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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CVIA 065

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PATH

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OVERVIEW

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i. Table of Contents

ii. Abbreviations and Acronyms

Ab	Antibody
AE	Adverse Events
A/G ratio	Ratio of Albumin to Globulin
ALS	Antibodies in Lymphocyte Supernatant
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
APTT	Activated Partial Thromboplastin Time
ASC	Antibody-Secreting cells
AST	Aspartate Aminotransferase
AUC	Area under the Curve
BMGF	Bill and Melinda Gates Foundation
bOPV	Bivalent Oral Polio Vaccine
CBC	Complete Blood Count
CDC	(U.S.) Centers for Disease Control and Prevention
cGMP	Current Good Manufacturing Practice
CI	Confidence Interval
CRF	Case Report Form
CRP	C - reactive protein
CSR	Clinical Study Report
CssBA	Recombinant ETEC CssBA Protein
CVIA	Center for Vaccine Innovation and Access
dmLT	*E.coli* Double Mutant Heat Labile Toxin
DMP	Data Management Plan
EC	Ethics Committee
EDC	Electronic Data Capture
EIA	Enzyme Immunoassay
Emmes	The Emmes Company, LLC
ETEC	Enterotoxigenic *Escherichia coli*
FAMHP	Federal Agency for Medicines and Health Products
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practice
GMFR	Geometric Mean Fold Rise
GMT	Geometric Mean Titer
GPEI	Global Polio Eradication Initiative
GVAP	Global Vaccine Action Plan
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICH	International Council on Harmonisation
ICF	Informed Consent Form
ICMJE	International Committee of Medical Journal Editors
ID	Intradermal
IHR	International Health Regulations

IM	Intramuscular
IPV	Inactivated Poliovirus Vaccine
IRB	Institutional Review Board
LLN	Lower Limit of Normal
LT	Enterotoxigenic *E. coli* (ETEC) Labile Toxin
MAAE	Medically Attended Adverse Event
mcg	microgram
mg	milligram
mL	milliliter
mLT	Single Mutant LT
mOPV1	Monovalent Oral Poliomyelitis Vaccine Type 1
NAb	Neutralizing Antibody
NLM	National Library of Medicine
NZW	New Zealand White
OPV	Oral Polio Vaccine
PATH REC	PATH's Research Ethics Committee
PEFs	Poliovirus Essential Facilities
PBMCs	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PO	By Mouth
PT	Prothrombin Time
PV	Poliovirus
RI	Routine Immunization
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAGE	Strategic Advisory Group of Experts
SIE	Shedding Index Endpoint
SL	Sublingual
SOP	Standard Operating Procedure (s)
SRC	Safety Review Committee
SWFI	Sterile Water for Injection
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
VAPP	Vaccine-Associated Paralytic Polio
cVDPVs	Circulating Vaccine-Derived Polioviruses
WBC	White Blood Cells
WHO	World Health Organization
WPV	Wild Poliovirus

iii. Key Roles and Contact Information

[REDACTED]

INVESTIGATOR'S AGREEMENT

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

"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Council on Harmonisation Good Clinical Practice Guideline and FAMHP Regulations."

_____ _____

Signature of Principal Investigator **Date**

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iv. Protocol Summary

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
Protocol Number	CVIA 065
Trial Phase	Phase 1
Investigational 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)
Study Hypotheses	**Safety:** IPV administered intramuscular (IM) with dmLT is safe and well-tolerated **Immunogenicity:** Concomitant IM administration of IPV with dmLT, enhances mucosal responses to IPV compared to IPV alone, and provides greater mucosal immunity, assessed following bOPV challenge
Study Objectives	**Primary Objectives:** 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 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 **Secondary Objectives:** 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

	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 **Exploratory Objectives:** Immunogenicity: • To evaluate and compare mucosal and systemic immune responses to IPV + dmLT, administered IM, and bOPV, administered orally
Study Endpoints	**Primary Endpoints:** 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 adverse events (AEs) during the 28 days following study vaccination 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 **Secondary Endpoints:** 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 cells (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 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 PCR-negative for virus, with samples taken on separate days. **Exploratory Endpoints:** 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

<table>
<tr><td></td><td>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 and 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</td></tr>
<tr><td>Study Design</td><td>This first in human clinical trial is a randomized, partially blinded study in which subjects will receive a single dose of licensed trivalent IPV, administered IM, with or without dmLT, or bOPV, administered orally (PO). IPV or IPV + dmLT will be administered to groups of 30 subjects each. Both subjects and clinical staff will be blinded to group assignment (IPV alone vs IPV + dmLT). A positive unblinded control group will be included, composed of 20 subjects receiving bOPV. One month (28 days) after receiving study vaccine, all subjects will receive a standard oral dose of bOPV (0.1 mL) to assess relative impact of study vaccine on shedding of that challenge virus. For this study, study vaccine is described as IPV+dmLT.

Study Schema
<table>
<tr><th>Study Group#</th><th>N</th><th>IPV volume*</th><th>dmLT dose</th><th>bOPV</th></tr>
<tr><td>1</td><td>30</td><td>0.5 mL</td><td>0</td><td>0</td></tr>
<tr><td>2</td><td>30</td><td>0.5 mL</td><td>0.5 µg</td><td>0</td></tr>
<tr><td>3</td><td>20</td><td>0</td><td>0</td><td>0.1 mL</td></tr>
</table>
* 0.5 ml = 40 DU type 1, 8 DU type 2, and 32 DU type 3 poliovirus
# All subjects will receive bOPV challenge on Day 29</td></tr>
</table>

Study population	80 healthy 18 to 45-year-old, inclusive, male or female volunteers with exclusive IPV polio vaccination history
Number of participating sites	Single site
Study Duration	Approximately 9 months
Participant Duration	6 months

1 BACKGROUND AND RATIONALE

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 polio virus 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[11-13]. These studies have also shown impact from IPV in boosting intestinal immunity when given to children who had received prior OPV [5-6]. 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[7]. 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., 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.

