly on duration and titer of virus shed in stool, compared to OPV [5-7]. These studies have also shown impact from IPV in boosting intestinal immunity when given to children who had received prior OPV [8-9]. The current data on wild polio virus transmission in populations highly vaccinated with IPV highlight the potential limitations of global immunization solely with IPV in the event of persistent cVDPV or a resurgence of WPV. An improved IPV that is more effective against fecal shedding could lessen or eliminate the need for reintroduction of OPV for emergency polio outbreak use [10]. Limiting the potential future need for OPV for polio outbreak use post-eradication would decrease the demands on the global stockpiles and supply chain and allow scarce resources to be reallocated to other vaccination programs.

E. coli heat-labile toxin (LT) has long been known to be a powerful adjuvant for mucosal immune responses following mucosal administration. Clinical tests of genetically detoxified variants of LT, termed mutant LT (mLT), and more recently double mutant LT (dmLT), have demonstrated their ability to stimulate not only mucosal but also systemic immune responses in humans. Remarkably, dmLT has been shown in animal models to also stimulate mucosal immune responses following parenteral—e.g., intramuscular (IM), intradermal (ID)—administration, suggesting that administration of dmLT as an adjuvant for IPV to stimulate mucosal immune responses in the gut following parenteral administration may be expected to improve the effectiveness of IPV immunization against fecal shedding of poliovirus.

In this study, the safety and tolerability of IPV co-administered with dmLT will be assessed, as well as whether co-administration of dmLT with IPV enhances mucosal responses compared to those with IPV alone.

The goal of the GPEI is to complete the eradication and containment of all wild, vaccine-related and Sabin polioviruses, such that no child ever again suffers paralytic poliomyelitis [2]. A major

component of the strategy aimed at worldwide eradication of polio advanced by the WHO is based on the replacement of OPV with IPV; however, IPV is not efficient in preventing person-to-person poliovirus transmission, particularly in settings of poor hygiene, due to limited impact on intestinal mucosal immunity compared to OPV. The addition of an adjuvant, in particular one that may direct the response towards mucosal homing, may offset that deficiency. The novel dmLT mucosal adjuvant has been evaluated in several animal and human studies in which it has been administered orally or parenterally with promising results. In this study, the safety and tolerability of IPV co-administered with dmLT will be assessed, as well as whether co-administration of dmLT with IPV enhances mucosal responses compared to those with IPV alone.

2.1. Purpose of the Analyses

The main purpose of this study is to assess in healthy adults the safety and tolerability of IPV co-administered with dmLT. The study will also evaluate whether co-administration of dmLT with IPV enhances mucosal responses compared to those with IPV alone.

Up to eighty, healthy participants 18-45 years of age will be enrolled and randomized to one of three different dose groups. Two groups of 30 participants will receive a single dose of licensed trivalent IPV, administered IM, either with or without dmLT, in a blinded fashion. A positive unblinded control group of 20 participants will receive bOPV administered orally (PO). One month (28 days) after receiving study vaccine, all participants will receive a standard oral dose of bOPV (0.1 milliliter [mL]) to assess the relative impact of study vaccine on shedding of the challenge virus. For this study, study vaccine is described as IPV+dmLT. All safety data will be summarized and reviewed by a blinded Safety Review Committee (SRC) prior to study initiation, following vaccination of all subjects (post Day 8), and one month after all subjects have received their bOPV challenge (post Day 57).

This SAP describes the statistical methodology and summaries required to assess the safety and efficacy of this vaccine approach to achieve better poliovirus-specific gut immunity via addition of this novel adjuvant when administered to 18 through 45 year-old healthy participants.

3. STUDY OBJECTIVES AND ENDPOINTS

Study objectives are listed here as in the protocol.

3.1. Study Objectives

The study will test the hypotheses that IM administration of IPV together with dmLT adjuvant is safe and well-tolerated and enhances mucosal responses to polioviruses types 1, 2, and 3 in comparison with administration of IPV alone and provides greater mucosal immunity, assessed following oral bOPV challenge.

3.1.1. Primary Objective

3.1.1.1. Safety and Tolerability

- To evaluate and compare the safety and tolerability of a single dose of IPV + dmLT and IPV alone, administered IM in healthy adults

3.1.1.2. Viral Shedding

- To evaluate and compare the rate of stool viral shedding following bOPV challenge 28 days after IM administration of IPV + dmLT and IPV alone

3.1.2. Secondary Objectives

3.1.2.1. Immunogenicity

- To evaluate and compare intestinal mucosal immune responses (IgA and poliovirus neutralization in stool) to IPV + dmLT and to IPV alone, administered IM
- To evaluate and compare other mucosal and systemic immune responses to IPV + dmLT and IPV alone, administered IM

3.1.2.2. Viral Shedding

- To additionally evaluate and compare the extent of stool viral shedding following bOPV challenge 28 days after administration of either IPV alone or IPV + dmLT
- To evaluate the rate and extent of stool viral shedding following bOPV challenge 28 days after administration of bOPV

3.1.3. Exploratory Objectives

3.1.3.1. Immunogenicity

- To evaluate and compare mucosal and systemic immune responses to IPV + dmLT, administered IM, and bOPV, administered orally

3.2. Study Endpoints

3.2.1. Primary Endpoints

3.2.1.1. Safety

- Frequency and incidence of serious adverse events (SAEs) throughout the study
- Frequency and incidence of severe adverse events (AEs) during the 28 days following study vaccination
- Frequency and incidence of local and systemic reactions during the 7 days following study vaccination
- Frequency and incidence of AEs during the 28 days following study vaccination

3.2.1.2. Viral Shedding

- Proportion of participants without detectable fecal shedding of bOPV vaccine virus on Day 7 following bOPV challenge, 28 days after study vaccination in IPV alone and IPV + dmLT arms

3.2.2. Secondary Endpoints

3.2.2.1. Immunogenicity

- Proportion of participants developing type-specific poliovirus fecal IgA and poliovirus fecal neutralization responses (minimum 4-fold increase from baseline) 28 days after administration of study vaccination, and 14 days after bOPV challenge
- Median and geometric mean titer (GMT) of type-specific poliovirus fecal IgA and neutralization before and 28 days after study vaccination and 14 days after bOPV challenge, and geometric mean fold rise (GMFR) between baseline and post-baseline measurements
- Serum neutralizing antibody seroconversion rate, defined as the proportion of participants demonstrating a minimum four-fold increase in type-specific poliovirus serum neutralizing antibody titers between baseline and 28 days post each vaccination, or post-vaccination titer >1:8 if seronegative at baseline
- Median and GMT of type-specific poliovirus serum neutralizing antibodies before and after study vaccination, and GMFR between baseline and post-baseline measurements
- Seroprotection rate of serum neutralizing antibodies at baseline as well as 28 days following vaccination, defined as type-specific poliovirus serum neutralizing antibody titer ≥1:8
- Proportion of participants developing type-specific poliovirus antibody secreting cell (ASC) responses defined as ≥8 ASC/10^6 peripheral blood mononuclear cell (PBMC) at any time point following both study vaccination and bOPV challenge, and overall (following any dose of study product)
- Median and GMT of the frequency of type-specific poliovirus ASCs before and after study vaccination, and GMFR between baseline and post-baseline measurements

3.2.2.2. Viral Shedding

- Area under the curve (AUC) of viral shedding in stool assessed at 7, 14, 21 and 28 days following bOPV challenge
- Time to cessation of viral shedding in stool, including types 1 or 3 as well as each type separately, defined as the study day of the first instance of 3 consecutive samples polymerase chain reaction (PCR)-negative for virus, with samples taken on separate days.

3.2.3. Exploratory Endpoints

3.2.3.1. Immunogenicity

- Frequency of poliovirus-specific CD4+ T cells before and after vaccination
- Frequency of poliovirus-type-specific memory B-cells before and after vaccination and after bOPV challenge
- Proportion of participants with type-specific poliovirus IgA and IgG responses in saliva (minimum four-fold increase in ratio of specific/total IgA and IgG between baseline and 28 days post-vaccination) at any time point after vaccination or after bOPV challenge
- Median and GMT of type-specific poliovirus IgA and IgG (ratio of specific/total) in saliva before and after study vaccination and after bOPV challenge and GMFR between baseline and post-baseline measurements
- Frequency of type-specific poliovirus-specific ASC expressing gut-homing marker α4β7 after vaccination, and after bOPV challenge
- Multiplex assessment of cytokine levels at baseline, and following vaccination and bOPV challenge
- Proportion of participants with type-specific poliovirus serum IgA and IgG responses (minimum four-fold increase in antibody titers between baseline and 28 days post-vaccination)
- Median and GMT of type-specific poliovirus serum IgA & IgG before and after study vaccination, and GMFR between baseline and post-baseline measurements
- Proportion of participants demonstrating at least a two-fold increase in type-specific poliovirus IgA or IgG in lymphocyte supernatant (ALS) at any time point after study vaccination or after bOPV challenge
- Median and GMT of type-specific poliovirus ALS IgG or IgA before and after study vaccination, and after bOPV challenge, and GMFR between baseline and post-baseline measurements

3.3. Study Definitions and Derived Variables

3.3.1. Adverse Event (AE)

An adverse event is any untoward medical occurrence in a participant after administration of the investigational vaccine and that does not necessarily have a causal relationship with the investigational vaccine. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptoms, physical examinations, or disease temporally associated with the use of the investigational vaccine, whether or not related to the investigational vaccine.

This definition includes exacerbations of pre-existing conditions. Stable pre-existing conditions which do not change in nature or severity during the study are not considered AEs; however, these should be reported as part of the medical history. The investigator will assess whether the AE is related to the inoculation using the following guidelines: related or not related as described in Section 3.3.5.

3.3.1.1. Solicited Local and Systemic Reactions

Solicited AEs are pre-specific local and systemic adverse events that are common or known to be associated with vaccination and that are actively monitored as indicators of vaccine reactogenicity. Solicited AEs with onset after the solicitation period will be captured as unsolicited AEs.

The following specific solicited AEs will be monitored for this trial:

- Local/injection site reactions: Pain, erythema/redness, swelling, induration, hyperpigmentation
- Systemic reactions: Fever (defined as oral temperature ≥38.0°C), chills, fatigue, headache, muscle aches/myalgia, joint ache/arthralgia, rash, nausea, vomiting, diarrhea.

3.3.1.2. Unsolicited Adverse Events

Unsolicited AEs are any AEs reported spontaneously by the participant, observed by the study personnel during study visits, or identified during review of medical records or source documents.

3.3.1.3. Adverse Reactions

An adverse reaction is any AE which is considered to have been caused by the investigational vaccine. A suspected adverse reaction is one in which the causal relationship to the investigational vaccine is at least a reasonable possibility, i.e., there is evidence to suggest a causal relationship between the study product and the AE. The concept of “reasonable causal relationship” is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship.

An unexpected adverse event is one that is not identified in nature, severity, specificity, or frequency in the risk profile described in the protocol or investigator’s brochure.

3.3.2. Serious Adverse Event (SAE) or Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SAE, including a serious suspected adverse reaction or serious adverse reaction as determined by the Principal Investigator (PI) or the Sponsor, is any event that results in any of the following outcomes:

- Death
- Is life-threatening (life-threatening means that the study participant was, in the opinion of the site PI or Sponsor, at 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
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital abnormality or birth defect
- Important medical event that may not result in one of the above outcomes but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of SAE

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Hospitalization for a pre-existing condition, including elective procedures, which has not worsened, does not constitute an SAE.

Suspected unexpected serious adverse reaction (SUSAR) is any suspected adverse reaction that is both unexpected and serious.

3.3.3. Reporting Period and Parameter

Safety events are reported from the time of study vaccination through completion of the study at 6 months after vaccination (Day 169). Specifically, unsolicited AEs from Day 1 through Day 57 and SAEs from Day 1 through (end of study) Day 169 will be followed until satisfactory resolution or until the Investigator deems the event to be chronic or the participant to be stable. Abnormal laboratory values will be repeated and/or investigated as appropriate. Attempts will be made to follow the participant at least monthly to determine the outcome and duration of an AE.

For this trial, solicited AEs will be assessed by study staff at least 30 minutes after the study vaccination then daily for 7 days by the participants. Participants will be provided a memory aid to record the presence or absence of solicited AEs, severity of the solicited AE and use of

concomitant medication. If a solicited AE started during the 7 days post vaccination and continues beyond the 7 days, it will continue to be reported as a solicited AE. Unsolicited AEs will be collected from Day 1 to 57 days after the study vaccination (Day 57), inclusive.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE unless there is a worsening of the condition. AEs characterized as intermittent require documentation of onset and duration of each episode.

Laboratory values of Grade 2 or higher will be reported as AEs. Grade 1 laboratory test results will be entered as AEs, if the site PI determines them to be clinically significant.

3.3.4. Severity of Adverse Event

The severity of all AEs will be assessed by the investigator and participant (as applicable) based on the severity grading criteria provided in Table 4 grade AEs from mild (Grade 1) to severe (Grade 3). All AEs leading to death are SAEs. AEs are graded with the worst severity grade during the illness/symptoms.

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity:

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

3.3.5. Causality of Adverse Events

The study investigators will determine the causal relationship between the study product and the AE. The causality assessment is made on the basis of the available information at the time of reporting and can be subsequently changed according to follow-up information. Determination of causality is based on clinical judgment and should take into consideration the following factors:

- Is there a temporal (time-based) relationship between the event and administration of the investigational product?
- Is there a plausible biological mechanism for the investigational product to cause the AE?
- Is there a possible alternative etiology for the AE such as concurrent illness, concomitant medications?
- Are there previous reports of similar AEs associated with the investigational product or other vaccines in the same class?

For this study, the investigator/s must classify the causality of the AE according to the categories defined below:

- **Related**: There is a reasonable possibility that the product caused the event. "Reasonable possibility" means that there is evidence to suggest a causal relationship between the study product and the AE.
- **Not Related:** There is not a reasonable possibility that the administration of the study product caused the event

3.3.6. Definitions and Derivations Used in this Study

- A baseline value will be defined as the last value obtained prior to the first vaccination of study product.
- Age will be calculated from the date of enrollment and will be presented in whole years.
- Fever: oral temperature ≥ 38.0°C or 100.4°F.
- Seropositive is defined as a baseline titer greater than a pre-defined threshold (e.g., Titer ≥ 1:8).
- Seroprotection is defined as a post-vaccination titer greater than a pre-defined threshold (e.g., Titer ≥ 1:8).
- Seroconversion is defined as a fold-rise in titer from baseline or pre-challenge above a pre-defined threshold, typically a minimum 4-fold increase.

4. INVESTIGATIONAL PLAN

4.1. Overall Study Design and Plan

This first in human Phase 1 clinical trial is a randomized, partially blinded, single-center outpatient study in which participants will receive a single dose of licensed trivalent IPV, administered IM, with or without dmLT, or bOPV, administered PO. IPV or IPV + dmLT will be administered to groups of 30 healthy participants each. Both participants and clinical staff will be blinded to group assignment (IPV-alone vs IPV + dmLT). A positive control group (unblinded) will be included, composed of 20 healthy participants receiving bOPV. The positive control arm is included in order to confirm the level of shedding observable following a dose of an oral vaccine known to develop intestinal immunity. However, due to likely persistence of ongoing shedding of vaccine virus at the date of challenge in the bOPV arm, a comparison of post-challenge shedding between the IPV + dmLT and bOPV arms may not be possible. The bOPV arm, however, will still provide a useful description of duration of shedding following vaccine dose, as well as reduction of viral shedding following the challenge dose, in participants who cease shedding the vaccine dose prior to challenge. Participants will be randomized 3:3:2 to receive either a single, standard dose of IPV IM, a single, standard dose of IPV IM co-administered with a 0.5 µg dose of dmLT or a single oral dose of bOPV (0.1 mL). 28 days after receiving study vaccine, all participants will receive a standard oral dose of bOPV (0.1 mL) to assess relative impact of study vaccine on shedding of that challenge virus.

The study will test the hypotheses that IM administration of IPV together with dmLT adjuvant is safe and well-tolerated and increases mucosal responses to polioviruses types 1, 2, and 3 in comparison with administration of IPV alone.

Demonstration of safety and evidence that dmLT enhances mucosal immune responses after IM administration may qualify IPV + dmLT for further studies, including descending age trials to reach the target population for IPV administration (infants).

Table 1: Study Schema – Protocol Table 2

Study group[a]	N	IPV volume[b]	dmLT dose	bOPV
1	30	0.5 mL	0	0
2	30	0.5 mL	0.5 µg	0
3	20	0	0	0.1 mL

[a] All participants will receive bOPV challenge on Day 29.
[b] 0.5 mL = 40 D-Antigen Units type 1, 8 D–Antigen Units type 2, and 32 D-Antigen Units type 3 poliovirus.

4.2. Discussion of Study Design

Protocol CVIA 065 is a randomized, partially blinded, single-center outpatient study designed to test the hypotheses that IM administration of IPV together with dmLT adjuvant is 1) safe and well-tolerated; 2) enhances mucosal responses to polioviruses types 1, 2, and 3 in comparison with administration of IPV alone; and 3) provides greater mucosal immunity, assessed following oral bOPV challenge when administered to healthy 18 through 45-year-old participants. The positive control arm is included in order to confirm the level of shedding observable following a dose of an oral vaccine known to develop intestinal immunity, and to provide a comparison of this gold standard to the level of shedding of the challenge observed by vaccination with IPV + dmLT. The bOPV control arm is also included to assess the proportion of participants shedding either Sabin type 1 or 3 after 28 days, which is expected to be informative for future studies, in addition to providing useful immunogenicity data.

4.3. Selection of Study Population

4.3.1. Description of Study Population

Eighty healthy 18 to 45-year-old (all inclusive) male or female participants with appropriate polio vaccination history who meet all the inclusion and exclusion criteria and reside within the screening population of the University of Antwerp catchment area will be enrolled into the study.

Up to two back up volunteers per day will be selected that may be enrolled in the event a subject becomes ineligible prior to receipt of the investigational product.

4.3.2. Inclusion Criteria for Enrollment

Participants are eligible for this study if they fulfill the inclusion criteria below:

- Adult male or female, ages 18–45, inclusive
- Healthy as defined by absence of clinically significant medical condition, either acute or chronic, as determined by medical history and clinical assessment
- History of prior receipt of at least 3 doses of IPV
- Willing and able to provide written informed consent and willing to comply with study requirements
- Intention to remain in the area during the study period
- If female and of childbearing potential*, not breastfeeding and not pregnant (based on a negative serum pregnancy test at screening and negative urine pregnancy tests prior to vaccine administration and bOPV challenge), planning to avoid pregnancy until at least three months after bOPV challenge and willing to use an adequate method of contraception consistently. Effective methods include intrauterine device or hormonal contraceptives (oral, injectable, patch, implant, vaginal ring). Women with credible history of abstinence or in monogamous relationship with a vasectomized partner are also eligible. Highly effective contraception should be maintained for three months after the administration of bOPV challenge.

* Females can be considered not of childbearing potential only with current bilateral tubal ligation or occlusion, or post-hysterectomy, or post-bilateral ovariectomy, or post-menopause.