1.1 Burden of Disease

The main risk for contracting polio from wild type polio virus is concentrated in the three countries that are still endemic (Nigeria, Afghanistan and Pakistan). During 2017, new cVDPV outbreaks were also detected in the Democratic Republic of the Congo (two emergences) and Syria (one emergence). Residual circulation of a previous cVDPV2 emergence in Nigeria was detected in 2016 and low-level detection of new emergences in Nigeria and Pakistan occurred during 2016. Fourteen newly identified persons in 10 countries were found to excrete immunodeficiency-associated VDPVs[1].

The main risk factor for the VDPV is low routine immunization coverage with OPV that leads to population vulnerability to circulation of revertant Sabin strains. The elimination of VDPVs as well as other OPV-like viruses depends on improving immunization coverage for the near-term, and eventually on complete cessation of use of OPV. Wild type poliovirus 2 (WPV2) transmission was last reported in 1999 and was declared eradicated in December 2015. Moreover, type 2 component of the OPV is known to cause more than 90% of all cVDPVs and approximately 30% of all vaccine associated paralysis (VAPP) cases. Based on this, a "global switch" was implemented in May 2016 with cessation of the Sabin 2 containing trivalent OPV (tOPV) and replacing it with bOPV. Thus, all previously OPV-using countries are now using a combined IPV–bOPV schedule. This shift in polio vaccination practices is supported by the results from several clinical trials investigating new bOPV and IPV combination schedules [8-12]. Since the switch, the number of isolated Sabin 2 strains from both acute flaccid paralysis patients and environmental surveillance systems has steadily declined[14]. To succeed with this transition, the supply and cost constraints of IPV urgently need to be overcome. Many initiatives are ongoing to meet the increasing demand for IPV. The addition of an adjuvant—in particular one like dmLT that may direct the response towards mucosal immunity—may offset the relative deficiency in primary intestinal mucosal response induced by IPV and help limit the transmission and circulation of wild and/or vaccine-derived poliovirus globally. Use of such an IPV with enhanced mucosal immunogenicity would also eliminate any risk of re-introduction of new vaccine-derived circulation when homotypic mOPVs are used in outbreak response.

1.2 Pathogen

Poliovirus is a member of the enterovirus subgroup, family Picornaviridae. Enteroviruses are transient inhabitants of the gastrointestinal tract and are stable at acid pH. Picornaviruses are small, non-enveloped, ether-insensitive viruses with a single (+ strand) RNA genome. There are three poliovirus serotypes (PV1, PV2, and PV3) in nature and there is minimal heterotypic immunity between the three serotypes. That is, immunity to one serotype does not produce significant immunity to the other serotypes[9]. It is primarily spread by the fecal-oral route of transmission but may also spread by the pharyngeal route.

Polio is a disease of the anterior horn motor neurons that occurs as a consequence of a generalized poliovirus infection, causing acute lower motor neuron dysfunction.

1.3 Description of Study Vaccine Components

1.3.1 IMOVAX® Polio, Inactivated Poliomyelitis Vaccine

IMOVAX® Polio [Inactivated Poliomyelitis Vaccine (Vero Cell Origin)] is a licensed vaccine, produced by Sanofi Pasteur (France) that 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 wildtypes 1, 2, and 3 of poliovirus cultured on Vero cells, purified and then inactivated by formaldehyde. IMOVAX® Polio induces the production of humoral immunity particularly neutralizing antibodies against each type of poliovirus, which are antibodies related to protective efficacy.

1.3.2 dmLT

LT (R192G/L211A), or "dmLT," is a protein toxoid derived from wild-type enterotoxigenic *Escherichia coli* (ETEC) labile toxin (LT). The LT toxin has been shown to have inherent mucosal adjuvant properties for co-administered antigens and thus has potential as a mucosal adjuvant for different co-administered vaccines. LT 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) or dmLT and has been extensively evaluated in pre-clinical animal studies and clinical human trials for its ability to adjuvant the immune responses for co-administered antigens [15-18]. Recombinant dmLT, produced by IDT Biologika (Germany) is produced by *E. coli*, purified, and lyophilized in a neutral buffer.

1.3.3 Bivalent Oral Poliomyelitis Vaccine Types 1 and 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, manufactured by GSK. Each dose (0.1 mL) contains not less than 106.0 $CCID_{50}$ of Type 1 and 105.8 $CCID_{50}$ 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.

1.4 Summary of Nonclinical Studies of Study Vaccine

1.4.1 Toxicology

IPV adjuvanted with dmLT has been evaluated in a Good Laboratory Practice (GLP) compliant nonclinical toxicology study in New Zealand White (NZW) rabbits. Animals were administered three biweekly IM injections of the full clinical dose (0.5 mL) of IPV with 0.1 or 0.5 µg dmLT. Standard vaccine toxicology endpoints, including measurement of body weight, food consumption, and body temperature; evaluation of the injection sites for irritation, inflammation, toxicity, and/or pain; assessment of clinical pathology; and detailed physicals, were assessed. Animals were sacrificed either two or 28 days (n = 6/sex or 5/sex, respectively) following the final

dose of vaccine. A full necropsy was performed on each animal, including measurement of organ weights, and tissues were collected and preserved for microscopic evaluation by a pathologist.
There were no adverse effects observed at either dose of dmLT with IPV. Slight elevations of C-reactive protein were observed, primarily in females, along with some changes in fecal output shortly after dosing, likely due to stress of handling and procedures along with a slight inflammatory response to vaccination. Microscopic changes at the injection site were attributed to the injection procedure; however, a slight exacerbation of these microscopic effects was observed at the terminal necropsy, two days after the third and final injection, in animals receiving IPV and dmLT. These changes were not considered to be adverse, that is not having an effect on life or function nor being linked to significant clinical findings and appeared to resolve by the recovery necropsy.
In conclusion, it was determined that IPV adjuvanted with 0.1 or 0.5 µg dmLT was well-tolerated in NZW rabbits.