4.3.3. Exclusion Criteria for Enrollment

Participants will be ineligible for this study for any of the following conditions or reasons:

- History of receiving any OPV at any time
- Receipt of IPV in the last five years
- History of or planned household contact with an individual receiving OPV in prior 4 weeks, or at any point during the study
- Unable to avoid contact with children younger than six months (and thus not yet fully vaccinated against polio) or immunocompromised individuals until two consecutive negative stool PCR results, post challenge
- Presence of fever on the day of vaccination (oral temperature ≥38°C)
- Received an investigational product within 30 days prior to randomization or planning to participate in another research study involving investigational product during the conduct of this study
- Presence of any systemic disorder (cardiovascular, pulmonary, hepatic, renal, gastrointestinal, hematological, endocrine, immunological, dermatological, neurological, cancer or autoimmune diseases) as determined by medical history and/or physical examination that would compromise the participant's health or is likely to result in nonconformance to the protocol or would interfere with the evaluation of responses according to the opinion of the investigator

- History of allergic disease or known hypersensitivity to any component of the study vaccine
- History of anaphylactic reaction
- Receipt of any immunoglobulin therapy and/or blood products in the last 6 months or planned administration during the study period
- History of chronic administration (defined as more than 14 days) of immunosuppressant medications, including oral steroids, parenteral steroids, or high-dose inhaled steroids (>800 µg/day of beclomethasone dipropionate or equivalent), in the last 6 months to either the study participant or their close household contacts (those on nasal or topical steroids may be permitted to participate in the study)
- Symptoms of an acute self-limited illness, such as an upper respiratory infection or gastroenteritis, including a temperature ≥38.0°C, within the 7 days prior to study vaccines administration
- Positive test for Human Immunodeficiency Virus (HIV), Hepatitis B surface antigen (HBsAg) or Hepatitis C virus (HCV) antibody
- Clinically significant screening laboratory value*
- History of receipt of experimental *E. coli*, LT, or cholera vaccines or live *E. coli* or *Vibrio cholerae* challenges
- Receipt of any licensed vaccine within 28 days before enrollment in this study or plans to receive any licensed vaccine between enrollment and 28 days after the bOPV challenge
- History of alcohol or drug abuse in the last 5 years
- Any condition that in the opinion of the investigator would pose a health risk to the participant if enrolled, or could interfere with the evaluation of the study vaccine

* Grade 1 laboratory abnormalities (see toxicity table in Appendix II) will not be considered to be exclusionary at screening unless judged to be clinically significant by the PI. Potential participants with laboratory values of grade 2 or higher are not to be enrolled.

4.4. Treatments

4.4.1. Treatments Administered

4.4.1.1. Trivalent IPV (IMOVAX® Polio) Vaccine

IMOVAX® Polio [Inactivated Poliomyelitis Vaccine (Vero Cell Origin)] is a sterile suspension of three types of inactivated poliomyelitis vaccine: type 1 (Mahoney), type 2 (MEF1) and type 3 (Saukett). This vaccine is prepared from types 1, 2 and 3 of poliovirus cultured on Vero cells, purified and then inactivated by formaldehyde.

4.4.1.2. dmLT

LT(R192G/L211A), or dmLT, is a derivative of wild-type Enterotoxigenic *Escherichia coli* (ETEC) heat-labile enterotoxin that has been genetically modified by replacing the arginine at amino acid position 192 with glycine and the leucine at amino acid position 211 with alanine. These two amino acid substitutions take place in proteolytic cleavage sites which are critical for activation of the secreted toxin molecules. The protein has been designated LT(R192G/L211A) and has been extensively evaluated in pre-clinical animal studies for its ability to induce anti-dmLT antibody responses, as well as adjuvant the immune responses for co-administered antigens.

4.4.1.3. bOPV

Polio Sabin™ One and Three (oral) is a bivalent, live attenuated poliomyelitis virus vaccine of the Sabin strains Type 1 (LSc, 2ab) and Type 3 (Leon 12a, 1b), propagated in MRC5 human diploid cells.

Each dose (0.1 mL) contains not less than 106.0 CCID50 of Type 1 and 105.8 CCID50 of Type 3. Magnesium chloride is used as a stabilizer. Polio Sabin™ One and Three (oral) contains trace amounts of neomycin sulphate and polymyxin B sulphate.

4.4.2. Identity of Investigational Product

IMOVAX® Polio [Inactivated Poliomyelitis Vaccine] is manufactured by Sanofi Pasteur SA Lyon, France.

The bulk LT (R192G/L211A), or dmLT, was produced to current Good Manufacturing Practice (cGMP) specifications by IDT Biologika in Dessau-Roßblau, Germany.

Polio Sabin™ One and Three (oral), Bivalent Oral Poliomyelitis Vaccine Types 1 and 3 (bOPV) is produced by GlaxoSmithKline Biologicals s.a. in Belgium.

4.4.3. Method of Assigning Participants to Treatment Groups (Randomization)

The randomization sequence was generated using a permuted-block design, with 80 participants randomized to one of the three treatment groups in a 3:3:2 ratio. The randomization scheme was generated and maintained by the Statistical Data Coordinating Center (SDCC) at The Emmes Company, LLC (Emmes).

A subset of 10 participants per group were randomly selected to participate in an assessment of ASC homing marker assay results. Due to site restrictions, 8 participants per day were to be selected for enrollment, in a 3:3:2 ratio of IPV, IPV+dmLT and bOPV, respectively, with one participant per group (3 total) selected for the subset analysis.

To expedite site processes on the day of vaccination, randomization of each block of 8 participants will occur on the day before vaccination (except participants who are to be vaccinated on a Monday will be randomized on the prior Friday). Due to this, there is a possibility that some participants may not attend on vaccination day or may no longer be eligible. To allow for such cases, two

additional potential participants will be at the site on the day of vaccination as replacements. Each replacement would be randomized to the same treatment group as a participant being replaced. If a participant selected for the ASC homing marker subset needs replacing, then the replacement will also be selected for the subset. Note that, due to the restriction on number of participants in the ASC homing marker subset, the 8 participants randomized each day must be in sequence. In the unlikely event that fewer than 8 out of 10 participants appear for the vaccination visit there will be no available replacements and those missing participants will be replaced at the end of enrollment (i.e., not the next day). Pre-randomization of the next 8 participants must not occur before the current 8 participants (or replacements) have been successfully vaccinated.

4.4.4. Selection of Doses in the Study

dmLT has been shown in animal models to also stimulate mucosal immune responses following parenteral—e.g., IM, ID—administration, suggesting that administration of dmLT as an adjuvant for IPV to stimulate mucosal immune responses in the gut following parenteral administration may be expected to improve the effectiveness of IPV immunization against fecal shedding of poliovirus. In addition, dmLT has recently been given to humans by the IM route up to 0.5 µg dose with a candidate vaccine and demonstrated no unexpected safety concerns. Selection of exact dmLT dose for this study made based on this safety data.

The standard licensed trivalent IPV and standard bOPV (0.1 mL) doses will be used in the study.

4.4.5. Selection and Timing of Dose for Each Participant

CVIA 065 is a randomized, partially blinded, single-center outpatient study where participants are randomized to receive IPV alone, IPV together with dmLT adjuvant or bOPV, in a 3:3:2 ratio, respectively. Each participant in Groups A and B will receive a single dose IPV with or without dmLT followed 28 days later by a standard oral dose of bOPV. Group C will receive a standard oral dose of bOPV followed 28 days later by another standard oral dose of bOPV.

4.4.6. Blinding

This is a partially-blind study: study participants, study personnel who perform study assessments after vaccine administration, data entry personnel at the sites, and laboratory personnel (including those performing immunology assays) will be masked to assignment of IPV or IPV + dmLT, which are administered intramuscularly, but not to orally administered bOPV. The Emmes statistician and other designated staff will have access to the unblinded treatment assignments.

The study product will be prepared by the unblinded pharmacist who refers to a Treatment Key Listing, provided for the trial by Emmes, to determine the treatment for the participant. The pharmacist maintains the Treatment Key Listing under locked/secured conditions and does not reveal the randomization code to any other study staff member or participant. The investigational study product (IPV or IPV+dmLT) prepared by the qualified unblinded research pharmacist is witnessed by another unblinded study staff member then dispensed in a syringe, labeled with the blinded treatment code number, and administered by the study product administrator who may be

blinded if the pharmacy team is able to provide the study product in a manner that retains blinding. If a site chooses to utilize an unblinded study product administrator, he/she will not be involved in study-related assessments or have any participant contact for data collection following study injection. All follow-up safety and efficacy evaluations will be performed by blinded clinic staff.

Randomization data are kept strictly confidential, and should be accessible only to authorized persons, until the time of unblinding.

4.4.6.1. Unblinding Procedure

The site investigator may require that the blind be broken for any participant experiencing an emergency when knowledge of the participant's treatment assignment may be necessary for subsequent clinical care. Unblinding will occur by accessing the protocol randomization list saved in electronic form in Subversion (an Emmes internal version controlled restricted access location) or in the Antwerp University Hospital UZA Pharmacy in a restricted access location, to which there will be 24-hour access, to determine the Treatment Assignment associated with the subjects Sequence Number or Treatment Number.

Details and documentation surrounding such unblinding will be described in the Unblinding Operational Manual. Every effort should be made to maintain the blind. Prior to unblinding, the site Investigator is encouraged (to the extent possible, without jeopardizing the participant's health) to contact the Sponsor (or designee) to discuss the decision to break the blind. The site PI will be expected to provide a rationale for the necessity of unblinding based on the expectation that knowledge of the participant's treatment assignment will have a meaningful impact on the participant's medical care in the short term. If a participant's treatment assignment is unblinded, the participant will remain in the study and continue with protocol-defined study visits, but not receive further study vaccines. The decision to unblind will be communicated to the regulatory bodies (e.g., ethics committees) as required. At the end of the study, documentation of all unblinded participants (and the rationale for unblinding) will be incorporated into the Trial Master File.

4.4.7. Prior and Concomitant Therapy

Administration of rabies, tetanus or other types of vaccine for post-exposure indications will take priority over the study vaccine. All vaccines other than study vaccine used during the study will be reported.

4.4.8. Treatment Compliance

All participants should receive one study vaccination followed by a challenge at Day 29. All vaccinations will be administered or witnessed by a study investigator, clinical research coordinator, or designee. Each participant will be observed for at least 30 minutes after administration in case of any immediate adverse reactions. If a participant experiences an immediate adverse reaction, he/she will be treated and the event will be recorded in the eCRF. A listing of all vaccinations received and dates of challenge will be presented in Listing 7.

4.4.9. Protocol Deviations

All participant-specific deviations from the protocol are to be documented on the Protocol Deviation report form, including, but not limited to, the start date, reason for deviation (e.g., participant declined vaccination or other study procedures), and whether the deviation resulted in an AE or subject termination from the study.

4.5. Safety, Immunogenicity and Efficacy Variables

The following section describes the collection of safety, immunogenicity and efficacy variables. For a detailed schedule of activities, refer to Table 2 below (sample collection) and Table 3 in Appendix 1. For a list of the primary, secondary and exploratory variables, refer to Section 3.2 and Section 8 of this report.

4.5.1. Safety Variables

4.5.1.1. Reactogenicity Events

Reactogenicity events are solicited AEs that are pre-specified local and systemic adverse events that are common and known to occur or are of particular interest following administration of the study vaccine. For this trial, solicited AEs will be assessed by study staff 30 minutes after vaccination and then by study participants daily up to 7 days post-vaccination. Participants are provided a diary card for recording the presence or absence and severity of solicited AEs and instructed to measure and record their oral body temperature every evening regardless of the occurrence of any symptoms. Should additional temperature measurements be performed at other times of day, participants are instructed to record the highest temperature in the diary card.

Investigators will review diary cards with the participant when the participant returns to the clinic on Day 8 to ensure the solicited AEs are appropriately documented.

The following specific solicited AEs will be monitored for this trial:

- Local/injection site reactions: Pain, erythema/redness, swelling, induration, hyperpigmentation
- Systemic reactions: Fever (defined as oral temperature ≥38.0°C), chills, fatigue, headache, muscle aches/myalgia, joint ache/arthralgia, rash, nausea, vomiting, diarrhea.

4.5.1.2. Unsolicited Adverse Event

Refer to Section 3.3.1 for a more detailed definition of AE. The occurrence of an AE might come to the attention of study personnel during study visits or during interviews of a study subject who presents separately for medical care. Information to be collected on AEs includes event description, time of onset, assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event.

4.5.1.3. Serious Adverse Event (SAE)

Refer to Section 3.3.2 for the definition of SAE.

4.5.1.4. Severity of Adverse Event

Refer to Section 3.3.4 for the details describing severity.

4.5.1.5. Causality Adverse Event

Refer to Section 3.3.5 for details on determining causality.

4.5.2. Immunogenicity Variables

Multiple samples will be assayed at various laboratories and time points to assess the immunologic responses to the investigational product (Table 2). Assays include:

- Type-specific poliovirus fecal IgA responses
- Type-specific neutralizing antibodies in serum
- Type-specific serum IgG and IgA by ELISA
- Type-specific circulating IgA and IgG ASC and ALS
- Type-specific circulating IgA and IgG-secreting α4β7 ASC Homing Marker- a subset of samples
- Type-specific poliovirus neutralizing antibody in stool
- Type-specific and total IgA and IgG in saliva by ELISA
- Type-specific memory B-cells
- Type-specific CD4+ T-cells.
- Multiplex cytokine assay

Table 2: Summary of Laboratory Sample Collection and Analysis (by Date and Collaborating Lab) – Protocol Table 3

dmLT-IPV - Summary of assay timing and location		Study Day											
Assay	**Lab Conducting Assay**	**-28 to 0**	**1†**	**8**	**29‡**	**33**	**36**	**39**	**43**	**46**	**50**	**57**	**169**
Safety blood specimens	AML-Riatol	X		X									
Serum for IgA/IgG (ELISA)	UMB CVD		X	X	X		X		X		X	X	X
Serum for neutralizing antibody	CDC		X		X								
Blood for ASC/ALS*	ULB		X	X	X		X						
Blood for ASC α4β7 *	ULB			X			X						
Blood for memory B and CD4+ T cells#	ULB		X		X							X	X
PBMC preparations & multiplex cytokine assay**	Cools Lab		X		X							X	X
Stool for IgA	Wright Lab	X		X	X		X		X		X	X	X
Stool for neutralizing activity	Wright Lab	X		X	X		X		X		X	X	X
Saliva specimen for IgA/IgG (ELISA)	UMB CVD	X		X	X		X						
Stool specimen for viral shedding (bOPV challenge shedding)	CDC				X	X	X	X	X	X	X	X	

*Assays requiring fresh cells, all other assays can be conducted on preserved samples.
Memory B cell and T cell assays will be conducted on the PBMC preparation.
† D1: Vaccination
‡ D29: Challenge
** includes detection of Eotaxin, Eotaxin-3, GM-CSF, IFN-γ, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, TNF-α, TNF-β, VEGF-A

4.5.3. Microbiological / Virological Variables

Stool specimens for the evaluation of bOPV challenge shedding will be collected as indicated in Table 2.

- bOPV and type-specific poliovirus stool shedding positivity via PCR following bOPV challenge
- $CCID_{50}$/g of poliovirus in stool following bOPV challenge

5. SAMPLE SIZE CONSIDERATIONS

Rates of solicited and unsolicited AEs, including SAEs will be determined for each experimental group. With 30 subjects per treatment group, this study has an 80% probability of detecting at least 1 AE that occurs at a rate of 5.3%. If no SAEs are observed among the 30 subjects, an approximation to the one-sided upper 95% confidence bound on the rate of SAE occurrence would be 9.5%.

5.1. Virologic Assessment

5.1.1. Primary Viral Shedding Endpoint

With 27 evaluable subjects per treatment arm, this study is designed to provide at least 96% power to detect ≥60% reduction in shedding rate in the IPV+ dmLT group assuming the shedding rate in the IPV alone group is at least 80% (see protocol Section 11.3, Table 3).

5.1.2. Secondary Viral Shedding Endpoint

Sample size calculations were based on prior experience of OPV shedding from IPV-vaccinated participants. Data from two previous studies were used to estimate the relationship between mean AUC and variability in AUC and in simulations to provide sample size/power computations for the 2 two-group comparisons of the AUC ratio of interest ($AUC(\mu_d)/AUC(\mu_I)$ <0.5, where $AUC(\mu_d)$ is the center of the distribution of AUC for the IPV + dmLT arm and $AUC(\mu_I)$ is the center of the distribution of AUC for the IPV only arm). See Protocol Section 11.3 for more details.

The minimum 50% reduction selected for comparison of the IPV+dmLT to the IPV only arm was selected by programmatic considerations as well as to reflect the information that may be garnered from the limited sample size required and chosen for the safety and primary viral shedding outcomes.

For comparison of shedding of challenge virus between the IPV+dmLT and IPV arms:

It was assumed that the AUC ratio $AUC(\mu_d)/AUC(\mu_I)$ would be ≤0.35. Estimated variances of the AUC of each group were obtained from the mean-variance relationship described above, and the power of the comparison, facilitated by a Wilcoxon rank-sum test on the log-transformed AUC values, was estimated via repeated simulation and testing.

For evaluation of shedding of bOPV vaccination virus:

There is ongoing uncertainty about the expected time until shedding cessation of Sabin types 1 and/or 3 after the first bOPV dose for IPV-vaccinated adults. In addition to providing useful immunogenicity data, the bOPV control arm provides an opportunity to assess the proportion of subjects shedding either Sabin type 1 or 3 after 28 days, which is expected to be informative for future studies. Varying levels of precision regarding the rate of vaccine virus shedding at the day of challenge in the bOPV arm are available, under varying assumptions of the shedding rate. In the table below, the precision for estimating the rate of Day 28 shedding with 18 evaluable subjects in the bOPV arm is displayed under varying assumptions of the shedding rate.

Rate of Day 28 shedding	Lower 95% CI bound	Upper 95% CI bound
10%	1.0%	33.3%
20%	5.2%	45.2%
30%	11.1%	55.7%
40%	18.1%	65.3%
50%	26.0%	74.0%
60%	34.7%	81.9%

Resultant calculations:

Under the assumptions and boundary values described above, approximately 80% power would be available for the primary comparison if 24 subjects per group are evaluable among the IPV+dmLT and the IPV-only groups and adequate precision would be achieved with 18 subjects in the bOPV arm.

6. GENERAL STATISTICAL CONSIDERATIONS

6.1. General Principles

All analyses will be grouped by product received (IPV, IPV + dmLT, bOPV), including a column for the total across all participants (except for immunogenicity). Except where otherwise indicated, descriptive statistics for continuous measures will include mean, standard deviation, median, 1st and 3rd quartiles, and range (min, max); for categorical measures: frequencies, proportions, and exact Clopper-Pearson 95% confidence intervals (CIs). All percentages will be presented to one decimal place.

All data will be listed, sorted by treatment group and participant, and when appropriate by time point within participant.

For all immunological and viral shedding assays with predefined limits of quantitation, all statistics which achieve the lower limit of quantitation (LLOQ) or upper limit of quantitation (ULOQ) will be replaced with "<LLOQ" or ">ULOQ", as appropriate, where "<LLOQ" and ">ULOQ" are replaced by the numerical limits. Analysis of immunological and viral shedding assays will be supplemented by reverse cumulative distribution (RCD) curves with each group for a given time point displayed on the same plot and a different panel for different each time point.

Baseline demographics and characteristics, including age, height, weight, gender, race/ethnicity, body mass index (BMI), and baseline serology (for HIV, Hepatitis B, and HCV) will be summarized for both the total vaccinated and per protocol populations. Summaries of participant disposition will be prepared for all subjects, including the number and percent screened, enrolled, and within each study population, as well as a CONSORT diagram (Figure 1 in Appendix 2) describing study participation and dropout. The reasons not enrolled will be summarized, along with the withdrawal rate and the reasons for withdrawal, as well as listed. A summary and listing of visit attendance will be prepared, in addition to a summary and listing of vaccine administration, and a sample collection/availability for each sample type (blood, stool).

6.2. Timing of Analyses

Upon collection, shipment, and analysis of the last stool sample from the last subject necessary for viral shedding objectives, at 28 days post-challenge, a topline analysis will be initiated. Topline analysis will include primary safety results through 28 days post-challenge, and both primary and secondary viral shedding including cell culture infectious dose 50% assay (CCID50) results, if available at the time of the analysis. These blinded primary safety results will consist of serious

and/or severe unsolicited and solicited AEs through 28 days post-challenge. Only the sponsor will receive the report, without listings or individual treatment assignments. Viral shedding results will be unblinded to group and safety results will be presented in aggregate only (blinded to IPV vs IPV+dmLT group). Tables included in the topline analysis are in Appendix 1: Table 56, Table 57, Table 59, and Table 60.

Throughout the study, all clinical assay laboratories will remain blinded to group assignment and time point of the samples, except ULB for the homing marker assay. ULB will be specifically unblinded in GlobalTrace for subject and visit number in order to be able to identify whether or not the subject is assigned to the sub-set for the homing marker assay. Following collection of the remainder of the data and database lock, all results will be described in the final CSR.

6.3. Analysis Populations

A summary of the analysis populations by study group will be prepared (Table 7, Appendix 1).

6.3.1. Enrolled Population

All screened participants who provide informed consent, regardless of the participant's randomization and treatment status in the trial.

6.3.2. Total Vaccinated Population

All participants in the enrolled population who were randomized and received a study vaccination. All safety analyses will be performed using this population. Treatment groups for safety analysis will be assigned according to the actual treatment received at Day 1.

6.3.3. Per Protocol Population

The Per Protocol (PP) population will include all participants in the total vaccinated population who have no protocol violations determined to potentially interfere with the immunogenicity assessment of the study vaccine. This population will serve as the primary analysis population for the immunogenicity and shedding endpoints. The population will be adapted by time point to include all eligible subjects' data up to the time of the disqualifying protocol deviation. The membership in this study population will be determined in a blinded fashion at a Data Review Meeting (DRM) attended by the sponsor, investigator, and SDCC representatives.

6.4. Covariates and Subgroups

No covariates will be used in the analyses. Within the bOPV arm we will consider the subset of the subjects who are not PCR positive at the time of bOPV challenge for exploratory summaries of shedding cessation.

6.5. Missing Data and Outliers

In this Phase I study, missing data will be assumed to be missing completely at random, and only observed data collected from participants and available in the appropriate study population will be used for analysis.

The analysis of immunogenicity will be performed primarily on the PP set. If 10% or more of the vaccinated subjects are eliminated from the PP set (for a given endpoint), a second analysis will be performed on the total vaccinated population. This percentage will be applied to missing data across study groups simultaneously.

Some analyses defined herein rely on all values from repeated sampling to be available for a given subject (e.g., shedding index endpoint [SIE] and AUC). Should the amount of missing data be extensive, alternative AUC computation methods that incorporate one or more valid interpolation techniques may be explored. If performed, this will be clearly justified and described in study reports.

6.6. Interim Analyses and Data Monitoring

6.6.1. Interim Analysis

Not applicable.

6.6.2. Data Monitoring

A safety review committee (SRC), composed of the PI, the PATH Medical Officer and three independent vaccine and infectious disease experts will periodically review the conduct and safety of the study. The responsibilities and procedures of the SRC are defined in the SRC Charter.

The SRC will convene prior to study initiation, following vaccination of all subjects (post Day 8), and a final safety data review meeting will occur one month after all subjects receive their bOPV challenge (post-Day 57). The Emmes statistician with assistance of the data management staff will prepare safety reports for review by the SRC. These reports will provide at a minimum the following information: 1) accrual and participant status data with regard to completion of study vaccination and study visits and 2) summaries of solicited and unsolicited AEs including safety labs. Cumulative safety data will be available continuously for review by SRC members who will also be guided by a set of predetermined stopping criteria.

The SRC may elect to discuss trial conduct issues that could affect study integrity and participant safety. These may include but are not limited to data quality, critical monitoring findings, study product, research specimens, etc. Emmes will also notify the sponsor and the SRC of the need for ad hoc safety reviews whenever the observation of SUSARs or AEs meet pre-specified study pause criteria as per Section 9.2.1 of the protocol.

In addition to routinely scheduled calls, if there are serious safety concerns or study pause criteria are met, the SRC will convene by teleconference to jointly review the data. The SRC reviews will

be summarized with recommendations to the study sponsor as to whether there are safety concerns and whether the study should continue without change, be modified, or be terminated.

If at any time, a decision is made to permanently discontinue administration of study product in all participants, the Sponsor will notify the Federal Agency for Medicines and Health Products (FAMHP) and PATH's research ethics committee (PATH REC). The site investigator of record will notify the responsible local institutional review boards/ethics committees (IRB/EC) expeditiously.

6.7. Multicenter Studies

Not applicable, this is a single center study.

6.8. Multiple Comparisons/Multiplicity

Despite the large number of statistical tests, due to the exploratory nature of these evaluations, no adjustment for multiplicity will be performed.

7. STUDY PARTICIPANTS

7.1. Disposition of Participants

The disposition of participants and exposure to study vaccinations will be tabulated by study group for all participants. Summary of participant disposition will include, but is not limited to, the number of participants screened/enrolled, received study vaccination, received bOPV challenge, completed the study and terminated early, together with reasons for not enrolled, vaccinated or terminated early (Table 6, Appendix 1).

The composition of analysis populations, including reasons for subject exclusion, by study group, will be presented in Table 7.

Table 8 will present a summary of the reasons that subjects were screened but not enrolled.

A CONSORT diagram of the study will also be prepared (Figure 1, Appendix 2). This figure will present the number of subjects screened, enrolled, lost to follow-up, and analyzed by study group.

7.2. Protocol Deviations

A summary of participant-specific protocol deviations will be presented by the deviation category, the deviation type, and study group for all participants in the total vaccinated population (Table 5, Appendix 1). All participant-specific and non-participant-specific protocol deviations will be included as data listings (Listing 2 and Listing 3, Appendix 3). Protocol deviations may lead to exclusion from the PP Population. Determination of exclusion from the PP population will be established before breaking the blind, based on a blinded review of protocol violations and other criteria.

8. SAFETY EVALUATION

Safety and tolerability is a primary objective of this study. All safety assessments will take place in the total vaccinated population, according to the treatment received. For continuous measures, descriptive statistics will include mean, standard deviation, median, 1st and 3rd quartiles and range; for categorical measures: frequencies, percentages and, where indicated, exact two-sided Clopper-Pearson 95% CIs. All percentages will be presented to one decimal place. Means and medians will be presented to one decimal place more than the raw value, standard deviations and confidence intervals will be presented to 2 extra decimals. For safety assessments presented by time point, unscheduled assessments will be summarized as separate time points in chronological order with scheduled study visits.

8.1. Demographic and Other Baseline Characteristics

Baseline demographics and characteristics, including age, height, weight, gender, race/ethnicity, BMI, and baseline serology (for HIV, Hepatitis B, and HCV) will be summarized for both the total vaccinated (Table 9 and Table 10 Appendix 1) and per protocol populations (Table 11 and Table 12 Appendix 1). Demographic and baseline characteristic summaries will be supplemented with two-sided level $\alpha = 0.05$ statistical assessment of differences between groups, using Fisher's exact test for categorical variables, and the Kruskal-Wallis test for continuous variables. Demographic listing will also be prepared (Listing 4, Appendix 3).

8.1.1. Medical History

An individual participant listing will be presented for all medical history terms reported at screening (Listing 5).

8.1.2. Prior and Concomitant Medications

A summary of medications that were taken prior to dosing and throughout the study will be presented by WHO Drug Anatomic Therapeutic Chemical (ATC) classification Level 1 and Level 4 (Table 13, Appendix 1). Individual participant listings will be prepared for all concomitant medications (Listing 6, Appendix 3).

8.2. Measurements of Visit Compliance and Research Sample Collection

A summary of the number of participants completing each scheduled visit and the number providing research sample results (e.g., stool, saliva, blood) at each designated visit (depends on sample type/use) will be prepared (Table 14, Appendix 1). In addition, a listing of all completed visits and samples collected and analyzed will be prepared (Listing 8, Appendix 3).

8.3. Adverse Events

All adverse events will be summarized for the total vaccinated population, according to treatment received and overall. All subject-level percentages (solicited/unsolicited AEs, laboratory abnormalities, etc.) will be supplemented with two-sided 95% CIs computed via the Clopper-Pearson method. Summaries will include all events occurring on or after the date of vaccination. Individual summaries (denominators for percentages) will be limited to the number of subjects within the appropriate analysis population with data available for analysis for the given endpoint.

8.3.1. Solicited Events and Symptoms

Reactogenicity will be assessed according to immediate (at least 30 minutes post-vaccination) and delayed reactogenicity (within 7 days post-vaccination), as well as combined. Solicited AEs will be summarized overall, by category (local/systemic), by reaction, and by severity within reaction and within category, and by severity and reaction according to post-vaccination day, where each subject is counted once under the maximum severity of each reaction and/or category, where relevant. Analyses involving severity will include those graded ≥2. Summaries of solicited AEs observed across the solicitation period will be accompanied by a two-sided Fisher exact test p-value for a difference among groups, for both local and systemic events (overall and by reaction), as well as across these events (overall). Reactogenicity events ongoing at 7 days post-vaccination will be listed. Duration of reactogenicity events will be summarized by reaction.

Measured injection site features will be summarized by group and post-vaccination time point, including but not limited to pain, erythema/redness, swelling/induration and hyperpigmentation.

Severity is graded on a scale of 0 (normal), 1 (mild), 2 (moderate) and 3 (severe). Local and systemic reactions are listed below.

Vaccination Site Reactions:

- Site pain, induration, swelling, induration diameter, swelling diameter, erythema(redness) diameter and hyperpigmentation diameter.

Systemic Reactogenicity:

- Fever (oral temperature), chills, fatigue, headache, muscle aches/myalgia, joint ache/arthralgia, rash, nausea, vomiting, diarrhea.

Solicited adverse events will be summarized and presented in Table 15 through Table 36. Each table summarizes events by treatment group, severity and time point (immediate - within 30 minutes, and delayed - within 7 days, at any time up to 7 days, and by day). Table 15 is a summary of participants with any reactogenicity (local or systemic), Table 16 is a summary of participants with any local reactogenicity, Table 17 is a summary of participants with any systemic reactogenicity and Table 18 through Table 34 each summarize an individual reaction. Table 35 presents descriptive statistics of the duration (days) of each reaction and Table 36 presents a listing of all reactogenicity events that were ongoing at Day 7.

All solicited adverse events will be listed by participant and included in Listing 9 (Appendix 3).

8.3.2. Unsolicited Adverse Events

All unsolicited AEs, including serious and/or severe AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) version 20.1 or later. A summary table will be prepared for unsolicited AEs presenting incidence of any AE, any related AE, any serious AE, any severe AE, any AE of grade ≥2, any related AE of grade ≥2, and any AE leading to study withdrawal where a subject only contributes once. Additional tabulations among these categories will be presented by severity, where relevant, where a subject only contributes once under the maximum severity event recorded. Summary tables will also be prepared for each of these categories presenting summaries across System Organ Class (SOC), and across preferred terms (PT) within SOC, where again each subject only contributes once per SOC/PT combination, and once per SOC. An additional table will include PT occurring in ≥2 subjects (across group, regardless of seriousness, severity, or relationship), sorted in descending order of incidence. Each of these tables will use the form "*n (%) m*", where *n* is the number of subjects with an event, *(%)* is the percent of subjects experiencing that event, using the number in the total vaccinated population for the specific group as the denominator, and *m* is the number of events of that type within that group, regardless of the number of subjects from which they originate. Listings will be prepared including each of the categories of unsolicited AEs listed above including the verbatim term, the SOC, PT, type, the date and study day of onset and resolution, as well as the seriousness, severity, causality assessment, actions taken, and the outcome.

Unsolicited adverse events will be summarized for the total vaccinated population and presented in Table 37 through Table 40.

All unsolicited AEs will be listed by participant and included in Listing 9 (Appendix 3).

The following summaries of unsolicited adverse events will be presented by study group and overall:

- Summary of unsolicited AEs grouped by participants with (1) any AE, (2) any SAE, (3) any severe AE, (4) any AE ≥ grade 2 and (5) any AE leading to withdrawal from the study. Separate summaries will be presented covering AEs throughout the study, AEs within 28 days of vaccination and AEs related to study product within 28 days of vaccination (Table 37, Appendix 1).
- Summary of unsolicited AEs by MedDRA SOC, PT and severity (Table 38, Appendix 1).
- Summary of unsolicited AEs related to study product, by MedDRA SOC, PT and severity (Table 39, Appendix 1).
- Summary of unsolicited AEs that occurred in ≥ 2 participants within the same MedDRA PT classification within 28 days of vaccination (Table 40, Appendix 1).

8.4. Deaths, Serious Adverse Events and Other Significant Adverse Events

A listing of all data related to deaths, SAEs and other significant AEs will be presented (Table 41, Appendix 1). Refer to Section 3.3.2 for details of SAEs and other important medical events.

8.5. Pregnancies

For any participants in the total vaccinated population who become pregnant during the study, every attempt will be made to follow them through completion of pregnancy to document the outcome, including information regarding any complications with pregnancy and/or delivery. A listing of pregnancies and outcomes will be presented (Listing 10, Appendix 3).

8.6. Clinical Laboratory Evaluations

Screening serology assessments for HIV, Hepatitis B, and HCV will be described as categorical variables on the demographics table.

Screening and post-vaccination (Day 8) serum chemistry and hematology will be graded by severity, according to Table 4 (Appendix 1). Grade 2 or higher were automatically considered as clinically significant and reported as AE, while Grade 1 abnormalities were subject to investigator opinion.

Continuous summaries of laboratory parameters and the change from baseline will be computed by time point (including unscheduled visits, in chronological order), for all observations regardless of abnormality status, and separately for values less than the lower limit of normal (<LLN) and greater than the upper limit of normal (>ULN), as appropriate. Summaries will also be produced presenting the proportion of each severity grading using the number of subjects that provided a sample as denominator, as well as those considered clinically significant, using the maximum severity for a given subject, where relevant. A summary of safety laboratory abnormalities will be prepared by parameter, severity (by grade and any abnormality), and time point. Fisher's exact 2-tailed test will be used to test the differences in abnormality rates across the groups, by time point for scheduled visits, and overall. For each parameter and post-baseline time point, shift tables will be prepared detailing the number and percent of subjects with observations mild/moderate/ severe according to the grade of their baseline grade of the same parameter.

Figures will be prepared for each laboratory parameter using boxplots to describe the distribution of values and changes from baseline across visits, by group, incorporating unique points and/or colors for abnormalities.

A listing of all lab results will be prepared, in addition to a listing only for abnormalities, and each will include the parameter, study day, value, units, change from baseline, LLN/ULN, and abnormality grade.

Clinical laboratory values are collected at screening and Day 8 post-vaccination. They will be summarized as described above for the total vaccinated population and presented in Table 42 through Table 53 (Appendix 1).

The distribution of each laboratory value and change from baseline will be presented by time point and treatment group (Table 42, Appendix 1), including mean, standard deviation, median, 1st and 3rd quartiles (IQR) and range. The severity of each laboratory value will be summarized by time point and compared between treatment groups (Table 43 through Table 52, Appendix 1). A shift table of the change in severity from baseline to Day 8 will be presented (Table 53, Appendix 1). Changes in laboratory values will be presented in Figure 2 through Figure 10. Finally, a listing of all clinical laboratory values will be prepared (Listing 11, Appendix 3).

8.7. Vital Signs and Physical Evaluations

Vital sign measurements including systolic and diastolic blood pressure (mmHg), oral temperature (°C), pulse (beats/min) and respiratory rate (breaths/minute) will be assessed at each visit (screening and days 1, 8, 29), including change from pre-vaccination baseline. Vital signs will be tabulated by visit and study product (Table 54, Appendix 1), including mean, standard deviation, median and range. A full listing will be prepared (Listing 12, Appendix 3) and will include study day, value, units, and change from baseline.

Physical exam results, including number and proportion of participants with abnormalities, will be summarized for the total vaccinated population and presented in Table 55 (Appendix 1), by body system, visit and treatment group. A full listing will be prepared (Listing 13, Appendix 3).

9. VIRAL SHEDDING

9.1. Primary Viral Shedding Endpoint

Viral shedding will be assessed in the per protocol population. The number and proportion of subjects with stool samples positive for viral shedding (via PCR; type-specific and non-specific) will be summarized by time point and group, including corresponding 95% CIs computed via the Clopper-Pearson method (Table 56, Figure 12). The denominator for each of these percentage calculations will reflect the number of subjects providing a sample to be assayed, per time point.

Table 57 presents the percent reduction in proportion of subjects shedding challenge virus on study Day 36 (7 days post-challenge). Proportions will be compared for each serotype and overall between the IPV + dmLT and IPV-alone arms via one minus the relative risk, and this computation will be accompanied by a 2-sided 95% CI computed via the Farrington and Manning method. [Note: These are exact unconditional confidence limits based on the SCORE statistic. Include the statement EXACT RELRISK(METHOD=FM) in PROC FREQ. Can be a bit computer intensive.]

Additionally, viral shedding ($\log_{10}$ $CCID_{50}$/g, not type-specific) will be summarized descriptively (Table 58) as a continuous variable on the $\log_{10}$ scale, with LLOQ (2.75 $\log_{10}$) and ULOQ (8.25 $\log_{10}$) used as the observed value, whenever these limits are met. A value of 0 will be used when

a sample is PCR-negative for viral shedding. These results will be supplemented with a CI for the median ($\log_{10}$ scale), computed via the percentile bootstrap method, using n=10,000 replicates. Should multiple samples be taken on a single day, the arithmetic mean of results ($\log_{10}$ scale) will replace the intended individual measurement whenever necessary for summaries (but not for listings).

9.2. Secondary Viral Shedding Endpoint

In addition to the primary viral shedding endpoint and associated descriptive statistics, the AUC of $\log_{10}$ $CCID_{50}$/g will be computed and summarized (Table 59). The AUC will be computed via two methods:

1. Using the simple arithmetic mean of the $\log_{10}$ $CCID_{50}$/g samples collected on Days 36, 43, 50, and 57 (i.e., 7, 14, 21, and 28 days, respectively, following challenge), using the LLOQ and ULOQ as observed data, and assigning a value of zero for samples PCR-negative for poliovirus, as described above. This will be referred to as the Shedding Index Endpoint (SIE), and requires values to be available for each of the four time points and is considered the main analysis of this secondary endpoint. As a sensitivity analysis SIE will also be calculated as the arithmetic mean of all samples available for Days 33 through 57.
2. As a supplementary summary, the extent of viral shedding will be described using the linear trapezoidal rule to compute the true AUC using all available samples and same rules for LLOQ, ULOQ and PCR-negative results as described above; this will be referred to as the AUC. The AUC will be missing for any subject with 2 or more missing values (whether consecutive or not), or if the first or last sample is missing; if only one missing value is present (except for the first and last), then the AUC will be computed from the available samples, effectively using linear interpolation for the missing sample.

The SIE and AUC will be summarized (Table 59) as continuous variables according to the summaries defined above for the time point-specific summaries of $\log_{10}$ $CCID_{50}$/g, and both will be supplemented with the difference in medians (IPV + dmLT minus IPV alone) with corresponding two-sided 95% CIs computed via the percentile bootstrap method, with n=10,000 replicates. The ratio of the SIE and AUC (IPV + dmLT to IPV alone) will be computed via the difference on the log scale, with accompanying 90% CI computed via the same bootstrap method, then back-transformed using the antilog. Here, the 90% CI is used instead of 95%, to enable a one-sided level 0.05 non-inferiority test corresponding to the sample size calculations described above.

Additionally, analysis of this primary viral shedding endpoint will be supplemented with the type-specific time to cessation of OPV shedding, which will be analyzed with Kaplan-Meier (KM) methods, including right-censoring where appropriate. The day of cessation of shedding will be defined as the day of the first PCR-negative stool sample for challenge virus after which the next two consecutive stool samples are also PCR negative and will be conducted for any virus (1, 3, or both) and either virus in a type-specific manner. The estimated quartiles of time to cessation of shedding with corresponding 95% CIs will be computed, and a figure will be prepared denoting

the KM estimate, with symbols used to indicate censored data points. The shedding cessation rate at each post-challenge day will be computed, along with corresponding 95% CI, via the Greenwood method. The log-rank test will be used to provide a global test for a difference among survival curves. This analysis will be considered supplementary to the shedding analyses described above.

Viral shedding results are collected pre-challenge (Day 29) and on Days 33, 36, 43, 50, and 57 (i.e., 4, 7, 14, 21, and 28 days, respectively, following challenge). They will be summarized as described above for the per-protocol population and presented in Table 56 through Table 60 (Appendix 1), Figure 11, Figure 12 and Figure 13 (Appendix 2), and in Listing 14 (Appendix 3).