1.4.2 Preclinical Immunogenicity

In previous preclinical studies of IPV adjuvanted with dmLT, Norton (et al., 2015) demonstrated that addition of 0.1 µg of dmLT to IPV vaccine resulted in higher neutralizing antibody responses against poliovirus type 1 (PV1) after an initial dose of vaccine compared to responses after vaccine alone. Responses against PV2 and PV3 were robust initially regardless of addition of dmLT. Duration of the responses was substantially better in animals receiving both IPV and dmLT compared to those receiving vaccine alone for all three serotypes, but particularly for PV1. This response is notable as PV1 is the least immunogenic of the virus strains in the vaccine as well as the serotype that represents the highest disease burden for wild-type poliomyelitis. Addition of dmLT to the vaccine formulation may enhance germinal center formation in the spleen and explain the improved magnitude and duration of immunity after vaccination with IPV adjuvanted with dmLT compared to IPV alone. Improved mucosal responses were also observed in mice vaccinated with IPV adjuvanted with 1 µg dmLT, as demonstrated by enhanced fecal IgA responses and *ex vivo* secretion of antibodies by Peyer's patch cells, a secondary lymphoid organ in intestinal tissue.

1.5 Summary of Clinical Studies of dmLT

1.5.1 Safety

Reactogenicity:
A first in human Phase 1 dose-escalation study evaluating the safety and tolerability of dmLT administered via the ID route is currently underway at Cincinnati Children's Hospital Medical Center (CCHMC) (CTN NCT02531685), in which four groups (N=16) of healthy adult subjects will receive 0.1 mcg, 0.3 mcg, 1.0 mcg, or 2.0 mcg of dmLT, and a fifth confirmatory cohort of up to 35 subjects is targeted to receive all three doses of dmLT or placebo. Thus far, cohorts 1, 2, 3 and 4 have completed the vaccination series with no product-related SAEs to report to date. The confirmatory Cohort 5 is targeted to be dosed in Q1 2019.

Additionally, dmLT has been administered IM in a Phase 1, open-label clinical trial (NCT03404674). This is the first in human study evaluating dmLT administered via IM. In this trial, a total of 50 healthy adult subjects received three IM injections of either CssBA alone, dmLT alone, or CssBA + dmLT on Days 1, 22, and 43. Dose escalation of CssBA from 5 mcg to 15 mcg to 45 mcg, and dmLT from 1.0 mcg to 0.5 mcg took place in 5 separate cohorts.

Additionally, LT, LTR1925 (single mutant LT (mLT)) and dmLT have been safely administered via multiple routes in prior clinical studies. An overview of these prior volunteer studies is provided below:

Table 1: Previous human experience with LT- and LT mutant-based adjuvants

Vaccine(s) Evaluated	**Study Phase**	**Route of Administration**	**Status**
LT (native)	Phase 1 safety and immunogenicity	Transcutaneous	Completed
mLT	Phase 1 safety and immunogenicity	Transcutaneous	Completed
CS6+LT or mLT	Phase 1 safety and immunogenicity	Transcutaneous	Completed
CfaE ETEC subunit + mLT	Phase 1 safety and immunogenicity	Intradermal	Completed
mLT	Phase 1 safety and immunogenicity	Oral	Completed
mLT + oral microencapsulated CS6	Phase 1 safety and immunogenicity	Oral	Completed (NCT00090688)
dmLT	Phase 1 safety and immunogenicity	Oral	Completed (NCT02052934)
dmLT	Phase 1 safety and immunogenicity	Sublingual	Completed (NCT02052934)
dmLT	Phase 1 safety and immunogenicity	Intradermal	Ongoing (NCT02531685)
Live attenuated ETEC (ACE527) ± dmLT	Phase 1/2b safety, immunogenicity and efficacy	Oral	Completed (NCT01739231)
CssBA ± dmLT	Phase 1 safety and immunogenicity	Intramuscular	Ongoing (NCT03404674)

Adverse Events:
Based on human studies with single mutant LT (mLT) alone and co-administered ID with ETEC subunit vaccine candidates, local site reactogenicity symptoms that were solicited from subjects

included pain, pruritus, tenderness, erythema, induration, plaques, edema, hypopigmentation, hyperpigmentation, and vesicles. Vaccine site reactions (rash) and pruritus were common with all subjects reporting these complaints at some point in the vaccination series. Severity was generally mild and resolved in all subjects. In terms of the characteristics and appearance of the vaccine site reactions, erythema and induration was present in all subjects who received mLT at some point over follow-up. Erythema was generally an early finding in the days after vaccination and resolved rapidly. Small areas of hyperpigmentation were often detected among subjects and these improved over the study period. Induration was present early and diminished over the study follow-up period but remained present in at least one vaccine site in all subjects. Hyperpigmentation was still present on a number of subjects by the last study follow up visit but was not significant enough to require any further follow up.