A summary of the proportion of participants PCR-positive for viral shedding on each day is presented in Table 56 and Figure 12. The reduction in the proportion of participants shedding the challenge virus on Day 7 in the IPV + dmLT group as a proportion of those shedding the virus in the IPV group is presented in Table 57. Descriptive statistics of viral shedding ($\log_{10}$ $CCID_{50}$/g), by time point, are presented in Table 58 and in reverse distribution curves in Figure 11. Table 59 presents descriptive statistics, difference in medians with 95% CIs via the percentile bootstrap method using 10,000 replicates, and the ratio with 90% CI of the SIE and AUC measures. The type-specific time to cessation of OPV shedding, analyzed by KM methods, including estimated quartiles of time to cessation of shedding and the shedding cessation rate at each post-challenge day along with corresponding 95% CI, via the Greenwood method, are presented in Figure 13 and Table 60.

10. IMMUNOGENICITY

Immunogenicity assessments will take place within the per protocol population. For estimation of the GMT, GMT ratio, and corresponding confidence limits, analysis will be conducted using SAS PROC LIFEREG, incorporating censoring where appropriate at LLOQ and ULOQ, and a Normal error distribution on the log scale; the SAS LSMEANS of the log-scale coefficients will be back-transformed in order to compute the estimate and corresponding confidence limits for the relevant quantity. The GMFR will be computed using standard two-sample methods for the $\log_2$ difference of the paired samples, with corresponding CIs computed via the *t*-distribution, utilizing the antilog transformation to present the ratio. Refer to the paper by Wang, et al. [1] for a justification of the use of PROC LIFEREG for these data.

10.1. Fecal IgA and Neutralization

Samples for Fecal IgA and Neutralization will be presented in Table 61 through Table 83 (Appendix 1), Figure 14 to Figure 21 (Appendix 2) and Listing 15 to Listing 16 (Appendix 3).

A positive fecal IgA (total IgA and type-specific IgA) and neutralization response is defined as a minimum 4-fold rise from the pre-vaccination value. Responses will be assessed following vaccine administration, as well as after bOPV challenge (using both pre-dose values, separately, to compute the post-challenge response rate, i.e. from baseline and from pre-challenge). For each

endpoint, the number and proportion exhibiting a type-specific response will be summarized overall and for each serotype by post-vaccination time point and group, including corresponding 95% CIs computed via the Clopper-Pearson method. Additionally, for all time points, serotype-specific fecal IgA and stool neutralization titer will be summarized as a continuous variable on the log scale, with one-half the LLOQ (LLOQ = 0.03 ng/mL for type-specific fecal IgA, LLOQ = 2 $\log_2$ for neutralization) and with the ULOQ (42.7 ng/mL for fecal IgA, LLOQ = 10 $\log_2$ for neutralization) used as the observed value, whenever these limits are met, except for the GMT and corresponding CI, to be computed as described above with SAS PROC LIFEREG. These results will be supplemented with a CI for the median (log scale), computed via the percentile bootstrap method, using n=10,000 replicates.

Similarly, for each serotype, post-baseline time point, and group, the GMFR and accompanying two-sided 95% CI will be computed using SAS PROC LIFEREG. For time points following bOPV challenge, this will additionally be computed using the last sample prior to challenge as the "baseline". A figure will be prepared displaying the geometric mean and accompanying CI for each group for each visit with a corresponding set of figures for the GMFR. Only subjects with both a pre- and post-vaccination/challenge measurement will contribute to the GMFR computations.

The type-specific difference in response proportion between IPV + dmLT and IPV alone (IPV + dmLT minus IPV alone) and between IPV + dmLT and bOPV (IPV + dmLT minus bOPV) will also be computed at each post-vaccination visit, with corresponding 95% CI computed via the Miettinen and Nurminen score method. The GMT ratio and corresponding two-sided 95% CI for each of these comparisons will be computed following fit of an ANCOVA-style model (separately for each comparison) to the log-transformed titer with SAS PROC LIFEREG. For time points following bOPV challenge, this will again be recomputed using the last sample prior to challenge.

The proportion (95% CI) of participants with positive (≥4-fold increase from baseline and from pre-challenge) fecal total IgA responses are summarized by serotype (any serotype and serotypes 1 – 3), visit and treatment group in Table 61 (Appendix 1). Descriptive statistics of titer and fold-rise from baseline (including fold-rise from pre-challenge) are presented by serotype in Tables 62 – 64 and Tables 65 – 67, respectively (Appendix 1), respectively. Statistical comparisons of positive responses and of GMTs are presented by serotype in Tables 68 – 70 (Appendix 1), where group comparisons are between IPV + dmLT and IPV alone and between the IPV + dmLT and bOPV, by visit.

The proportion (95% CI) of participants with positive (≥4-fold increase from baseline and from pre-challenge) fecal neutralization responses are summarized by serotype (any serotype and serotypes 1 – 3), visit and treatment group in Table 71 (Appendix 1). Descriptive statistics of titer and fold-rise from baseline (including fold-rise from pre-challenge) are presented by serotype in Tables 72 – 74 and Tables 75 – 77, respectively (Appendix 1), respectively. Statistical comparisons of positive fecal neutralization responses and of GMTs are presented by serotype in Tables 78 –

80 (Appendix 1), where group comparisons are between IPV + dmLT and IPV alone and between the IPV + dmLT and bOPV, by visit.

10.2. Serum Neutralizing Antibodies (NAb)

Samples for serum NAbs are collected prior to vaccination (Day 1) and prior to challenge (Day 28) and will be presented in Table 81 through Table 85 (Appendix 1) and Listing 17 (Appendix 3).

For each serotype and serum sample time point, the type-specific titer of serum neutralizing antibody (NAb) will be summarized as a continuous variable on the $\log_2$ scale using the same statistical methods as described for the primary immunogenicity variables, where the ULOQ is 10.5 $\log_2$ and LLOQ is 2.5 $\log_2$.

The rates of seroprotection (NAb reciprocal titer ≥1:8) and seroconversion (minimum four-fold increase or greater in serum NAbs between baseline and post-vaccination time point, or post-vaccination reciprocal titer ≥1:8 if seronegative at baseline) will be summarized as categorical variables and supplemented with Clopper-Pearson two-sided 95% CIs. Because it is possible that some IPV-vaccinated subjects have high serum NAb titers at baseline, the rate of seroconversion will secondarily be computed among the subset of subjects with baseline values low enough that seroconversion is possible to observe ($\log_2$ reciprocal titer ≤ 8.5).

The type-specific differences in rates of seroprotection and seroconversion between groups (IPV + dmLT minus IPV alone and IPV + dmLT minus bOPV) will be computed and accompanied with two-sided 95% CIs computed via the Miettinen and Nurminen score method. Additionally, GMT ratios between these groups will be computed using SAS PROC LIFEREG as described previously, to account for censored data.

Seroprotection (titer ≥ 1:8) and seroconversion (≥4-fold increase from baseline) are summarized by the proportion (95% CI) of participants with a response, by virus type, visit and treatment group in Table 81. Descriptive statistics of titer and fold-rise from baseline are presented in Table 82 (PV1), Table 83 (PV2) and Table 84 (PV3). Statistical comparisons using estimates and confidence intervals of the differences in seroprotection, seroconversion and GMT on Day 29 are presented in Table 85, by virus type and visit, where group comparisons are between IPV + dmLT and IPV alone and between the IPV + dmLT and bOPV. Pairwise comparison estimates will be obtained from a single model, separately for each virus type.

10.3. IgA and IgG Antibody-Secreting Cells (Non-Homing)

Samples for IgA and IgG ASCs (non-homing) will be collected on study Days 1, 8, 29 and 36. IgA and IgG ASCs will be presented in Table 86 through Table 90 (Appendix 1) and Listing 18 to Listing 19 (Appendix 3).

An ASC response is defined as an ASC count at a post-vaccination sample that is ≥ 8 cells per 10^6 PBMC. The proportion of subjects with type/antibody-specific ASC response both following vaccination and following bOPV challenge will be summarized by type, group, and time point as

a categorical variable and accompanied by two-sided Clopper Pearson 95% CIs in Table 86. In addition, these frequencies will be summarized for ASCs as a continuous variable across type, group and time point in Table 87 through Table 89. The total ASC counts will be summarized as continuous variables and will be accompanied by a two-sided 95% CI for the median, computed via the percentile bootstrap method, using n = 10,000 replicates.

The type-specific differences in proportion of responders (IPV + dmLT minus IPV alone and IPV + dmLT minus bOPV) will be computed and accompanied with two-sided 95% CIs computed via the Miettinen and Nurminen score method. The difference in counts between IPV + dmLT and IPV alone, as well as IPV + dmLT and bOPV at each time point will be compared via the Wilcoxon test in Table 90.

Circulating IgA ASC response (post-vaccination count ≥ 8 cells per 10^6 PBMC) is summarized by the proportion (95% CI) of participants with a response, by virus type, visit and treatment group in Table 86 (Appendix 1). Descriptive statistics (e.g., mean, geometric mean count) of ASC counts are presented in Table 87 (PV1), Table 88 (PV2) and Table 89 (PV3). Statistical comparisons of response are presented in Table 90, by virus type and visit, where group comparisons are between IPV + dmLT and IPV alone and between the IPV + dmLT and bOPV.

Circulating IgG ASC response (post-vaccination count ≥ 8 cells per 10^6 PBMC) is summarized by the proportion (95% CI) of participants with a response, by virus type, visit and treatment group in Table 91 (Appendix 1). Descriptive statistics (e.g., mean, geometric mean count) of ASC counts are presented in Table 92 (PV1), Table 93 (PV2) and Table 94 (PV3). Statistical comparisons of positive response and ASC count are presented in Table 95, by virus type and visit, where group comparisons are between IPV + dmLT and IPV alone and between the IPV + dmLT and bOPV.

10.4. Mucosal Antibody-Secreting Cells (ASC) (Homing Marker)

Mucosal samples for ASC (homing marker) counts will collected on Day 7 post-vaccination and Day 7 post-challenge. Results will be presented in Table 96 through Table 98 (Appendix 1) and Listing 20 (Appendix 3).

The proportion of subjects with type/antibody-specific mucosal ASC (those expressing gut-homing marker, e.g., α4β7) response both following vaccination and following bOPV challenge will be summarized by type, group, and time point as a categorical variable and accompanied by two-sided Clopper Pearson 95% CIs. In addition, these frequencies will be summarized for mucosal ASCs as a continuous variable across type, group and time point. The total mucosal ASC counts will be summarized as continuous variables and will be accompanied by a two-sided 95% CI for the median, computed via the percentile bootstrap method, using n = 10,000 replicates.

The proportion (95% CI) of participants with an antibody-specific (α4β7) mucosal ASC response (ASC>0) seven days after vaccination and after bOPV challenge is summarized by virus type, antibody, visit and treatment group in Table 96. Descriptive statistics (e.g., mean, median, SD) by virus type are presented in Table 97. Statistical comparisons of response (ASC>0) and ASC count

are presented in Table 98 by virus type, where group comparisons are between IPV + dmLT and IPV alone and between the IPV + dmLT and bOPV.

10.5. IgA and IgG Immune Response in Saliva

Saliva samples for the estimation of type-specific IgA and IgG immune responses will be collected at screening, pre-vaccination, 7 days post-vaccination and 7 days post-challenge (Days -28, 1, 8, and 36).

Analyses of these data are anticipated, but will not be included in the CSR.

10.6. Cellular Immune Response

The cell-mediated immune response will be assessed via continuous-variable summary of the frequency of poliovirus-specific CD4+ T cells (via flow cytometry) before and after vaccination, and memory B-cells (via ELISPOT) before and after vaccination and after bOPV challenge. In addition, vaccine-specific cytokine responses will be studied before and after vaccination using an *in vitro* antigen re-stimulation assay followed by a multiplex cytokine assay (including 30 [pro]inflammatory T helper 1 and 2 cytokines as well as chemokines) on the supernatants of the stimulated cell cultures. Type-Specific CD4+ T Cell Response (Flow-Cytometry), Type-Specific B-Cells, and Cytokine Responses will be listed in Appendix 3 (Listing 21, Listing 22 and Listing 23, respectively). Analyses of these data are anticipated and may be included in the CSR depending on data availability prior to database lock. Tables and listings for these data will be finalized if and when data availability is confirmed, but if included will use the following format.

- Poliovirus type-specific CD4+ T-Cells will be analyzed on study Days 1, 29, 57 and 169. Descriptive statistics (e.g., mean, GMT) of each CD4+ T-Cell parameter will be presented as in Table X1 (Appendix 1).
- Poliovirus type-specific B-Cell ELISpots will be analyzed on study Days 1, 29, 57 and 169, by antigen. Descriptive statistics (e.g., mean, GMT) will be presented as in Table X2 (Appendix 1).
- Poliovirus type-specific cytokine responses will be analyzed on study Days 1, 29, 57 and 169, by cytokine. Descriptive statistics (e.g., mean, GMT) will be presented as in Table X3 (Appendix 1).

10.7. Serum IgA and IgG

Samples for serum IgA and IgG will be collected on study Days 1, 8, 29, 36, 43, 50, 57 and 169. Type-specific serum IgA and IgG will be summarized using the same methods as the primary immunogenicity variable, using assay-specific LLOQ/ULOQ as appropriate, except that no measure of seroprotection is available and seroconversion will be replaced with serum IgA/IgG response, defined as a minimum four-fold increase in antibody titers between baseline and post-vaccination time point, and for pre-/post challenge time points.

Analyses of these data are anticipated but will not be included in the CSR.

10.8. IgA and IgG Antibodies in Lymphocyte Supernatant (ALS)

The proportion of participants demonstrating at least a two-fold increase in type-specific poliovirus IgA and IgG in lymphocyte supernatant at any time point following vaccination or following bOPV challenge will be summarized as a categorical variable and accompanied by two-sided Miettinen and Nurminen score-based 95% CIs. The ALS will also be summarized as continuous variables as for serum IgA and IgG, described above.

Analyses of these data are anticipated but will not be included in the CSR.

11. REPORTING CONVENTIONS

P-values will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001" and p-values greater than 0.999 will be reported as ">0.999". Means and medians will be presented to one decimal place more than the raw value, standard deviations and confidence intervals will be presented to 2 extra decimals. Percentages will be reported to one decimal and corresponding 95% CIs will be to two decimals. Percentages less than 0.1% (<0.01%) will be reported as "<0.1" ("<0.01"), percentages >99.9% (>99.99%) will be reported as ">99.9" (">99.99") and 100% will be reported as "100".

12. TECHNICAL DETAILS

SAS version 9.4 or above will be used to generate all tables, figures and listings.

13. SUMMARY OF CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Immunogenicity analyses typically include GMT, GMFR, and other statistics on a log scale when the raw data consist of titer values, with appropriate imputation of values <LLOQ or >ULOQ. ASC responses (homing and non-homing) are count data, so summary statistics will not be based on log counts.

14. REFERENCES

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APPENDIX 1. TABLES

LIST OF TABLES

Appendix 1.A. Demographics

Tables copied from the protocol:

Table 1: Study Schema – Protocol Table 2 (see Table 1 in Section 4.1)

Table 2: Summary of Laboratory Sample Collection and Analysis – Protocol Table 3 (see Table 2 in Section 4.5.2)

Table 3: Schedule of Study Visits and Evaluations – Protocol Appendix I

Day	-28 to 0	1	8	29	33	36	39	43	46	50	57	169
Window (days)	N/A	N/A	±1	±2	±1	±1	±1	±2	±1	±2	±2	±14
Study Visit	Screening (00)	1	2	3	4	5	6	7	8	9	10	11
Informed Consent and Demographics	√											
Clinic Visit	√	√	√	√		√		√		√	√	√
Medical History	√	√[A]	√[A]	√[A]		√[A]		√[A]		√[A]	√[A]	√[A]
Prior and Concomitant Medications	√	√	√	√		√		√		√	√	
Physical Examination	√	√[B E]	√[B]	√[B E]		√[B]		√[B]		√[B]	√[B]	
Pregnancy Test[F] (Female only)	√	√[E]		√[E]								
Vital Signs	√	√[E]	√	√								
Safety Laboratory Evaluation[C]	√		√									
Screening for HIV, Hepatitis B and C[D]	√											
I/E Criteria	√	√										
Randomization		√										
Study Vaccination		√										
bOPV Challenge				√[G]								
Visual Inspection of Injection Site and/ or Reactogenicity Assessment		√	√	√								
Memory Aid Review and Collection			√									
Diary Card Review				√		√		√		√	√	
Saliva for IgA and IgG	√		√	√[E]		√						

Day	-28 to 0	1	8	29	33	36	39	43	46	50	57	169
Window (days)	**N/A**	**N/A**	**±1**	**±2**	**±1**	**±1**	**±1**	**±2**	**±1**	**±2**	**±2**	**±14**
Study Visit	**Screening (00)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Memory B and CD4+ T cells (Blood) & Cytokine Assay[H]		80 mL		80 mL[E]							80 mL	80 mL
ASC Homing Marker-a subset of samples (α4β7) (Blood)			30 mL			30 mL						
ALS/ASC (Blood)		20 mL	20 mL	20 mL[E]		20 mL						
Serum Neutralizing Antibody		5 mL		5 mL[E]								
Serum IgA and IgG		5mL[E]	5 mL	5 mL[E]		5 mL		5 mL		5 mL	5 mL	5 mL
Provide stool collection kit for the next visit		√	√	√	√	√	√	√	√	√	√	
Stool IgA	√		√	√[E]		√		√		√	√	√
Stool Neutralizing and IgA/IgG Antibody	√		√	√[E]		√		√		√	√	√
Stool Specimen for Viral Shedding				√[E]	√	√	√	√	√	√[j]	√[j]	
Cumulative Blood Volume	13.5 mL	110mL[E]	68.5mL	110 mL		55 mL		5 mL		5 mL	85 mL	85 mL
Adverse Event Reporting		Solicited local and systemic reaction [I]										
		Unsolicited Adverse Events										
		Serious Adverse Events										

A Updated medical history only
B Targeted physical examination, as indicated
C Complete blood count (CBC): WBC, ANC, Hemoglobin, Platelets
Chemistry panel: ALT, AST, CRP, Creatinine, Albumin, Total Bilirubin
D HBsAg, anti-HCV (if positive, by PCR), HIV EIA
E Before vaccination.
F Only in case of female participants with childbearing potential. At the time of screening a serum pregnancy test will be performed whereas on the day of vaccination, urine pregnancy test will be performed
G All study subjects
H Cytokine assays will be done using the same blood sample as for memory B and CD4+ T cells
I Solicited local and systemic reactions could be still ongoing after Day 8
J Subjects with positive stool samples on Day 50 or 57 to be further followed up until they provide two consecutive negative stool samples.

ALT = Alanine Aminotransferase; ASC=Antibody-Secreting cell; CRP=C-reactive protein

Table 4: Grading Reactogenicity, Memory Aid Symptoms and AEs (Toxicity Table) – Protocol Appendix II

ESTIMATING SEVERITY GRADE: For abnormalities NOT found elsewhere in the Toxicity Tables, use the scale below to estimate grade of severity:				
GRADE 1	Mild	Transient or mild discomfort; does not interfere with activities		
GRADE 2	Moderate	Mild to moderate limitation in activity; no or minimal medical intervention/therapy required		
GRADE 3	Severe	All normal activity is prevented for 24 hours or more		
		Grade 1 (Mild)	**Grade 2 (Moderate)**	**Grade 3 (Severe)**
Systemic:				
Nausea, headache, fatigue		mild discomfort that does not interfere with activities	discomfort causing mild to moderate limitation in activity	symptom prevents all normal activity for ≥24 hours
Vomiting		2-3 episodes in 24 hours	4-5 episodes in 24 hours	≥6 episodes in 24 hours
Diarrhea		3-5 loose stools/day or meets minimal definition of diarrhea by volume but <1000 mL/day	6-9 loose stools/day or 1000-1999 mL output per 24 hours	≥10 loose stools/day OR orthostatic hypotension
Allergic Reaction		transient flushing or rash	Rash, flushing, urticaria	symptomatic bronchospasm, parenteral medication indicated, allergy-related edema/ angioedema, hypotension
Fever: oral		38.0˚ to 38.9˚C or 100.4˚ to 102.1˚F	39.0˚ to 39.9˚C or 102.2˚ to 103.9˚F	≥40.0˚C or ≥104˚F
Rash (Specify type, if Applicable)		Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site
Local:				
Pain		Does not interfere with activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Erythema/Redness *		2.5 - 5 cm	5.1 - 10 cm	> 10 cm
Induration/Swelling **		2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	> 10 cm or prevents daily activity
Injection site hyperpigmentation		1 - 4 cm	4.1 – 8 cm	> 8 cm
Laboratory:				
Albumin (g/dL)		3.0 - < LLN	≥ 2.0 - < 3.0	< 2.0
Total Bilirubin		1.1 - 1.5 x ULN	>1.5 - 2.0 x ULN	>2.0 x ULN
ALT		>1.25 - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 x ULN
AST		>1.25 - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 x ULN
CRP		>3-10 mg/L	10 - 100 mg/L	>100 mg/L
Creatinine		1.1 - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 x ULN or dialysis needed
Hemoglobin		9.5 gm/dL - <LLN	7.0 - 9.4 gm/dL	<7.0 gm/dL
Absolute Neutrophil count		1000/mm^3 - <LLN	500 - 999/mm^3	<500/mm^3
Platelets		75,000/mm^3 - <LLN	30,000 - 74,999/mm^3	<30,000/mm^3
WBCs		11,000 - 30,000/ mm^3	>30,000 - 100,000/mm^3	>100,000/mm^3

ULN=Upper limit of normal; LLN = Lower limit of normal; WBCs=White blood cells; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRP: C - reactive protein

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration\Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Table 5: Distribution of Protocol Deviations by Category, Type and Study Group

Category	Deviation Type	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)	
		n	%	n	%	n	%	n	%
Eligibility/enrollment	Any type								
	Did not meet inclusion criterion								
	Met exclusion criterion								
	ICF not signed prior to study procedures								
	Other								
Vaccine administration schedule	Any type								
	Out of window visit								
	Missed visit/visit not conducted								
	Missed vaccine administration								
	Delayed vaccine administration								
	Other								
etc.	etc.								

N = number of participants in the total vaccinated population.

Table 6: Participant Disposition by Study Group

Subject Disposition	IPV	IPV + dmLT	bOPV	Total
	n (%)	n (%)	n (%)	n (%)
Screened	NA	NA	NA	xxx
Not enrolled				
Reasons not enrolled [1]				
[Reason 1]				
[Reason 2]				
[etc.]				
Enrolled				
Received vaccination				
Reasons vaccination not received [1]				
[Reason 1]				
[etc.]				
Completed Day 29 visit				
Received bOPV Challenge on Day 29				
Completed the Study				
Early Termination				
Reasons for early termination [1]				
Lost to follow-up				
Investigator decision				
SAE				
AE				
Etc.				

[1] Participants can have multiple reasons.
Percentages are out of number enrolled.

Table 7: Analysis Populations by Study Group and Visit

Visit	Reason Subjects Excluded	IPV (N=X) n (%)	IPV + dmLT (N=X) n (%)	bOPV (N=X) n (%)	Total (N=X) n (%)
Total Vaccinated Population[1]					
NA	Number vaccinated				
NA	Number not vaccinated				
NA	[Reason 1]				
NA	[Reason 2]				
NA	[etc.]				
Per Protocol Population[2]					
Day 8	Included				
	Excluded				
	[Reason excluded]				
	[etc.]				
Day 29	Included				
	Excluded				
	[Reason excluded]				
	[etc.]				
Etc.					

N = Number of participants in enrolled population.

[1] Denominator for percentage is N.

[2] Denominator for percentage is number vaccinated. Reasons for exclusion do not include missing data, which is specific to each parameter.

[For the PPP include all visits 8, 29, 33, 36, 39, 43, 46, 50, 57, 169]

Table 8: Summary of Screen Failures

Inclusion/Exclusion Category	Inclusion/Exclusion Criterion	n[a]	%[b]
Inclusion and Exclusion	Number of subjects failing any eligibility criterion	x	100
Inclusion	Any inclusion criterion	x	xx
	[inclusion criterion 1]	x	xx
	[inclusion criterion 2]	x	xx
	[inclusion criterion 3]	x	xx
Exclusion	Any exclusion criterion	x	xx
	[exclusion criterion 1]	x	xx
	[exclusion criterion 2]	x	xx
	[exclusion criterion 3]	x	xx

[a] More than one criterion may be marked per subject.
[b] Denominator for percentages is the total number of screen failures.

[Note: include all inclusion and exclusion criteria listed in the protocol]

Table 9: Summary of Categorical Baseline Demographics and Characteristics by Study Group, Total Vaccinated Population

	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)	P-Value[1]
Gender					**x.xxx**
Male, n (%)					
Female, n (%)					
Ethnicity					**x.xxx**
Hispanic or Latino, n (%)					
Hispanic or Latino, n (%)					
Not Reported, n (%)					
Unknown, n (%)					
Race					**x.xxx**
American Indian or Alaskan Native, n (%)					
Asian, n (%)					
Native Hawaiian or other Pacific Islander, n (%)					
Black or African American, n (%)					
White, n (%)					
Positive Serology Tests					
Number of samples					
HBV surface antigen, n (%)					x.xxx
HCV antigen, n (%)					x.xxx
HIV antibody, n (%)					x.xxx

N = number of participants in the total vaccinated population.

[1] Two-sided level $\alpha = 0.05$ statistical assessment of differences between groups, using Fisher's exact test.

Table 10: Summary of Continuous Baseline Demographics and Characteristics by Study Group, Total Vaccinated Population

	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)	P-Value[1]
Age (years)					**x.xxx**
Mean (SD)					
Median					
Min – Max					
Height (cm)					**x.xxx**
Mean (SD)					
Median					
Min – Max					
Weight (kg)					**x.xxx**
Mean (SD)					
Median					
Min – Max					
BMI					**x.xxx**
Mean (SD)					
Median					
Min – Max					

N = number of participants in the total vaccinated population

[1] Two-sided level $\alpha = 0.05$ statistical assessment of differences between groups, using the Kruskal-Wallis test.

Table 11: Summary of Categorical Baseline Demographics and Characteristics by Study Group, Per-Protocol Population

Table 12: Summary of Continuous Baseline Demographics and Characteristics by Study Group, Per-Protocol Population

Table 13: Summary of Prior and Concomitant Medications by Study Group, Total Vaccinated Population

WHO Drug Anatomic Therapeutic Chemical (ATC) classification		Study Group			
Level 1	**Level 4**	**IPV (N=X)**	**IPV + dmLT (N=X)**	**bOPV (N=X)**	**Total (N=X)**
		n (%)	**n (%)**	**n (%)**	**n (%)**
Classification 1	**Any Classification 4**				
	Classification 1				
	Classification 2				
	etc				
Classification 2	**Any Classification 4**				
	Classification 1				
	Classification 2				
	Etc				
Etc	Etc				

N = number of participants in the total vaccinated population.
Each participant contributes only once per drug category.

Table 14: Summary of Visit Attendance and Sample Collection[1], Total Vaccinated Population

	Study Day											
Day	**Screening**	**1[2]**	**8**	**29[3]**	**33**	**36**	**39**	**43**	**46**	**50**	**57**	**169**
Completed Visits												
IPV (N=X)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)
IPV+dmLT (N=X)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)
bOPV (N=X)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)
Total (N=X)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)
Saliva for IgA and IgG												
IPV (N=X)	x (%)		x (%)	x (%)		x (%)						
IPV+dmLT (N=X)	x (%)		x (%)	x (%)		x (%)						
bOPV (N=X)	x (%)		x (%)	x (%)		x (%)						
Total (N=X)	x (%)		x (%)	x (%)		x (%)						
Memory B and CD4+ T cells (Blood) & Cytokine Assay												
IPV (N=X)		x (%)		x (%)							x (%)	x (%)
IPV+dmLT (N=X)		x (%)		x (%)							x (%)	x (%)
bOPV (N=X)		x (%)		x (%)							x (%)	x (%)
Total (N=X)		x (%)		x (%)							x (%)	x (%)
ASC Homing Marker (α4β7)(Blood)												
IPV (N=X)			x (%)			x (%)						
IPV+dmLT (N=X)			x (%)			x (%)						
bOPV (N=X)			x (%)			x (%)						
Total (N=X)			x (%)			x (%)						
ALS/ASC (Blood)												
IPV (N=X)		x (%)	x (%)	x (%)		x (%)						
IPV+dmLT (N=X)		x (%)	x (%)	x (%)		x (%)						
bOPV (N=X)		x (%)	x (%)	x (%)		x (%)						
Total (N=X)		x (%)	x (%)	x (%)		x (%)						

	Study Day											
Day	**Screening**	**1[2]**	**8**	**29[3]**	**33**	**36**	**39**	**43**	**46**	**50**	**57**	**169**
Serum Neutralizing Antibodies												
IPV (N=X)		x (%)		x (%)								
IPV+dmLT (N=X)		x (%)		x (%)								
bOPV (N=X)		x (%)		x (%)								
Total (N=X)		x (%)		x (%)								
Serum IgA and IgG												
IPV (N=X)		x (%)	x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
IPV+dmLT (N=X)		x (%)	x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
bOPV (N=X)		x (%)	x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
Total (N=X)		x (%)	x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
Stool IgA												
IPV (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
IPV+dmLT (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
bOPV (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
Total (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
Stool Neutralizing and IgA/IgG Antibody												
IPV (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
IPV+dmLT (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
bOPV (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
Total (N=X)	x (%)		x (%)	x (%)		x (%)		x (%)		x (%)	x (%)	x (%)
Stool Specimen for Viral Shedding												
IPV (N=X)				x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	
IPV+dmLT (N=X)				x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	
bOPV (N=X)				x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	
Total (N=X)				x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	x (%)	

N = number of participants in the total vaccinated population.

[1] Samples collected and assay results available.

[2] Day of vaccination.

[3] Day of bOPV challenge (prior to challenge)

[Note: data are only expected at visits indicated by x (%)]

Appendix 1.B. Safety – Reactogenicity

Table 15: Summary of Overall Reactogenicity, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)		
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	P-Value[1]
Immediate reactions (within 30 minutes of vaccination)									
None									
Mild									
Moderate									**x.xxx**
Severe									
Moderate or Severe									**x.xxx**
Delayed reactions (within 7 days of vaccination)									
None									
Mild									
Moderate									**x.xxx**
Severe									
Moderate or Severe									**x.xxx**
At any time									
None									
Mild									
Moderate									**x.xxx**
Severe									
Moderate or Severe									**x.xxx**
Day 1 (day of vaccination)									
None									
Mild									
Moderate									**x.xxx**
Severe									
Moderate or Severe									**x.xxx**

N = number of participants in the total vaccinated population. n (%) = Number (%) of participants experiencing the event. 95% CI = Clopper-Pearson 95% confidence interval for the percent (%).

[1] Two-sided Fisher exact test p-value for a difference among groups in the distribution of severity and in the proportion of participants with moderate or severe reactogenicity.

[Note: Include separate assessments of days 1 to 7]

The following will be in the same format as Table 15:

Table 16: Summary of Overall Local Reactions, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Table 17: Summary of Overall Systemic Reactions, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Table 18: Summary of Vaccination Site Pain, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Table 19: Summary of Vaccination Site Induration, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Table 20: Summary of Vaccination Site Swelling, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Table 21: Summary of Vaccination Site Induration Diameter, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Mild: 2.5 - 5 cm	Moderate: 5.1 - 10 cm	Severe: > 10 cm

Table 22: Summary of Vaccination Site Swelling Diameter, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Mild: 2.5 - 5 cm	Moderate: 5.1 - 10 cm	Severe: > 10 cm

Table 23: Summary of Vaccination Site Erythema (Redness) Diameter, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Mild: 2.5 - 5 cm	Moderate: 5.1 - 10 cm	Severe: > 10 cm

Table 24: Summary of Vaccination Site Hyperpigmentation Diameter, by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Mild: 1 - 4 cm	Moderate: 4.1 - 8 cm	Severe: > 8 cm

Table 25: Summary of Fever (Oral Temperature), by Time-Post Vaccination, Severity and Study Group – Total Vaccinated Population

Mild: 38.0˚ to 38.9˚C or 100.4˚ to 102.1˚F	Moderate: 39.0˚ to 39.9˚C or 102.2˚ to 103.9˚F	Severe: >40.0˚C or >104˚F

Table 35: Duration of Post-Vaccination Reactogenicity Events, by Study Group – Total Vaccinated Population

	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)
Vaccination Site Pain				
n				
Mean (SD)				
Median				
Min, Max				
Vaccination Site Induration				
n				
Mean (SD)				
Median				
Min, Max				
Vaccination Site Swelling				
Vaccination Site Erythema (Redness)				
Vaccination Site Hyperpigmentation				
Fever (Oral Temperature)				
Chills				
Fatigue				
Headache				
Myalgia				
Arthralgia				
Rash				
Nausea				
Vomiting				
Diarrhea				

[Duration is in number of days from first to last occurrence regardless of severity.]

Table 36: Listing of Reactogenicity Events Ongoing at 7 Days Post-Vaccination – Total Vaccinated Population

	Time/Day								Post-Day 7 Resolution	
Reactogenicity Event	**30 mins**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Day**	**Max Severity**
Participant ID, Treatment Group										
Participant ID, Treatment Group										
Etc.										

Yellow: Mild (Grade 1), Orange: Moderate (Grade 2), Red: Severe (Grade 3)

[cells will be color-coded only, except for day of resolution]

Appendix 1.C. Safety – Unsolicited Adverse Events

Table 37: Summary of Unsolicited Adverse Events by Study Group – Total Vaccinated Population

	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)	
Event	**n (%) m**	**95% CI**	**n (%) m**	**95% CI**	**n (%) m**	**95% CI**	**n (%) m**	**95% CI**
All adverse events								
Any AE								
Any SAE								
Any severe AE								
Any AE ≥Grade 2								
Any AE leading to withdrawal								
All adverse events within 28 days of study vaccination[1]								
Any AE								
Any SAE								
Any severe AE								
Any AE ≥Grade 2								
Any AE leading to withdrawal								
All adverse events related to study treatment, within 28 days of study vaccination[1]								
Any AE								
Any SAE								
Any severe AE								
Any AE ≥Grade 2								
Any AE leading to withdrawal								

N = number of participants in the total vaccinated population. n (%) = Number (%) of participants experiencing the event.
m = total number of events, including repeats within participant. 95% CI = Clopper-Pearson 95% confidence interval for the percent (%).
[1] Includes AEs within 28 days of study vaccination and prior to bOPV challenge.

Table 38: Summary of Unsolicited Adverse Events Within 28 Days of Study Vaccination[1], by MedDRA System Organ Class (SOC), Preferred Term (PT), Severity and Study Group – Total Vaccinated Population

	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)	
	n (%) m	95% CI	n (%) m	95% CI	n (%) m	95% CI	n (%) m	95% CI
Any SOC								
Any PT								
Mild (Grade 1)								
Moderate (Grade 2)								
Severe (Grade 3)								
Any AE								
[SOC-1]								
Any PT								
Mild (Grade 1)								
Moderate (Grade 2)								
Severe (Grade 3)								
Any AE								
[PT-1]								
Mild (Grade 1)								
Moderate (Grade 2)								
Severe (Grade 3)								
Any AE								
[PT-2]								
Mild (Grade 1)								
Moderate (Grade 2)								
Severe (Grade 3)								
Any AE								
[Etc]								
Mild (Grade 1)								
Moderate (Grade 2)								
Severe (Grade 3)								
Any AE								

N = number of participants in the total vaccinated population. n (%) = Number (%) of participants experiencing the event.
m = total number of events, including repeats within participant. 95% CI = Clopper-Pearson 95% confidence interval for the percent (%).
[1] Includes AEs within 28 days of study vaccination and prior to bOPV challenge.

Table 39: Summary of Unsolicited Adverse Events Related to Study Vaccine, Within 28 Days of Study Vaccination[1], by MedDRA System Organ Class (SOC), Preferred Term (PT), Severity and Study Group – Total Vaccinated Population

[Similar to previous table. Only include SOC/PT terms with any events related to study treatment.]

Table 40: Summary of Unsolicited Adverse Events that Occurred in at Least 2 Participants[1] Within 28 Days of Study Vaccination[2], by MedDRA Preferred Term (PT) and Study Group – Total Vaccinated Population

	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)	
MedDRA Preferred Term	**n (%) m**	**95% CI**	**n (%) m**	**95% CI**	**n (%) m**	**95% CI**	**n (%) m**	**95% CI**
[PT-1]								
[PT-2]								
[PT-3]								
[Etc]								

N = number of participants in the total vaccinated population. n (%) = Number (%) of participants experiencing the event.
m = total number of events, including repeats within participant. 95% CI = Clopper-Pearson 95% confidence interval for the percent (%).
[1] Preferred terms occurring in ≥2 subjects (across group, regardless of seriousness, severity, or relationship).
[2] Includes AEs within 28 days of study vaccination and prior to bOPV challenge.

[Note: table will be sorted by descending order of Total incidence]

Table 41: Listing of Serious Adverse Events

Group, Participant ID, Gender, Age (years)

SAE description	
MedDRA System Organ Class	
MedDRA Preferred Term	
Associated with vaccine or challenge	
Onset date (and day post-vaccine or challenge)	
End date (and duration in days)	
Severity	
Relation to vaccine or challenge	
Did the SAE cause the subject to be discontinued from the study?	
Outcome	
Date event became an SAE	
Did the SAE result in death?	
If death, date of death	
If death, was an autopsy performed?	
Was the SAE life-threatening?	
Did the SAE result in inpatient hospitalization or prolongation of existing hospitalization for the subject?	
Hospital admission date	
Hospital discharge date	
Did the SAE result in persistent or significant disability or incapacity?	
Was the SAE associated with a congenital anomaly or birth defect?	
Was the SAE a medically important event not covered by other serious criteria?	
If 'Yes', specify	
Comments	

[Include only the fields with data. Repeat for each SAE, Death and Other significant AE]

Appendix 1.D. Safety – Laboratory Parameters

Table 42: Descriptive Statistics of Laboratory Parameters, by Visit and Study Group – Total Vaccinated Population

			IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)
Hemoglobin (g/dL)						
Screening (baseline)	**All values**	**n**				
		Mean (SD)				
		Median				
		Min, Max				
	Values <LLN	**n**				
		Mean (SD)				
		Median				
		Min, Max				
	Values >ULN	**n**				
		Mean (SD)				
		Median				
		Min, Max				
Day 8	**All values**	**n**				
		Mean (SD)				
		Median				
		Min, Max				
	Values <LLN	**n**				
		Mean (SD)				
		Median				
		Min, Max				
	Values >ULN	**n**				
		Mean (SD)				
		Median				
		Min, Max				
Change from Baseline		**n**				
		Mean (SD)				
		Median				
		Min, Max				
Repeat for each parameter*						

N = number of participants in the total vaccinated population. n = number non-missing. SD = standard deviation.

* WBC (cells/ul), Absolute Neutrophils (cells/ul), Platelets (cells/ul), Creatinine (mg/dL), Albumin (g/dL), Total bilirubin (mg/dL), Aspartate Aminotransferase (AST) (u/L), Alanine Aminotransferase (ALT) (u/L), C-Reactive Protein (mg/L)

[Add unscheduled visits if available]

Table 43: Summary of Laboratory Parameter Severity, by Visit and Study Group – Hemoglobin (g/dL) - Total Vaccinated Population

	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)		P-Value[1]
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	
Screening (baseline)									
Number of samples									
Normal									
Mild									
Moderate									x.xxx
Severe									
Any Abnormal									x.xxx
Day 8									
Number of samples									
Normal									
Mild									
Moderate									x.xxx
Severe									
Any Abnormal									x.xxx
Maximum Post-Vaccination Severity [include if data from unscheduled visits are available]									
Number of samples									
Normal									
Mild									
Moderate									x.xxx
Severe									
Any Abnormal									x.xxx

N = number of participants in the total vaccinated population. n (%) = Number (%) of participants experiencing the event.
95% CI = Clopper-Pearson 95% confidence interval for the percent (%).
Grading scale: Normal (≥LLN), Mild (9.5 - <LLN), Moderate (7.0 – 9.4), Severe (<7.0).
LLN = xxx (males) and yyy (females) [as appropriate]

[1] Fisher's exact 2-tailed tests of differences in abnormality rates across groups.

[Tables with the same format as the above].