The first in human Phase 1 dose-escalation study evaluating the safety and tolerability of dmLT administered via the ID route is currently underway. The majority of adverse events were reported by subjects in the 1.0 mcg ID dmLT cohort. Symptoms of moderate severity were reported for induration/swelling, pain and tenderness at the injection site. The most frequent symptoms of mild severity were reported for induration/swelling and erythema/redness at the injection site. Both hyperpigmentation and pruritis increased and became more severe with each cohort; but none have been observed as necrotic. One subject complained of intense itching and was prescribed hydrocortisone cream; the subject subsequently improved rapidly after the cream was applied. There have been no other concomitant symptoms present, such as fever or any other symptoms, to suggest more systemic involvement.

In addition to local site reactions, systemic systems such as feverish chills, headache, arthralgia, myalgia, loose stools, and malaise were solicited from study participants. Approximately 3-11% of subjects experienced systemic side effects that were self-limiting. Severity of all reactogenicity proved generally mild and resolved in all subjects. No diarrhea was observed in any of the participants.

In the CssBA+dmLT IM trial, vaccinations were safe and well tolerated among the healthy adult subjects in this study. Administration of the vaccine products induced predominately mild to moderate symptoms. The most common vaccine-related symptom across all groups was vaccination site erythema (70%). Vaccination site induration and pain appeared to increase in frequency with advancing groups and increasing doses. No SAEs related to the vaccine products occurred during the study, and no subjects were withdrawn due to AEs. Based on the small number of volunteers (N=50) studied to date, CssBA + dmLT appear to be safe and well-tolerated when administered IM, with no severe or serious AEs recorded over the active study period. Additionally, 6 and 12 month follow-up has been completed for first 40 subjects with no Medically Attended Adverse Events (MAAEs) recorded. While the 12 month follow-up is pending for last group of 10, no MAAEs were recorded at the 6 month follow-up. Additionally, no safety concerns were noted that limited any of the planned dose escalations. The majority (97.0 %) of AEs considered at least possibly related to the vaccine were coded as mild severity.

1.5.2 Immunogenicity:

A series of phase 1 trials with the CFA/I adhesin prototype candidate have recently been completed (Riddle and Gutierrez et al, unpublished data, CssBA Phase 1 Protocol CT01382095, NCT01644565)

in which the prototype vaccine was co-administered transcutaneously and ID with LTR192G, or mLT, an *Escherichia coli* mutant heat labile toxin with a mutation at amino acid 192. While 50 μg of LTR192G administered transcutaneously was safe and induced serum IgG and IgA responses in 75-100% of subjects, the magnitude of the responses was relatively low across all immune parameters. In contrast, anti-LT responses in subjects administered 100 ng of mLT intradermally was safe and demonstrated both a higher rate of serologic responses and ALS responses. Local pruritus was also seen but somewhat less frequently, and mLT given alone at 100 ng gave similar local reactogenicity results to the mLT plus CFA/I adhesin prototype vaccine combination. Given the tolerable safety profile and immunogenicity results, the ID vaccine was moved into a Phase 2/2b challenge study in which a total of 56 subjects underwent vaccination. Similar to the phase 1 trial, there were no vaccine-attributable systemic adverse events (AEs) while vaccine site reactions (rash) and pruritus were common.

A first in human Phase 1 dose-escalation study evaluating the safety and tolerability of dmLT administered via the ID route is currently underway at CCHMC (DMID Protocol 13-0013; NCT02531685) and oral dmLT administered alone in a dose of up to 100 μg and together with an inactivated ETEC vaccine and with a live attenuated ETEC vaccine in a dose of up to 25 μg were all safe in their respective Phase 1 studies. Finally, comparative preclinical data in mice indicate that dmLT may be less reactogenic at the injection site than mLT.

In addition to being well-tolerated, intramuscular immunization with CssBA+dmLT induced robust systemic and mucosal antibody responses in a dose-dependent manner with the majority of responses observed in 5, 15, or 45 mcg CssBA and 0.5 mcg dmLT groups. The vaccine induced robust LT-specific IgG ALS response in dmLT included groups (100.0%). Anti-CS6 IgG ALS responses were also robust and observed in 80.4% of all subjects. At low doses, CssBA alone was poorly immunogenic, inducing a low-level serum and ALS IgG response only. The addition of as little as 0.1 mcg of dmLT increased anti-CS6 response rates across every immune parameter assessed and yielding a 100% rate as assessed by IgG ALS. Furthermore, most anti-LT responses seemed to increase significantly following the first vaccination then plateau. Anti-CS6 responses continued to increase in magnitude and frequency with an increased dose of dmLT to 0.5 mcg and with increasing doses of CssBA.

1.6 Potential Risks and/or Benefits of Study Vaccine

For the purpose of this study, the study vaccine is defined as the combination of IMOVAX® Polio with dmLT. The local reactogenicity of IMOVAX® Polio alone, was evaluated in two multicenter randomized clinical trials involving a total of 395 patients and local reactions were uncommonly to very commonly reported: injection site redness: in 0.7% to 2.4% of subjects in each trial, injection site pain: 0.7% to 34%, injection site mass: 0.4%. There are no known interactions of IMOVAX® Polio with drugs or foods. An extensive review by the (U.S.) Institute of Medicine of AEs associated with vaccination suggested that no SAEs have been associated with IPV.