Table 44: Summary of Laboratory Parameter Severity, by Visit and Study Group – WBC (cells/ul) - Total Vaccinated Population

Table 45: Summary of Laboratory Parameter Severity, by Visit and Study Group – Absolute Neutrophils (cells/ul) - Total Vaccinated Population

Table 46: Summary of Laboratory Parameter Severity, by Visit and Study Group – Platelets (cells/ul) - Total Vaccinated Population

Table 47: Summary of Laboratory Parameter Severity, by Visit and Study Group – Creatinine (mg/dL) - Total Vaccinated Population

Table 48: Summary of Laboratory Parameter Severity, by Visit and Study Group – Albumin (g/dL) - Total Vaccinated Population

Table 49: Summary of Laboratory Parameter Severity, by Visit and Study Group – Total bilirubin (mg/dL) - Total Vaccinated Population

Table 50: Summary of Laboratory Parameter Severity, by Visit and Study Group – Aspartate Aminotransferase (AST) (u/L) - Total Vaccinated Population

Table 51: Summary of Laboratory Parameter Severity, by Visit and Study Group – Alanine Aminotransferase (ALT) (u/L) - Total Vaccinated Population

Table 52: Summary of Laboratory Parameter Severity, by Visit and Study Group – C-Reactive Protein (mg/L) - Total Vaccinated Population

Table 53: Change from Baseline in Severity, by Laboratory Parameter and Study Group –Total Vaccinated Population

		IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)
Baseline	**Day 8**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Hemoglobin					
Normal (n=)	Normal Mild Moderate Severe	x (xx.x)	x (xx.x)	x (xx.x)	x (xx.x)
Mild (n=)	Normal Mild Moderate Severe				
Moderate (n=)	Normal Mild Moderate Severe				
Severe (n=)	Normal Mild Moderate Severe				
Repeat for all parameters *					

N = number of participants in the total vaccinated population.
n = number of participants.
Denominator for % is n.
* WBC, Absolute Neutrophils, Platelets, Creatinine, Albumin, Total bilirubin, AST, ALT, C-Reactive Protein

Appendix 1.E. Safety – Vital Signs

Table 54: Descriptive Statistics of Vital Signs Parameters, by Visit and Study Group – Total Vaccinated Population

		Parameter Values				Change From Baseline			
		IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)
Oral Temperature (°C)									
Screening (baseline)	**n**					-	-	-	-
	Mean (SD)					-	-	-	-
	Median					-	-	-	-
	Min, Max					-	-	-	-
Day 1	**n**								
	Mean (SD)								
	Median								
	Min, Max								
Day 8	**n**								
	Mean (SD)								
	Median								
	Min, Max								
Day 29	**n**								
	Mean (SD)								
	Median								
	Min, Max								
Systolic Blood Pressure (mmHg)									
Diastolic Blood Pressure (mmHg)									
Pulse (beats/min)									
Respiratory Rate (breaths/min)									

N = number of participants in the total vaccinated population. n = number non-missing. SD = standard deviation.

Table 55: Summary of Abnormal Physical Examination Results, by Body System, Visit and Study Group – Total Vaccinated Population

Body System	IPV (N=X) x/n (%)	IPV + dmLT (N=X) x/n (%)	bOPV (N=X) x/n (%)	Total (N=X) x/n (%)
Abdomen				
Screening				
Pre-vaccination, Day 1				
Day 8				
Pre-challenge, Day 29				
Day 36				
Day 43				
Day 50				
Day 57				
Cardiovascular/Heart				
Musculoskeletal				
HEENT				
Lymph nodes				
Skin				
Neurological				
Pulmonary/Chest				
Other: Specify Body System				

N = number of participants in the total vaccinated population.
x = number abnormal. n = number of participants examined.

Appendix 1.F. Safety – Viral Shedding

Table 56: Summary of Participants PCR-Positive for Viral Shedding (Type-Specific and Non-Specific), by Post-Challenge Visit and Study Group – Per-Protocol Population

Day Post- Challenge	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)	
	x/n (%)	95% CI	x/n (%)	95% CI	x/n (%)	95% CI	x/n (%)	95% CI
Non-Specific (1, 3 or both)								
Pre-challenge (Day 29)								
Day 33								
Day 36								
Day 43								
Day 50								
Day 57								
Day XX								
Poliovirus Type 1								
Poliovirus Type 3								

N = number of participants in the per-protocol population. CI = Clopper-Pearson confidence interval for the percent.
x = number positive. n = number of participants who provided a sample.

Table 57: Percent Reduction[1] in Proportion of Participants Shedding Challenge Virus on Study Day 36 (Day 7 Post-Challenge) – Per-Protocol Population

		IPV (N=X)	IPV + dmLT (N=X)	Relative Risk (RR)	Percent Reduction (1-RR)
Non-Specific	**x/n (%)**				
	95% CI				
Poliovirus Type 1	**x/n (%)**				
	95% CI				
Poliovirus Type 3	**x/n (%)**				
	95% CI				

N = number of participants in the per-protocol population.
x = number positive. n = number of participants who provided a sample.
CI = Confidence interval.
For the proportion of participants with viral shedding, exact Clopper-Pearson CI's will be calculated.
For RR (and % reduction), Farrington and Manning confidence intervals will be calculated.

[1] Difference in proportions between groups relative to the proportion in the IPV alone group.

[Notes:
Risk of shedding (IPVm) in the IPV+dmLT group relative to the risk (IPV) in the IPV group = IPVm / IPV = RR
Assuming IPV > IPVm
Percent reduction = (IPV – IPVm) /IPV = 1 – RR]

Table 58: Viral Shedding (log_{10} $CCID_{50}$/g, not type-specific) Descriptive Statistics – Per-Protocol Population

	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)	Total (N=X)
Study Day 29 (pre-challenge)				
n				
Mean (SD)				
Median				
CI for the median[5]				
Study Day 33 (Day 4 post-challenge)				
n				
Mean (SD)				
Median				
CI for the median[1]				
Study Day 36 (Day 7 post-challenge)				
Study Day 43 (Day 14 post-challenge)				
Study Day 50 (Day 21 post-challenge)				
Study Day 57 (Day 28 post-challenge)				
Study XX (Day xx post-challenge)				

N = number of participants in the per-protocol population.

LLOQ (2.75 log10) and ULOQ (8.25 log10) used as the observed value, whenever these limits are met. A value of 0 will be used when a sample is PCR-negative for viral shedding.

[1] Confidence intervals for all medians computed via the percentile bootstrap method using 10,000 replicates.

Table 59: Viral Shedding (log_{10} $CCID_{50}$/g, not type-specific) Descriptive Statistics for the Shedding Index Endpoint (SIE[1]) and Area Under the Curve (AUC[2]) – Per-Protocol Population

	IPV (N=X)	IPV + dmLT (N=X)	Difference in Medians[3] (N=X)	AUC Ratio[4] (N=X)
SIE (Complete data)				
n				
Mean (SD)			-	-
Median				
CI for the median[5]				
SIE (All available samples)				
n				
Mean (SD)			-	-
Median				
CI for the median[5]				
AUC				
n				
Mean (SD)			-	-
Median				
CI for the median[5]				

N = number of participants in the per-protocol population.

[1] Using the simple arithmetic mean of the log_{10} CCID50/g samples collected on Days 36, 43, 50, and 57 (i.e., 7, 14, 21 and 28 days, respectively, following challenge), using the LLOQ and ULOQ as observed data, and assigning a value of zero for samples PCR-negative for poliovirus. Two analyses are presented based on (1) complete data from all 4 visits and (2) all available samples from Day 33 through Day 57.

[2] Using the linear trapezoidal rule to compute the true AUC using all available samples. The AUC will be missing for any participant with 2 or more missing values (whether consecutive or not), or if the first or last sample is missing; if only one missing value is present (except for the first and last), then the AUC will be computed from the available samples, effectively using linear interpolation for the missing sample. [Assumption: all available samples refers to days 4, 7, 14, 21 and 28 post-challenge]

[3] IPV minus IPV+dmLT on a log_{10} scale. CI for the median is the 95% CI.

[4] As above (IPV minus IPV+dmLT) back-transformed to the original scale. CI for the ratio is the 90% CI.

[5] Confidence intervals for all medians and differences of medians computed via the percentile bootstrap method using 10,000 replicates.

Table 60: Type-Specific Shedding Cessation Rate, by Sub-Type, Post-Challenge Visit and Study Group – Per-Protocol Population

Day Post-Challenge	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)		Total (N=X)	
	x/n (%)	95% CI	x/n (%)	95% CI	x/n (%)	95% CI	x/n (%)	95% CI
Non-Specific								
Day 33								
Day 36								
Day 43								
Day 50								
Day 57								
Poliovirus Type 1								
Poliovirus Type 3								

N = number of participants in the per-protocol population. CI = confidence interval for the percent, via the Greenwood method.
x = number ceased shedding. n = number of participants who provided a sample.

Appendix 1.G. Immunogenicity – Poliovirus Fecal IgA Responses

Table 61: Positive Poliovirus Fecal IgA Response[1], by Serotype, Visit and Study Group – Per-Protocol Population – Total IgA Response

		IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)	
		n/m (%)	95% CI	n/m (%)	95% CI	n/m (%)	95% CI
Positive Response to Any Serotype							
From Pre-Vaccination	Day 8						
	Day 29						
	Day 36						
	Day 43						
	Day 50						
	Day 57						
	Day 169						
From Pre-Challenge	Day 36						
	Day 43						
	Day 50						
	Day 57						
	Day 169						
Positive Response to Serotype 1							
Positive Response to Serotype 2							
Positive Response to Serotype 3							

N = number of participants in the per-protocol population. n = number of participants with a positive response.
m = number of participants with data.
95% CI = Confidence interval computed via Clopper-Pearson method.
[1] Positive response is defined as a minimum 4-fold rise from the pre-vaccination or pre-challenge (Day 29) values.

Table 62: Poliovirus Serotype Type 1 Fecal IgA Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Total IgA Response

Visit [1]	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)
Baseline (Screening)			
n			
Mean (log_2)			
SD (log_2)			
Median (log_2)			
95% CI[2] (log_2)			
Range (Min – Max)			
GMT[3]			
95% CI for GMT			
Day 8			
Day 29 (pre-challenge)			
Day 36			
Day 43			
Day 50			
Day 57			
Day 169			

N = number of participants in the per-protocol population. n = number of participants with data.
GMT = Geometric mean titer.
LLOQ for fecal IgA = 42.7 ng/mL. Values <LLOQ are replaced with LLOQ/2 for mean and median but censored for GMT.

[1] Day post-vaccination (day post-challenge).
[2] Confidence interval for median (log_2) computed via the percentile bootstrap method, using n=10,000 replicates.
[3] GMT and confidence interval computed via the maximum likelihood method.

[GMT results are based on LSMEANS estimates from PROC LIFEREG (single model per visit including group as a covariate). Titers<LLOQ will be censored]

Table 63: Poliovirus Serotype Type 2 Fecal IgA Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Total IgA Response

Table 64: Poliovirus Serotype Type 3 Fecal IgA Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Total IgA Response

Table 65: Poliovirus Serotype 1 Fecal IgA Descriptive Statistics of Geometric Mean Fold-Rise (GMFR), by Visit and Study Group – Per-Protocol Population - Total IgA Response

Day post vaccination / challenge	IPV (N=X)			IPV + dmLT (N=X)			bOPV (N=X)		
	n	GMFR	95% CI	n	GMFR	95% CI	n	GMFR	95% CI
Fold-Rise from Baseline									
Day 8									
Day 29									
Day 36									
Day 43									
Day 50									
Day 57									
Day 169									
Fold-Rise from Day 29 (Pre-Challenge)									
Day 36									
Day 43									
Day 50									
Day 57									
Day 169									

N = number of participants in the per-protocol population. n = number of participants with visit and baseline data.
LLOQ for fecal IgA = 42.7 ng/mL. Value <LLOQ are censored.
Participant responses calculated as log2 (titer/baseline) or log2 (titer/pre-challenge). Baseline (or pre-challenge) titers <LLOQ will be replaced by LLOQ/2.
Observations with post-baseline (challenge) titers <LLOQ are left censored.
GMFR and 95% CI computed via the maximum likelihood method.

[GMFR results based on LSMEANS estimates from PROC LIFEREG. Single model per visit including group as a covariate]

Table 66: Poliovirus Serotype 2 Fecal IgA Descriptive Statistics of Geometric Mean Fold-Rise (GMFR), by Visit and Study Group – Per-Protocol Population - Total IgA Response

Table 67: Poliovirus Serotype 3 Fecal IgA Descriptive Statistics of Geometric Mean Fold-Rise (GMFR), by Visit and Study Group – Per-Protocol Population - Total IgA Response

Table 68: Poliovirus Serotype 1 Fecal IgA Group Comparisons, by Visit – Per-Protocol Population - Total IgA Response

			IPV+dmLT versus IPV alone		IPV+dmLT versus bOPV	
		n	Estimate	95% CI	Estimate	95% CI
Positive Response (≥4-fold rise)[1]						
From Baseline	Day 8					
	Day 29					
	Day 36					
	Day 43					
	Day 50					
	Day 57					
	Day 169					
From Day 29 (Pre-Challenge)	Day 36					
	Day 43					
	Day 50					
	Day 57					
	Day 169					
GMT Ratio[2]						
From Baseline	Day 8					
	Day 29					
	Day 36					
	Day 43					
	Day 50					
	Day 57					
	Day 169					
From Day 29 (Pre-Challenge)	Day 36					
	Day 43					
	Day 50					
	Day 57					
	Day 169					

n = number of participants with data. GMT = Geometric mean titer. CI = confidence interval.
LLOQ for fecal IgA = 42.7 ng/mL. Values <LLOQ are censored for GMT ratio calculations.
Baseline (or pre-challenge) titers <LLOQ will be replaced by LLOQ/2. Observations with post-baseline (challenge) titers <LLOQ are left censored.
GMT results based on ANCOVA-style model of log2 Titer with baseline (or pre-challenge) log2 titer as a covariate.

[1] 95% CI for difference in response proportions computed via the Miettinen and Nurminen score method.
[2] 95% CI computed following fit of an ANCOVA-style model (separately for each comparison) to the log-transformed titer.

[ANCOVA-style model using SAS PROC LIFEREG. A separate model will be fit for each comparison on each day.

Table 69: Poliovirus Serotype 2 Fecal IgA Group Comparisons, by Visit – Per-Protocol Population - Total IgA Response

Table 70: Poliovirus Serotype 3 Fecal IgA Group Comparisons, by Visit – Per-Protocol Population - Total IgA Response

a. Fecal Neutralization

Table 71: Positive Poliovirus Fecal Neutralization Response[1], by Serotype, Visit and Study Group – Per-Protocol Population – Total IgA Response *[same format as Table 61]*

Table 72: Poliovirus Serotype Type 1 Fecal Neutralization Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Total IgA Response *[same format as Table 62]*

Table 73: Poliovirus Serotype Type 2 Fecal Neutralization Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Total IgA Response *[same format as Table 62]*

Table 74: Poliovirus Serotype Type 3 Fecal Neutralization Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Total IgA Response *[same format as Table 62]*

Table 75: Poliovirus Serotype 1 Fecal Neutralization Descriptive Statistics of Geometric Mean Fold-Rise (GMFR), by Visit and Study Group – Per-Protocol Population - Total IgA Response *[same format as Table 65]*

Table 76: Poliovirus Serotype 2 Fecal Neutralization Descriptive Statistics of Geometric Mean Fold-Rise (GMFR), by Visit and Study Group – Per-Protocol Population - Total IgA Response *[same format as Table 65]*

Table 77: Poliovirus Serotype 3 Fecal Neutralization Descriptive Statistics of Geometric Mean Fold-Rise (GMFR), by Visit and Study Group – Per-Protocol Population - Total IgA Response *[same format as Table 65]*

Table 78: Poliovirus Serotype 1 Fecal Neutralization Group Comparisons, by Visit – Per-Protocol Population - Total IgA Response *[same format as Table 68]*

Table 79: Poliovirus Serotype 2 Fecal Neutralization Group Comparisons, by Visit – Per-Protocol Population - Total IgA Response *[same format as Table 68]*

Table 80: Poliovirus Serotype 3 Fecal Neutralization Group Comparisons, by Visit – Per-Protocol Population - Total IgA Response *[same format as Table 68]*

Appendix 1.H. Immunogenicity – Type-Specific Neutralizing Antibodies in Serum

Table 81: Type-Specific Neutralizing Antibodies in Serum, Seroprotection and Seroconversion by Visit and Study Group – Per-Protocol Population

		IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)	
		n/m (%)	95% CI	n/m (%)	95% CI	n/m (%)	95% CI
Poliovirus Type 1							
Pre-Vaccination	Seropositive[1]						
Pre-Challenge	Seroprotection[1]						
	Seroconversion (all participants)						
	Seroconversion (log_2 baseline≤8.5)[3]						
Poliovirus Type 2							
Pre-Vaccination	Seropositive[1]						
Pre-Challenge	Seroprotection[1]						
	Seroconversion (all participants)						
	Seroconversion (log_2 baseline≤8.5)[3]						
Poliovirus Type 3							
Pre-Vaccination	Seropositive[1]						
Pre-Challenge	Seroprotection[1]						
	Seroconversion (all participants)						
	Seroconversion (log_2 baseline≤8.5)[3]						

N = number of participants in the per-protocol population. n = number of participants with a response.
m = number of participants with data.
95% CI = Confidence interval computed via Clopper-Pearson method.

[1] NAb reciprocal titer ≥1:8.
[2] ≥4-fold increase in serum NAbs from baseline or post-vaccination reciprocal titer ≥1:8 if seronegative at baseline.
[3] Includes subjects with baseline values low enough (log_2 reciprocal titer ≤ 8.5) that seroconversion is possible to observe.

Table 82: Type-Specific Neutralizing Antibodies in Serum, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Poliovirus Type 1

Visit [1]	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)
Pre-Vaccination			
n			
Mean (log_2)			
SD (log_2)			
Median (log_2)			
95% CI[2] (log_2)			
Range (Min – Max)			
GMT[3]			
95% CI for GMT			
Pre-Challenge			
n			
Mean (log_2)			
SD (log_2)			
Median (log_2)			
95% CI[2] (log_2)			
Range (Min – Max)			
GMT[3]			
95% CI for GMT			
GMFR[4]			
95% CI for GMFR			

N = number of participants in the per-protocol population. n = number of participants with data.
GMT = Geometric mean titer.
LLOQ for NAb = 2.5 log_2. Values <LLOQ are replaced with LLOQ/2 for mean and median but censored for GMT.
ULOQ for NAb = 10.5 log_2. Values >ULOQ are replaced with ULOQ for mean and median but censored for GMT.
GMFR participant responses calculated as log2 (titer/baseline) or log2 (titer/pre-challenge), where baseline (or pre-challenge) titers <LLOQ are replaced by LLOQ/2 and those >ULOQ are replaced by ULOQ. Observations with post-baseline (challenge) titers <LLOQ or >ULOQ are left and right censored, respectively.
[1] Day post-vaccination (day post-challenge).
[2] Confidence interval for median (log_2) computed via the percentile bootstrap method, using n=10,000 replicates.
[3] GMT and confidence interval computed via the maximum likelihood method.
[4] GMFR and confidence interval computed via the maximum likelihood method.
[GMT results are based on LSMEANS estimates from PROC LIFEREG (single model per visit including group as a covariate)]

Table 83: Type-Specific Neutralizing Antibodies in Serum, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Poliovirus Type 2

Table 84: Type-Specific Neutralizing Antibodies in Serum, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population - Poliovirus Type 3

Table 85: Type-Specific Differences in Neutralizing Antibodies in Serum, Group Comparisons at Day 29 (Pre-Challenge) – Per-Protocol Population

		IPV+dmLT versus IPV alone		IPV+dmLT versus bOPV	
	n	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Poliovirus Type 1					
Seroprotection[1]					
Seroconversion[2] (all participants)					
Seroconversion (log_2 baseline≤8.5)[3]					
GMT ratio					
Poliovirus Type 2					
Seroprotection[1]					
Seroconversion[2] (all participants)					
Seroconversion (log_2 baseline≤8.5)[3]					
GMT ratio					
Poliovirus Type 3					
Seroprotection[1]					
Seroconversion[2] (all participants)					
Seroconversion (log_2 baseline≤8.5)[3]					
GMT ratio					

n = number of participants with data. GMT = Geometric mean titer. CI = confidence interval.
LLOQ for NAb = 2.5 log_2. Values <LLOQ are replaced with LLOQ/2 for mean and median but censored for GMT.
ULOQ for NAb = 10.5 log_2. Values >ULOQ are replaced with ULOQ for mean and median but censored for GMT.
GMT participant responses calculated as log2 (titer/baseline) or log2 (titer/pre-challenge), where baseline (or pre-challenge) titers <LLOQ are replaced by LLOQ/2 and those >ULOQ are replaced by ULOQ. Observations with post-baseline (challenge) titers <LLOQ or >ULOQ are left and right censored, respectively.