The study adjuvant, dmLT is a non-infectious product; the risk of direct transmission of dmLT from study participants to the community or research members is negligible. The study adjuvant has been given to humans as a stand-alone single-component antigen and as an adjuvant with other candidate vaccines. There have been few AEs reported. Any candidate vaccine has the risk of allergic reaction; these reactions are rare and unpredictable. The dmLT has recently been given to humans by the IM

route up to 500 ng dose with a candidate vaccine and demonstrated no unexpected safety concerns. The IM vaccination was associated with injection site reactogenicity solicited as erythema, pruritus or induration in a dose-dependent manner. Most vaccine site reactions were short-lived, lasting 1-2 days after vaccination. Most of these adverse events were mild in severity, only two of moderate severity. Doses of 100 ng of mLT by the ID route have also been safe and well-tolerated in a Phase 1 trial[11].

In the ongoing DMID Protocol 13-0013 study in which subjects are to be immunized with dmLT at levels no higher than 2.0 μg by the ID route, there is very little risk of intestinal reactogenicity based on human data with single mutant mLT. In other studies, 100 ng of mLT administered via the ID route in humans has resulted in mild to moderate, self-limiting local site reactogenicity. Based on the further attenuation of dmLT compared to mLT, as well as, the findings of the preclinical studies, any local site reactogenicity is expected to be similar or lower in frequency and magnitude.

In the vast majority of cases side effects are very rarely reported with the bOPV vaccine.

The attenuated poliomyelitis viruses have ability to multiply in the gut. The fecal excretion of the vaccine viruses may persist for several weeks and may also be transmitted to the contacts of the vaccines; contacts of vaccines should therefore be warned about the need for strict personal hygiene. In this study, the subjects will be reminded of the importance of the strict hygienic measures especially post bOPV challenge on Day 29.

Very rarely, vaccine-associated paralysis has been observed with oral poliomyelitis vaccines (less than one case per 1 million doses administered). The majority of post-vaccinal paralytic poliomyelitis occurred after the administration of the first dose. Persons in close contact with a recently vaccinated person may very rarely be at risk of VAPP. Fever, vomiting, diarrhea and allergic/anaphylactic reactions have been described after immunization with trivalent oral poliomyelitis vaccine.

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure to the venipuncture site for several minutes. Breaking the skin during venipuncture or ID vaccination may cause transient discomfort, fainting, or infection. The use of sterile technique and materials will minimize the risk of infection.

Participants will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential, within the limits of the local laws and regulations. There is the chance that unauthorized persons will gain access to PHI. To minimize this risk, all records will be maintained in locked file cabinets or locked rooms, when not in use. When in use, research records will be under the direct supervision of study team members. Electronic files will be password protected. Only persons who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected.

1.7 Overall Development Strategy

The ultimate goal of the IPV + dmLT clinical development plan is to demonstrate the safety and efficacy of this vaccine approach to achieve better poliovirus-specific gut immunity via addition of this novel adjuvant.

1.8 Study Rationale

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 HYPOTHESIS, OBJECTIVES AND ENDPOINTS

2.1 Study Hypothesis/Hypotheses

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.

2.2 Study Objectives

2.2.1 Primary Objectives

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

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

2.2.2 Secondary Objectives

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

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

2.2.3 Exploratory Objectives

Immunogenicity:

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

2.3 Study Endpoints

2.3.1 Primary Endpoints

Safety:

- Frequency and incidence of SAEs throughout the study
- Frequency and incidence of severe 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

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

2.3.2 Secondary Endpoints

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 $\geq$1:8

- Proportion of participants developing type-specific poliovirus antibody secreting cell (ASC) responses defined as ≥8 ASC/10^6 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

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 PCR-negative for virus, with samples taken on separate days.

2.3.3 Exploratory Endpoints

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 STUDY DESIGN

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 subjects 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 subjects 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 subjects 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 2: Study schema

Study group[#]	N	IPV volume*	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

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

4 STUDY POPULATION

4.1 Description of Study Population

Eighty healthy 18 to 45-year-old (all inclusive) male or female volunteers with appropriate polio vaccination history who meet all the inclusion and exclusion criteria and reside within the screening population of 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.

4.2 Inclusion Criteria

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

- Adult male or female, ages 18–45, all 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 Exclusion Criteria

Subjects 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 negative consecutive stool PCR results, post challenge
- Presence of fever on the day of vaccination (oral temperature $\geq 38^{o}$ 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 subject 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 HIV, HBsAg or 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 subject 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 Screen Failures

Screen failures are the participants who consent to participate in the trial but are not subsequently entered in the study. Individuals who do not meet the criteria for participation in this trial (screen failure) because of screening laboratory abnormalities may be rescreened. These screening labs may be repeated only once during the protocol-defined screening period if certain values are outside the acceptable ranges and if the value does not increase the risk to the subject in the opinion of the clinical investigator. Rescreened participants should be assigned the same participant number as for the initial screening.

5 STUDY PRODUCTS

5.1 Trivalent IPV (IMOVAX® Polio)

5.1.1 Product Description

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.

5.1.2 Manufacturer

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

5.1.3 Presentation and Formulation

IPV vaccine 0.5 mL per dose supplied as single use one dose per syringe.

IMOVAX® Polio is a clear, colorless solution. Each 0.5 mL dose contains:
Active Ingredients:
Inactivated Poliomyelitis Vaccine:

- Type 1 (Mahoney) 40 D-antigen units*
- Type 2 (MEF1) 8 D-antigen units*
- Type 3 (Saukett) 32 D-antigen units*

* or the equivalent antigen quantity, determined by suitable immunochemical method

Other Ingredients:
Excipients:

- 2-phenoxyethanol ≤1.0%

Manufacturing Process Residuals:

- Formaldehyde ≤0.02%
- Residual calf serum protein <1 ppm
- Trace amounts of neomycin, streptomycin and polymyxin B, Medium 199 Hanks (without phenol red) up to 0.5 mL

IMOVAX® Polio is a highly purified, inactivated poliovirus vaccine produced by microcarrier culture. The viruses are grown in cultures of Vero cells, a continuous line of monkey kidney cells, by the microcarrier technique. The cells are grown in Eagle MEM modified medium, supplemented with newborn calf serum tested for adventitious agents prior to use, obtained from countries believed to be free of bovine spongiform encephalopathy. For viral growth the culture medium is replaced by M-199 without calf serum.

After clarification and filtration, viral suspensions are concentrated by ultrafiltration, and purified by three liquid chromatography steps; one column of anion exchanger, one column of gel filtration and again one column of anion exchanger. After re-equilibration of the purified viral suspension, with Medium M-199 and adjustment of the antigen titer, the monovalent viral suspensions are inactivated at +37°C for at least 12 days with 1:4,000 formalin. This vaccine fulfills European Pharmacopoeia and WHO requirements.

5.1.4 Stability and Storage

Store at 2° to 8°C (35° to 46°F). Do not freeze. Discard product if exposed to freezing. Do not use after expiration date.

5.2 dmLT

5.2.1 Product Description

LT(R192G/L211A), or dmLT, is a derivative of wild-type 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.

5.2.2 Manufacturer

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

5.2.3 Presentation and Formulation

The product was formulated as a freeze-dried (lyophilized), white to off-white cake, containing ~500 µg of dmLT in a sodium phosphate buffer supplemented with 5% lactose to stabilize. The product is provided in a 2 mL injection vial, 2 R, made of clear borosilicate glass, and sealed by a 13 mm dark grey rubber freeze-drying stopper and a crimped 13 mm aluminum cap, color: silver/yellow. The label appears below (Figure 1).

Figure 1: Product Label for dmLT lot 001-08-16

Recombinant double mutant Heat Labile Toxin (dmLT)
LT (R192G/L211A) Expressed in E.coli
Contents: 0.5 mL (1 mg/mL, Lyoph.)
Storage ≤-10˚C
Caution: New drug limited by Federal Law to investigational use only
Manufactured by: IDT, Rockville, MD 20850
IDT Biologika GmbH, Am Pharmpark, 06861, Dessau Roßlau

Lot #: 001 08 16
MFG Date: 08/2016

5.2.4 Stability and Storage

Vials of lyophilized dmLT should be stored, as described on the label, at ≤-10˚C. Once rehydrated with sterile water for injection, as described in the pharmacy manual and preparation worksheets, dmLT should be held at 2-8˚C. The dmLT bulk solution expires 6 hours after preparation, and the vials need to be placed on ice until ready for use.

5.3 bOPV

5.3.1 Product Description

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**.**

5.3.2 Manufacturer

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

5.3.3 Presentation and Formulation

The bOPV is presented in multi-dose glass vials containing 10 doses or 20 doses; one immunizing dose (0.1 mL) is contained in two drops. Due to minor variation of its pH, Polio Sabin™ One and Three (oral) may vary in color from light peach to light red. Changes of the color of the vaccine within these ranges do not signify deterioration of the vaccine.

5.3.4 Stability and Storage

The vaccine is potent if stored at not higher than -20°C until the expiry date indicated on the vial. It can be stored for up to six months between +2°C and +8°C. The vaccine may present a color varying from light yellow to light red, due to a slight variation of pH; however, this color variation does not affect the quality of the vaccine. The shelf life is 24 months at storage temperature of -20°C. Vials contain type 2 vaccine vial monitors.

In order to preserve optimal potency of Polio Sabin™ One and Three (oral), exposure of the vaccine to ambient (non-refrigerated) temperatures should be kept to a minimum and exposure to sunlight should be avoided.

Shipment should be done under refrigerated conditions, particularly in hot climates. Freezing and thawing does not affect the titer of the vaccine. When distribution or administration is not imminent, it is advisable to store the vaccine, if possible, at temperatures of -20°C or less since this halts deterioration in vaccine potency. If the vaccine has been accidentally exposed to high environmental temperatures it is recommended that the vaccine be used immediately or stored at -20°C until administration. Store in the original package to protect from light.

5.4 Dose Preparation and Administration

IMOVAX® Polio (Inactivated Poliomyelitis Vaccine) is to be administered by the IM route. Group 1, will receive one dose, 0.5mL of IPV.

Group 2 will receive IPV along with dmLT as an adjuvant, as a single dose. The vaccine product will be prepared in the clinical research pharmacy from the components described above on each day of

vaccination as described in step by step formulation procedures summarized below and detailed in the Manual of Operating Procedures (Appendix D). The vaccine product preparation will be carried out by an unblinded qualified research pharmacist and witnessed by another study staff member. The research pharmacist will dispense the vaccine product in a blinded manner to the clinical staff.

Group 2 doses will be formulated separately under aseptic conditions from final pre-filled IPV syringes and vaccine adjuvant dmLT (Lot 001-08-16). On the day of dose administration, a single vial of lyophilized dmLT, lot 001-08-16, will be rehydrated with 0.5 mL of Sterile Water for Injection (SWFI) to produce a 1000 µg/mL stock solution of dmLT. Serial dilutions of dmLT will be performed with pooled IMOVAX® Polio vaccine, produced from combining the contents of single dose syringes (0.5 mL) in a sealed, sterile glass vial. This diluted dmLT will be added to a final container containing pooled IMOVAX® Polio vaccine in a quantity sufficient to vaccinate all scheduled subjects on the day of preparation.