[1] 95% CI for difference in response proportions computed via the Miettinen and Nurminen score method.
[2] 95% CI for GMT ratios computed following fit of an ANCOVA-style model (separately for each comparison) to the log-transformed titer.

[ANCOVA-style model using SAS PROC LIFEREG. A separate model will be fit for each comparison on each day.]

Appendix 1.I. Immunogenicity – Circulating IgA Antibody-Secreting Cells (ASC) (Non-Homing)

Table 86: Positive[1] Type-Specific Circulating IgA Antibody-Secreting Cells, by Visit and Study Group – Per-Protocol Population

	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)	
	n/m (%)	95% CI	n/m (%)	95% CI	n/m (%)	95% CI
Poliovirus Type 1						
Baseline						
Day 8						
Day 29						
Day 36						
Poliovirus Type 2						
Baseline						
Day 8						
Day 29						
Day 36						
Poliovirus Type 3						
Baseline						
Day 8						
Day 29						
Day 36						

N = number of participants in the per-protocol population. n = number of participants with a positive ASC count.
m = number of participants with data.
95% CI = Confidence interval computed via Clopper-Pearson method.

[1] ASC count at a post-vaccination visit that is ≥ 8 cells per 10^6 PBMC

Table 87: Type-Specific Circulating IgA Antibody-Secreting Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population – Poliovirus Type 1

Visit [1]	IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)
Baseline			
n			
Mean (log_{10})			
SD (log_{10})			
Median (log_{10})			
95% CI[2] (log_{10})			
Range (Min – Max)			
GM[3]			
95% CI for GM			
Day 8			
Day 29 (pre-challenge)			
Day 36 (7)			

N = number of participants in the per-protocol population. n = number of participants with data.
GM = Geometric mean count.

[1] Day post-vaccination (day post-challenge).
[2] Confidence intervals for median (log_{10}) computed via the percentile bootstrap method, using n=10,000 replicates.
[3] GM and confidence interval computed via the maximum likelihood method.

[GM results are based on LSMEANS estimates from PROC LIFEREG (single model per visit including group as a covariate).]

Table 88: Type-Specific Circulating IgA Antibody-Secreting Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population – Poliovirus Type 2

Table 89: Type-Specific Circulating IgA Antibody-Secreting Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population – Poliovirus Type 3

Table 90: Type-Specific Circulating IgA Antibody-Secreting Cells, Group Comparisons, by Visit – Per-Protocol Population

			IPV+dmLT versus IPV alone			IPV+dmLT versus bOPV		
		n	**Estimate**	**95% CI**	**P-Value[5]**	**Estimate**	**95% CI**	**P-Value[5]**
Poliovirus Type 1								
Positive Response[1, 2]	Day 8				na			na
	Day 29				na			na
	Day 36				na			na
ASC Count[3, 4]	Day 8				x.xxx			x.xxx
	Day 29				x.xxx			x.xxx
	Day 36				x.xxx			x.xxx
Poliovirus Type 2								
Poliovirus Type 3								

n = number of participants with data. CI = confidence interval.

[1] Positive response = ASC count at a post-vaccination visit that is ≥ 8 cells per 10^6 PBMC

[2] 95% CI for difference in response proportions computed via the Miettinen and Nurminen score method.

[3] Difference in median ASC counts.

[4] 95% CI for difference in median ASC count computed via the Bootstrap method (10,000 simulations).

[5] Wilcoxon test of a difference in ASC counts.

Appendix 1.J. Immunogenicity – Circulating IgG Antibody-Secreting Cells (ASC)

Table 91: Positive[1] Type-Specific Circulating IgG Antibody-Secreting Cells, by Visit and Study Group – Per-Protocol Population

(Same format as Table 86)

Table 92: Type-Specific Circulating IgG Antibody-Secreting Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population – Poliovirus Type 1

(Same format as Table 87)

Table 93: Type-Specific Circulating IgG Antibody-Secreting Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population – Poliovirus Type 2

(Same format as Table 87)

Table 94: Type-Specific Circulating IgG Antibody-Secreting Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol Population – Poliovirus Type 3

(Same format as Table 87)

Table 95: Type-Specific Circulating IgG Antibody-Secreting Cells, Group Comparisons, by Visit – Per-Protocol Population

(Same format as Table 90)

Appendix 1.K. Immunogenicity – Mucosal Antibody-Secreting Cells (α4β7 ASC) (Homing Marker)

Table 96: Positive[1] Type-Specific Mucosal Antibody-Secreting Cells (Homing Marker), by Visit and Study Group – Per-Protocol Population

Day 7 Post-Vaccination/Challenge	IPV (N=X)		IPV + dmLT (N=X)		bOPV (N=X)	
	n/m (%)	95% CI	n/m (%)	95% CI	n/m (%)	95% CI
Poliovirus Type 1						
Vaccination						
bOPV Challenge						
Poliovirus Type 2						
Vaccination						
bOPV Challenge						
Poliovirus Type 3						
Vaccination						
bOPV Challenge						

N = number of participants in the per-protocol population. n = number of participants with a positive ASC count. m = number of participants with data.
95% CI = Confidence interval computed via Clopper-Pearson method.
[1] Samples expressing gut-homing marker (ASC count > 0).

Table 97: Type-Specific Mucosal Antibody-Secreting Cells (ASC) (Homing Markers), Descriptive Statistics, by Visit and Study Group – Per-Protocol

Day 7 Post-Vaccination/Challenge		IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)
Poliovirus Type 1				
Post-Vaccination	n			
	Mean (log_{10})			
	SD (log_{10})			
	Median (log_{10})			
	95% CI[1] (log_{10})			
	Range (Min – Max)			
	GM[2]			
	95% CI for GM			
Post-bOPV Challenge	n			
	Mean (log_{10})			
	SD (log_{10})			
	Median (log_{10})			
	95% CI[1] (log_{10})			
	Range (Min – Max)			
	GM[2]			
	95% CI for GM			
Poliovirus Type 2				
Poliovirus Type 3				

N = number of participants in the per-protocol population. n = number of participants with data.
GM = Geometric mean count.

[1] Confidence intervals for median (log_{10}) computed via the percentile bootstrap method, using n=10,000 replicates.
[2] GM and confidence interval computed via the maximum likelihood method.

[GM results are based on LSMEANS estimates from PROC LIFEREG (single model per visit including group as a covariate).]

Table 98: Type-Specific Mucosal Antibody-Secreting Cells (ASC) (Homing Markers), Group Comparisons – Per-Protocol Population

		IPV+dmLT versus IPV alone		IPV+dmLT versus bOPV	
	n	**Estimate**	**95% CI**	**Estimate**	**95% CI**
Poliovirus Type 1					
Positive Response[1]					
7 days post-vaccination					
7 days post-bOPV challenge					
GM[2]					
7 days post-vaccination					
7 days post-bOPV challenge					
Poliovirus Type 2					
Poliovirus Type 3					

n = number of participants with data. GMT = Geometric mean titer. CI = confidence interval.
Positive response = ASC count > 0
GM participant responses calculated as log10 (count/baseline) or log10 (count/pre-challenge).

[1] 95% CI for difference in response proportions computed via the Miettinen and Nurminen score method.
[2] 95% CI computed following fit of an ANCOVA-style model (separately for each comparison) to the log-transformed count.

[ANCOVA-style model using SAS PROC LIFEREG. A separate model will be fit for each comparison on each day.]

Appendix 1.L. Immunogenicity – CD4+ T Cells (via Flow Cytometry)

[To be updated if and when data become available]

Table X1: Table 99: Type-Specific CD4+ T Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol

		IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)
Poliovirus Type 1				
Pre-Vaccination	n			
	Mean (log_{10})			
	SD (log_{10})			
	Median (log_{10})			
	95% CI[1] (log_{10})			
	Range (Min – Max)			
	GM[2]			
	95% CI for GM			
Post-Vaccination (Day 29)	n			
	Mean (log_{10})			
	SD (log_{10})			
	Median (log_{10})			
	95% CI[1] (log_{10})			
	Range (Min – Max)			
	GM[2]			
	95% CI for GM			
Day 57				
Day 169				
Poliovirus Type 2				
Poliovirus Type 3				

N = number of participants in the per-protocol population. n = number of participants with data.
GM = Geometric mean count.

[1] Confidence intervals for median (log_{10}) computed via the percentile bootstrap method, using n=10,000 replicates.
[2] GM and confidence interval computed via the maximum likelihood method.

[GM results are based on LSMEANS estimates from PROC LIFEREG (single model per visit including group as a covariate).]

Appendix 1.M. Immunogenicity – B-Cells (ELISpot)

[To be updated if and when data become available]

Table X2: Table 100: Type-Specific B-Cells, Descriptive Statistics, by Visit and Study Group – Per-Protocol

		IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)
Poliovirus Type 1				
Pre-Vaccination	n			
	Mean (log_{10})			
	SD (log_{10})			
	Median (log_{10})			
	95% CI[1] (log_{10})			
	Range (Min – Max)			
	GM[2]			
	95% CI for GM			
Pre-Challenge				
Day 57				
Day 169				
Poliovirus Type 2				
Poliovirus Type 3				

N = number of participants in the per-protocol population. n = number of participants with data.
GM = Geometric mean count.

[1] Confidence intervals for median (log_{10}) computed via the percentile bootstrap method, using n=10,000 replicates.
[2] GM and confidence interval computed via the maximum likelihood method.

[GM results are based on LSMEANS estimates from PROC LIFEREG (single model per visit including group as a covariate).]

Appendix 1.N. Immunogenicity – Cytokine Responses

[To be updated if and when data become available]

[In addition, vaccine-specific cytokine responses will be studied before and after vaccination using an in vitro antigen re-stimulation assay followed by a multiplex cytokine assay (including 30 [pro]inflammatory T helper 1 and 2 cytokines as well as chemokines) on the supernatants of the stimulated cell cultures.]

Table X3: Table 101: Vaccine-Specific Cytokines, Descriptive Statistics, by Visit and Study Group – Per-Protocol

		IPV (N=X)	IPV + dmLT (N=X)	bOPV (N=X)
Eotaxin				
Pre-Vaccination	n			
	Mean (log_{10})			
	SD (log_{10})			
	Median (log_{10})			
	95% CI[1] (log_{10})			
	Range (Min – Max)			
	GM[2]			
	95% CI for GM			
Post-Vaccination	n			
	Mean (log_{10})			
	SD (log_{10})			
	Median (log_{10})			
	95% CI[1] (log_{10})			
	Range (Min – Max)			
	GM[2]			
	95% CI for GM			
Continue for all other cytokines				

N = number of participants in the per-protocol population. n = number of participants with data.
GM = Geometric mean count.

[1] Confidence intervals for median (log_{10}) computed via the percentile bootstrap method, using n=10,000 replicates.
[2] GM and confidence interval computed via the maximum likelihood method.

[Complete list of cytokines: Eotaxin, Eotaxin-3, GM-CSF, IFN-γ, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IP-10, MCP-1, MCP-4, MDC, MIP-1α,MIP-1β, TARC, TNF-α, TNF-β, VEGF-A

GM results are based on LSMEANS estimates from PROC LIFEREG (single model per visit including group as a covariate).]

APPENDIX 2. FIGURES

LIST OF FIGURES

Appendix 2.A. Demographics

Figure 1 Consort Diagram

Appendix 2.B. Clinical Laboratory Values

Figures will be prepared for each laboratory parameter using boxplots to describe the distribution of values and changes from baseline across visits, by group, incorporating unique points and/or colors for abnormalities.

Figure 2 Clinical Laboratory Values – Safety Population – WBC (cells/ul)

Figure 3 Clinical Laboratory Values – Safety Population – Absolute Neutrophils (cells/ul)

Figure 4 Clinical Laboratory Values – Safety Population – Platelets (cells/ul)

Figure 5 Clinical Laboratory Values – Safety Population – Creatinine (mg/dL)

Figure 6 Clinical Laboratory Values – Safety Population – Albumin (g/dL)

Figure 7 Clinical Laboratory Values – Safety Population – Total bilirubin (mg/dL)

Figure 8 Clinical Laboratory Values – Safety Population – Aspartate Aminotransferase (AST) (u/L)

Figure 9 Clinical Laboratory Values – Safety Population – Alanine Aminotransferase (ALT) (u/L)

Figure 10 Clinical Laboratory Values – Safety Population – C-Reactive Protein (mg/L)

Appendix 2.C. Viral Shedding

Figure 11 Viral Shedding (log_{10} CCID50/g) Reverse Cumulative Distribution Curves

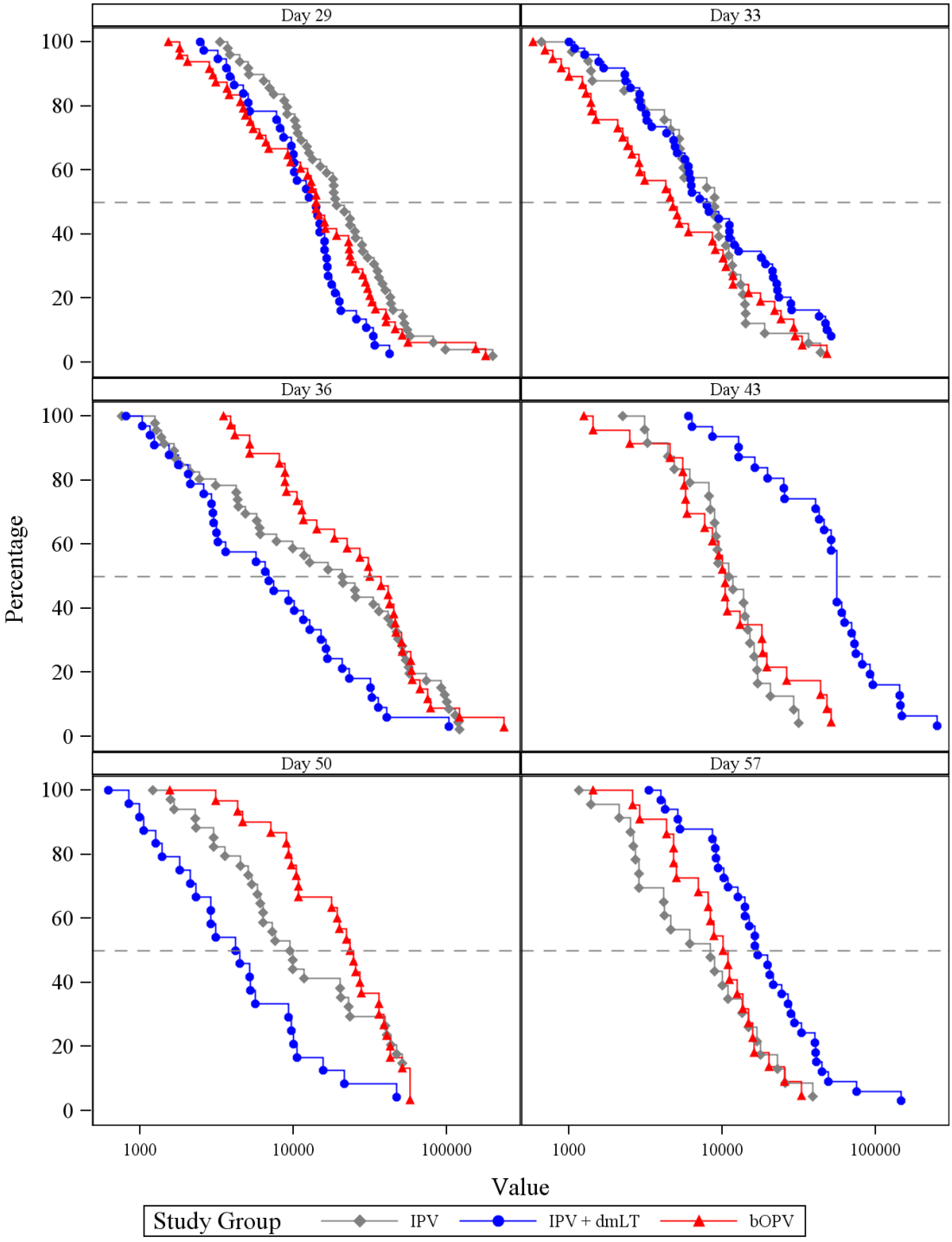
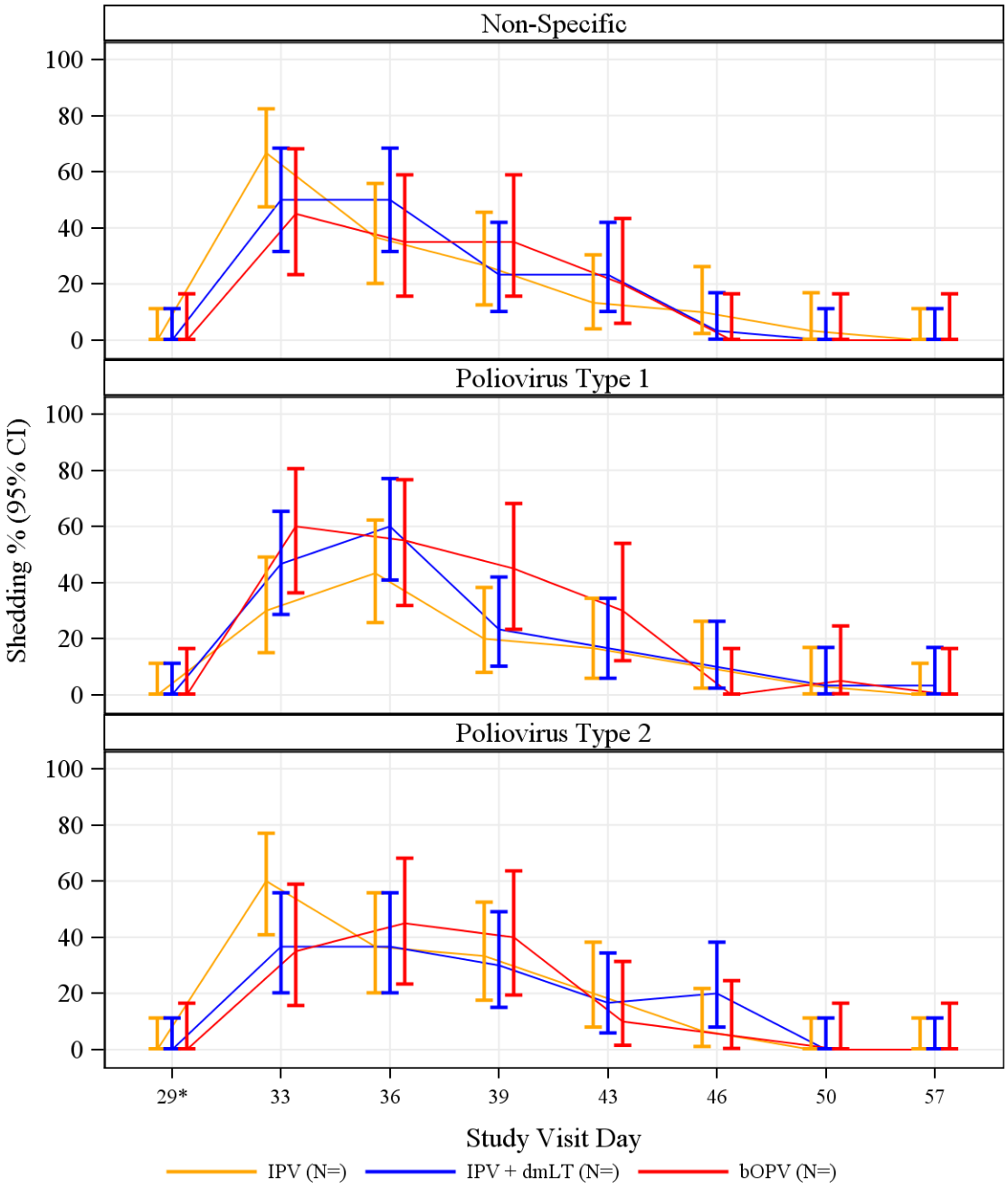