Group 3 and all the study participants later in the challenge phase of the study will receive one dose of bOPV vaccine in two drops, which are delivered from the polyethylene dropper supplied with the multi-dose container, for each subject a separate vial will be used.

5.5 Accountability and disposal

The Site Principal Investigator (PI) is responsible for ensuring study product distribution and disposition and has ultimate responsibility for study product accountability. The Site PI will delegate to an unblinded Site Research Pharmacist responsibility for study product accountability. Study product accountability records should include date received, date prepared, date administered, time of preparation, quantity administered, and the subject identification number to whom the study product was administered. The designated Research Pharmacist(s) will be responsible for maintaining accurate records of the shipments and dispensing the investigational vaccine product. The pharmacy records must be available for inspection by the CRO clinical monitor and is subject to inspection by the local regulatory agency at any time. Used and unused study product will be retained until monitored and released for disposition per the Sponsor.

6 STUDY PROCEDURES

6.1 Recruitment

Healthy subjects, ages 18 to 45 years (all inclusive), will be recruited from within the screening population of Antwerp area based on general good health, with an exclusive history of polio vaccination, expressed interest in the study as determined at a preliminary interview, and availability for required follow-up. 80 subjects will be randomized. 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.

6.2 Blinding Procedures

The study vaccine for Groups 1 and 2 (IPV alone and IPV + dmLT) will be administered IM in a blinded fashion. An unblinded positive control group (Group 3) will be administered bOPV.

The unblinded Research Pharmacist will perform all preparations of the study product for Groups 1 and 2. Preparation of the study product and administration to the subjects will occur in separate locations to preserve the blinding of staff except for Research Pharmacist. The study subjects, the study personnel who perform study assessments after administration of study product, data entry personnel at the site, and laboratory personnel performing immunologic assays will be blinded to the treatment assignment.

6.3 Study Visits

6.3.1 Screening Visit (Day -28 to Day 0)

Research staff must obtain written consent per the standard informed consent form (ICF) process before conducting protocol-specific screening activities.

After signing the ICF, the following procedures will be performed during the screening clinic visit.

- Confirm signed ICF
- Collect demographic information, medical history (including details of any previous vaccinations and reaction to vaccinations), and prior/concomitant medication history
- Record height and weight, oral temperature, pulse, respiratory rate and blood pressure
- Perform a brief physical examination, to include head, eyes, ears, nose, throat (HEENT); lymph nodes; skin; pulmonary; cardiovascular; abdominal; neurological; and musculoskeletal systems
- Collect approximately 13.5 mL of venous blood for screening labs: complete blood counts (CBC) with differential for white blood cell (WBC), Hemoglobin, absolute neutrophil count (ANC), platelets, creatinine, albumin, total bilirubin, alanine transaminase (ALT), aspartate aminotransferase (AST), C-Reactive Protein (CRP), HBsAg, HIV and HCV antibody
- Collect saliva for Poliovirus Secretory IgA and IgG
    - Saliva collection should be performed no earlier than 30 minutes after a meal, drinking coffee or oral intake of medications. Chewing gum is not allowed in the 30 minutes prior to saliva collection. This is to ensure that there will be no contamination of saliva by interfering substances.
- All women of childbearing potential will have a serum pregnancy test performed during screening (2 mL blood). To be eligible the result of the serum pregnancy test must be negative.

As a part of the screening procedures, prior to Day 1:

- Collect minimum 5–10 grams of screening stool for Poliovirus IgA and Poliovirus Neutralizing Antibodies
    - The study staff will provide a stool container and cooling bag to the potential participant and give instructions to collect stool. Participants should bring the stool sample back to the clinic, within 48 hours of collection, ideally, and a within a maximum of 4 days of collection, for the completion of screening stool collection process.

Prospective subjects will be carefully screened to ensure that they are in excellent physical and mental health. Screening tests will be conducted up to 28 days before Day 1 (the day of vaccination). Two

back up volunteers per day may be asked to go to the clinic on vaccination days in the event a planned subject cannot/does not enroll in the study.

6.3.2 Enrollment/First Vaccination Visit (Day 1)

- Review eligibility criteria prior to performing any study procedures, to include updating the medical history and concomitant medications
- Record oral temperature, pulse, respiratory rate and blood pressure
- Perform targeted physical examination, as indicated based on review of medical history
- Urine pregnancy test will be obtained from all women of childbearing potential, must be negative prior to dosing
- Collect 110 mL of venous blood for (prior to vaccination):
    - Blood Poliovirus Antibody-Secreting Cell (ASC) and Antibodies in Lymphocyte Supernatant (ALS)
    - Blood Memory B and CD4+ T cells
    - Serum IgA and IgG
    - Serum Poliovirus Neutralizing Antibodies
    - Multiplex cytokine assay: 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
- Administer vaccine, per the randomization code
- Provide and explain memory aid and other 7-day solicited and unsolicited adverse event (AE) and reactogenicity instructions
- Inspect the injection site and perform post-vaccination reactogenicity assessment at least 30 minutes after vaccination. This assessment is to include unsolicited AEs and SAEs.
- Provide stool collection kit for the Day 8 sample

6.3.3 Follow-up Visits

Day 8 (±1 day)