Figure 12 Viral Shedding (Type-Specific and Non-Specific), by Visit and Study Group – Per-Protocol Population

[Graph below is based on dummy data]



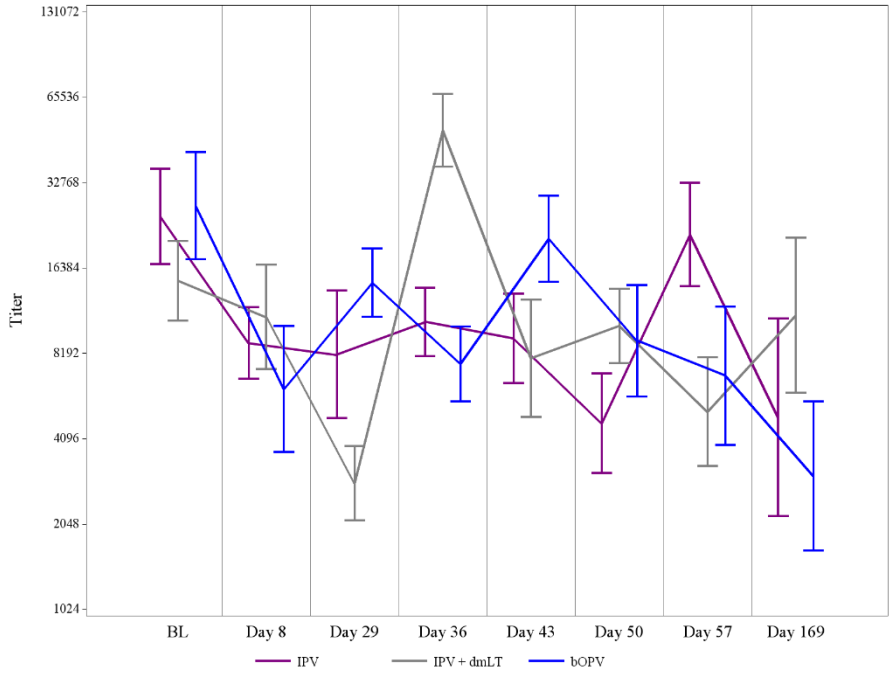
* Day of challenge

Figure 13 Type-Specific, Kaplan-Meier Time to Cessation of Viral Shedding

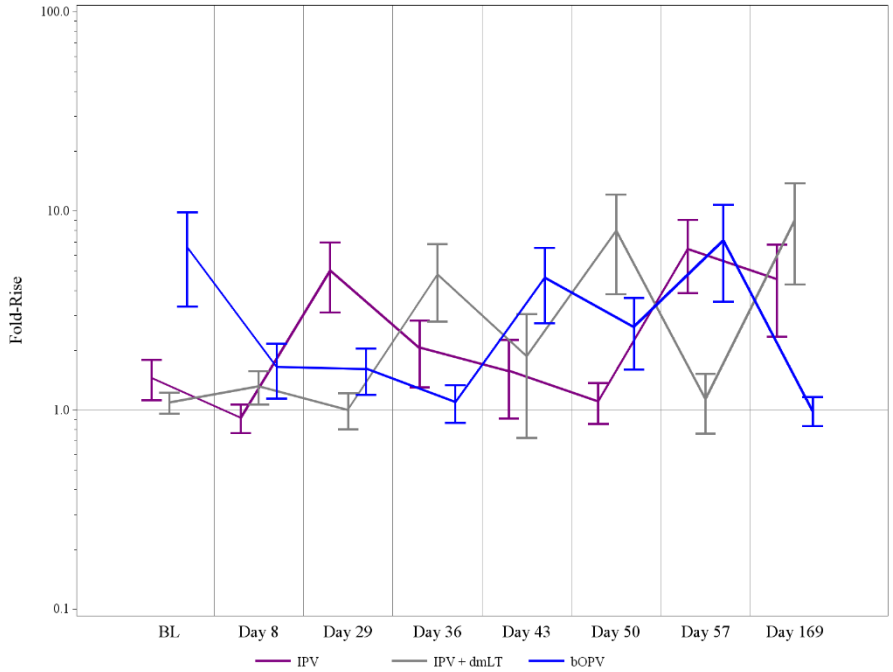
The figure will consist of 3 panels (or 3 separate graphs, depending on complexity), one for each poliovirus type. The estimated quartiles of time to cessation of shedding with corresponding 95% CIs will be computed along with the log-rank test and included in a figure denoting the KM estimate, with symbols used to indicate censored data points. The log-rank test will be used to provide a global test for a difference among survival curves.

Appendix 2.D. Immunogenicity

Figure 14 Fecal IgA by Visit and Treatment Group – Per-Protocol Population – Total IgA



Bars represent geometric mean titer and 95% CI



Bars represent geometric mean fold-rise and 95% CI

[GMT, GMFR and corresponding CI to be computed with SAS PROC LIFEREG.]

Figures similar to Figure 12:

Figure 15 Fecal IgA by Visit and Treatment Group – Per-Protocol Population – Poliovirus Type 1

Figure 16 Fecal IgA by Visit and Treatment Group – Per-Protocol Population – Poliovirus Type 2

Figure 17 Fecal IgA by Visit and Treatment Group – Per-Protocol Population – Poliovirus Type 3

Figure 18 Fecal Neutralization Geometric Mean Titer and Geometric Mean Fold Rise From 95% CI, Per-Protocol Population – Total IgA

Figure 19 Fecal Neutralization Geometric Mean Titer and Geometric Mean Fold Rise From 95% CI, Per-Protocol Population – Poliovirus Type 1 IgA

Figure 20 Fecal Neutralization Geometric Mean Titer and Geometric Mean Fold Rise From 95% CI, Per-Protocol Population – Poliovirus Type 2 IgA

Figure 21 Fecal Neutralization Geometric Mean Titer and Geometric Mean Fold Rise From 95% CI, Per-Protocol Population – Poliovirus Type 3 IgA

APPENDIX 3. LISTINGS

LIST OF LISTINGS

Listing 1 Withdrawals

Study Group	Subject ID	Reason for Early Termination	Early Termination Date	Last Completed Study Visit	Time Point[1]	Comments
IPV	[001]					
IPV+dmLT	[002]					
bOPV	[003]					

[1] Relative to vaccination or bOPV challenge. [e.g., "5 days prior to vac.", "6 days post-challenge."]

Listing 2 Subject Specific Protocol Deviation

	Study Group	Subject ID	DV Number	Deviation	Deviation Category	Study Visit	Reason for Deviation	Deviation Resulted in AE?	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Resolution	Excluded from PPP	Major/ Minor
Comment:													

Listing 3 Non-Subject-Specific Protocol Deviations

Start Date	Deviation	End Date	Reason for Deviation	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Category	Deviation Resolution	Comments

Listing 4 Demographics

									Serology (Positive/Negative)		
Participant ID	**Treatment Group**	**Gender**	**Age (years)**	**Height (cm)**	**Weight (kg)**	**BMI**	**Race**	**Ethnicity**	**HIV**	**Hepatitis B**	**HCV**

Listing 5 Medical History

Subject ID	MH Number	Area/System	Medical History Term	Medication Start Date	Medication End Date / Ongoing	Comment
Study Group: IPV						
[001]						
[002]						
Study Group: IPV + dmLT						
Study Group: bOPV						

Note: Area/System will be one of the following:

• HEENT • Cardiovascular • Respiratory • Gastrointestinal • Hepatobiliary/Pancreas • Urologic • Neurologic	• Blood/Lymphatic • Endocrine/Metabolic • Musculoskeletal • Genital/Reproductive • Dermatologic • Allergies	• Cancer • Immunodeficiency • Psychiatric • Drug or Alcohol Dependence • Autoimmune Disease • Any other significant medical history

Listing 6 Prior and Concomitant Medications

Subject ID	CM Number	Medication	Medication Start Day[1]	Medication End Day[1]/ Ongoing	Indication	Taken for an AE? (AE Description)	Taken for a condition on Medical History? (MH Description)	ATC Level 1 (ATC Level 4)
Study Group: IPV								
[001]								
[002]								
Study Group: IPV + dmLT								
Study Group: bOPV								

[1] Relative to vaccination or bOPV challenge. [e.g., "5 days prior to vac.", "6 days post-challenge."]

Listing 7 Vaccination and bOPV Challenge Compliance

Participant ID	Vaccination Date	bOPV Challenge Date	bOPV Challenge Day Post-Vaccination
IPV			
IPV + dmLT			
bOPV			

Missing dates indicate participants who were not vaccinated or did not receive bOPV challenge.

Listing 8 Visit Attendance and Sample Collection

	Scheduled Day	Visit Window	Completed Visit?	Date	Actual Study Day	Samples collected						
						IgA/IgG Saliva	Memory B and CD4+ T-cells (blood)	ASC Homing Marker a subset of samples (α4β7) (Blood)	ALS/ASC (Blood)	Serum Neutralizing Antibody	Stool IgA + Neutralizing and IgA/IgG Antibody (Stool)	Viral Shedding (Stool)
Participant ID, Treatment Group												
Screening	-28 to 0	-	Y/N			Y/N					Y/N	
Vaccination	1	-					Y/N		Y/N	Y/N		
	8	7 – 9				Y/N		Y/N	Y/N		Y/N	
Challenge	29	27 – 31				Y/N	Y/N		Y/N	Y/N	Y/N	Y/N
	33	32 – 34										Y/N
	36	35 – 37				Y/N		Y/N	Y/N		Y/N	Y/N
	39	38 – 40										Y/N
	43	42 – 44									Y/N	Y/N
	46	45 – 47										Y/N
	50	49 – 51									Y/N	Y/N
	57	55 – 59					Y/N				Y/N	Y/N
	169	155 – 183					Y/N				Y/N	
Participant ID, Treatment Group												
Participant ID, Treatment Group												

[will include any unscheduled visits]

Listing 9 All Solicited and Unsolicited Adverse Events

	MedDRA®			Onset		Resolution						
Verbatim Term	System Organ Class	Preferred Term	Type[1]	Date	Day[2]	Date	Day	SAE?	Severity Grade	Causality Assessment	Action Taken	Outcome
Participant ID, Treatment Group												
								Y/N				
Comment:												
								Y/N				
Comment:												
Participant ID, Treatment Group												
								Y/N				
Comment:												
								Y/N				
Comment:												
Etc..												

[1] Local, Systemic or Unsolicited AE. [2] Relative to vaccination [will include "30 mins" if appropriate].

Listing 10 Pregnancies

Format and content to be decided based on available data. All available data will be presented.

Listing 11 Clinical Laboratory Values

Participant ID, Treatment Group [show each participant on a new page]

Parameter	Units	Reference Range	Visit	Day	Value	Change from Baseline	Severity
Leukocytes (WBC)	cells/ul	aaa - bbb	Baseline	1		na	
			Day 8	X			
Absolute Neutrophils	cells/ul		Baseline				
			Day 8				
Hemoglobin	g/dL		Baseline				
			Day 8				
Platelets	cells/ul		Baseline				
			Day 8				
Creatinine	mg/dL		Baseline				
			Day 8				
Albumin	g/dl		Baseline				
			Day 8				
Total Bilirubin	mg/dl		Baseline				
			Day 8				
AST (SGPT)	u/L		Baseline				
			Day 8				
ALT (SGOT)	u/L		Baseline				
			Day 8				
C-Reactive Protein	mg/L		Baseline				
			Day 8				

Severity: Abnormal (outside the Reference Range but less than mild), Mild, Moderate, Severe

[Note: will include unscheduled visits if available]

Listing 12 Vital Signs

Parameter	Visit	Day	Value	Change from Baseline
Participant ID [001], Treatment Group				
Oral Temperature (°C)	Screening	-xx		na
	Day 1	xx		
	Day 8	xx		
	Day 29	xx		
Systolic Blood Pressure (mmHg)	Screening	-xx		na
	Day 1	xx		
	Day 8	xx		
	Day 29	xx		
Diastolic Blood Pressure (mmHg)	Screening	-xx		na
	Day 1	xx		
	Day 8	xx		
	Day 29	xx		
Pulse (beats/min)	Screening	-xx		na
	Day 1	xx		
	Day 8	xx		
	Day 29	xx		
Respiratory Rate (breaths/min)	Screening	-xx		na
	Day 1	xx		
	Day 8	xx		
	Day 29	xx		
Participant ID [002], Treatment Group				
Etc.				

[Note: will include unscheduled visits if available]

Listing 13 Physical Exam

Day	Exam Date	Abdomen	Cardiovascular/ heart	Musculo-skeletal	HEENT	Lymph nodes	Skin	Neurological	Pulmonary/ chest	Other
Participant ID, Treatment Group										
Screening										
Day 1										
Day 3										
Day 29										
Day 36										
Day 43										
Day 50										
Day 57										
Continue for all Participants										

[Note: will include unscheduled visits if available]

Listing 14 Type-Specific Viral Shedding

	Not Type-Specific		Poliovirus Type 1		Poliovirus Type 3		Poliovirus Type 3	
	PCR Positive	$CCID_{50}$/g	PCR Positive	$CCID_{50}$/g	PCR Positive	$CCID_{50}$/g	PCR Positive	$CCID_{50}$/g
Participant ID, Treatment Group								
Pre-challenge	Y/N							
Day 4								
Day 7								
Day 10								
Day 14								
Day 17								
Day 21								
Day 28								
SIE[1]	na		na		na		na	
AUC[2]	na		na		na		na	
Etc. for other participants.								

[1] Arithmetic mean of the $\log_{10}$ $CCID_{50}$/g samples collected on Days 7, 14, 21, and 28 days following challenge, using the LLOQ and ULOQ as observed data, and assigning a value of zero for samples PCR-negative for poliovirus, as described above.

[2] Linear trapezoidal rule to compute the true AUC using all available samples. The AUC is missing for any participants with 2 or more missing values (whether consecutive or not), or if the first or last sample is missing; if only one missing value is present (except for the first and last), then the AUC will be computed from the available samples, effectively using linear interpolation for the missing sample.

Listing 15 Type-Specific Poliovirus Fecal IgA

	Total IgA			Poliovirus Type 1 IgA			Poliovirus Type 2 IgA			Poliovirus Type 3 IgA		
	Titer	FR from Baseline	FR from Pre-Challenge	Titer	FR from Baseline	FR from Pre-Challenge	Titer	FR from Baseline	FR from Pre-Challenge	Titer	FR from Baseline	FR from Pre-Challenge
Participant ID, Treatment Group												
Screening												
Day 8												
Day 29												
Day 36												
Day 43												
Day 50												
Day 57												
Day 169												
Continue for all participants												

FR = Fold Rise.

[Titers <LLOQ will be reported as "<0.03". Titers >ULOQ will be reported as ">42.7".
[Data from unscheduled visits will be included if available]

Listing 16 Type-Specific Fecal Neutralization

	Total IgA			Poliovirus Type 1 IgA			Poliovirus Type 2 IgA			Poliovirus Type 3 IgA		
	Titer	FR from Baseline	FR from Pre-Challenge	Titer	FR from Baseline	FR from Pre-Challenge	Titer	FR from Baseline	FR from Pre-Challenge	Titer	FR from Baseline	FR from Pre-Challenge
Participant ID, Treatment Group												
Screening												
Day 8												
Day 29												
Day 36												
Day 43												
Day 50												
Day 57												
Day 169												
Continue for all participants												

[Titers <LLOQ will be reported as "<4" (<2 $\log_2$). Titers >ULOQ will be reported as ">1024" (>10 $\log_2$).
[Data from unscheduled visits will be included if available]

Listing 17 Type-Specific Neutralizing Antibodies in Serum

		Poliovirus Type 1		Poliovirus Type 2		Poliovirus Type 3	
Participant ID	**Time-Point**	**Titer**	**Fold-Rise**	**Titer**	**Fold-Rise**	**Titer**	**Fold-Rise**
IPV Alone							
[ID 001]	Pre-Vaccination						
	Pre-Challenge						
[ID 002]	Pre-Vaccination						
	Pre-Challenge						
[etc]	Pre-Vaccination						
	Pre-Challenge						
IPV + dmLT							
[ID 001]	Pre-Vaccination						
	Pre-Challenge						
[etc]	Pre-Vaccination						
	Pre-Challenge						
bOPV							
[ID 001]	Pre-Vaccination						
	Pre-Challenge						
[etc]	Pre-Vaccination						
	Pre-Challenge						

[Titers <LLOQ will be reported as "<6" (approx. <2.5 $\log_2$). Titers >ULOQ will be reported as ">1448" (>10.5 $\log_2$).
[Data from unscheduled visits will be included if available]

Listing 18 Type-Specific Circulating IgA Antibody-Secreting Cells (Non-Homing)

	Poliovirus Type 1 IgA			Poliovirus Type 2 IgA			Poliovirus Type 3 IgA		
	Cells/10^6 PBMC	FR from Baseline	FR from Pre-Challenge	Cells/10^6 PBMC	FR from Baseline	FR from Pre-Challenge	Cells/10^6 PBMC	FR from Baseline	FR from Pre-Challenge
Participant ID, Treatment Group									
Pre-Vaccination									
Day 8									
Pre-Challenge Day 29									
Day 36									
Continue for all participants									

[Data from unscheduled visits will be included if available]

Listing 19 Type-Specific Circulating IgG Antibody-Secreting Cells (Non-Homing)

	Poliovirus Type 1 IgG			Poliovirus Type 2 IgG			Poliovirus Type 3 IgG		
	Cells/10^6 PBMC	FR from Baseline	FR from Pre-Challenge	Cells/10^6 PBMC	FR from Baseline	FR from Pre-Challenge	Cells/10^6 PBMC	FR from Baseline	FR from Pre-Challenge
Participant ID, Treatment Group									
Pre-Vaccination									
Day 8									
Pre-Challenge Day 29									
Day 36									
Continue for all participants									

[Data from unscheduled visits will be included if available]

Listing 20 Mucosal antibody-secreting cells (α4β7 ASC) (Homing Marker)

Participant ID	Treatment Group	Poliovirus Type 1		Poliovirus Type 2		Poliovirus Type 3	
		Post-Vaccination	Post-Challenge	Post-Vaccination	Post-Challenge	Post-Vaccination	Post-Challenge
ID #1							
ID #2							
Etc.							

Listing 21 Type-Specific CD4+ T Cell Response (Flow-Cytometry)

Cytokine	Time Point	Poliovirus Type 1	Poliovirus Type 2	Poliovirus Type 3
Participant ID, Treatment Group				
[IFNg]	Pre-Vaccination			
	Pre-Challenge Day 29			
[TNFa]	Pre-Vaccination			
	Pre-Challenge Day 29			
[IL-2]	Pre-Vaccination			
	Pre-Challenge Day 29			
Continue for all participants				

[Exact format and contents of table to be decided.
Data from unscheduled visits will be included if available]

Listing 22 Type-Specific B-Cells

	Time Point	Poliovirus Type 1	Poliovirus Type 2	Poliovirus Type 3
IPV				
Participant ID	Pre-Vaccination			
	Pre-Challenge Day 29			
	Day 57			
	Day 169			
IPV+dmLT				
bOPV				

[Exact format and contents of table to be decided.
Data from unscheduled visits will be included if available]

Listing 23 Cytokine Responses

Cytokine	Pre-Vaccination	Pre-Challenge Day 29
Participant ID, Treatment Group		
Eotaxin		
Eotaxin-3		
Etc.		
Continue for all participants		
Eotaxin		
Eotaxin-3		
Etc.		

Complete list of cytokines:
Eotaxin, Eotaxin-3, GM-CSF, IFN-γ, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, TNF-α, TNF-β, VEGF-A

[Exact format and contents of table to be decided.
Data from unscheduled visits will be included if available]