- Collect and review memory aid/reactogenicity information and the occurrence of any unsolicited AEs and SAEs that have occurred since the last visit as well as update the interval medical history, and concomitant medications as necessary
- Provide and explain diary card for recording unsolicited AEs between day 8 and 57.
- Visually inspect the injection site
- Record oral temperature, pulse, respiratory rate, and blood pressure
- Perform a targeted physical examination, as indicated based on review of medical history
- Collect 13.5 mL of venous blood for follow up safety laboratory tests: complete blood counts (CBC) with differential for WBC, hemoglobin, ANC, platelets, creatinine, albumin, ALT, AST, CRP, and total bilirubin
- Collect 55 mL of venous blood for:
    - Serum IgA and IgG
    - Blood ASC Homing Marker (α4B7) (a subset of samples)
    - Blood ALS and ASC
- Collect saliva for Secretory IgA and IgG

- Collect minimum 5–10 grams of stool for Poliovirus IgA and Poliovirus Neutralizing Antibodies
    - Participants should bring the stool sample back to the clinic, within 48 hours of collection, ideally, and a within a maximum of 4 days of collection.
    - Provide stool collection kit for the Day 29 sample

Day 29 (± 2 days) (Day of bOPV Challenge)

- Review subject's diary card and occurrence of any unsolicited AEs and SAEs that have occurred since the last visit as well as update the interval medical history and concomitant medications as necessary
- Urine pregnancy test will be obtained from all women of childbearing potential, must be negative prior to dosing
- Record oral temperature, pulse, respiratory rate and blood pressure
- Perform a targeted physical examination, as indicated based on review of medical history
- Collect 110 mL of venous blood for (prior to vaccination):
    - Blood ALS and ASC
    - Blood Memory B and CD4+ T cells
    - Serum IgA and IgG
    - Serum Poliovirus Neutralizing Antibodies
    - Multiplex cytokine assay: 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
- Collect minimum 5–10 grams of stool for IgA, Poliovirus Neutralizing Antibodies and viral shedding
- Collect saliva for poliovirus secretory IgA and IgG
- Administer a standard 0.1mL oral dose of bOPV to all participants
- Perform post-vaccination reactogenicity assessment at least 30 minutes after vaccination. This assessment is to include unsolicited AEs and SAEs.
- Provide stool collection kit for the Day 33 sample

Days 33, 39 and 46 (±1 day)

- Collect stool for viral shedding only
    - Participants should bring the stool sample back to the clinic, within 48 hours of collection, ideally, and a within a maximum of 4 days of collection. During each above listed visit, a stool collection kit will be provided for the following visit

Day 36 (±1 day)

- Collect 55 mL of venous blood for:
    - Serum IgA and IgG
    - Blood ASC Homing Marker (α4B7)- (a subset of samples)
    - Blood ALS and ASC
- Collect minimum 5–10 grams of stool for IgA, Poliovirus Neutralizing Antibodies and viral shedding
- Collect saliva for poliovirus secretory IgA and IgG
- Provide stool collection kit for the Day 39 sample

- Perform a targeted physical examination, as indicated based on review of medical history, and update concomitant medications as necessary
- Review subject's diary card and occurrence of any unsolicited AEs and SAEs since the last visit as well as update the interval medical history and concomitant medications as necessary

Day 43 (±1 day) and Day 50 (±2 days)

- Collect 5 mL of venous blood for serum IgA and IgG
- Collect minimum 5–10 grams of stool for IgA, Poliovirus Neutralizing Antibodies and viral shedding
- Provide stool collection kit for the next visit
- Perform a targeted physical examination, as indicated based on review of medical history and update concomitant medications as necessary
- Review subject's diary card and occurrence of any unsolicited AEs and SAEs since the last visit as well as update the interval medical history and concomitant medications as necessary

Day 57 (±2 days)

- Collect 85 mL of venous blood for:
  - Serum IgA and IgG
  - Blood Memory B and CD4+ T cells
  - Multiplex cytokine assay
- Collect minimum 5–10 grams of stool for IgA, Poliovirus Neutralizing Antibodies and viral shedding
- Perform a targeted physical examination, as indicated based on review of medical history and update concomitant medications as necessary
- Collect and review subject's diary card and occurrence of any unsolicited AEs and SAEs since the last visit as well as update the interval medical history and concomitant medications as necessary
- Provide stool collection kit for the Day 169 sample and additional sampling if the subject is still shedding

If poliovirus shedding is detected by PCR on one of the last two scheduled stool samples, the study duration for that subject will be extended. As soon as the shedding results are known (anticipated approximately 3 weeks after the last stool sample provided for evaluation) the subject will be asked to collect additional stool samples (with a maximum of one sample per day) after the last per protocol sample, and to repeat this until shedding is PCR-negative for poliovirus on two consecutive stool samples collected on different days. They will be provided additional stool collection kit for each sampling day.

If the last stool sample is missing the subject will be asked to provide a new sample as soon as possible in order to determine their end of study or the need for further stool sample collection until poliovirus shedding is PCR negative on two consecutive stool samples.

6.3.4 Final Study Visit, Day 169 (±14 days)

- Review the occurrence of any SAEs that have occurred since the last visit as well as update the medical history and major medical events
- Collect 85 mL of venous blood for:
  - Serum IgA and IgG
  - Blood Memory B and CD4+ T cells
  - Multiplex cytokine assay
- Collect minimum 5-10 grams of stool for IgA, Poliovirus Neutralizing Antibodies

6.3.5 Interim Contacts and Unscheduled Visits

Interim contacts and visits (those between regularly scheduled follow up visits, including phone contact) may be performed at participant request or as deemed necessary by the investigator or designee at any time during the study. All interim contacts and visits will be documented in participants' study records and on applicable case report forms.

6.4 Discontinuation of Vaccination or Study Procedures

An enrolled/vaccinated participant may be terminated from the study for any of these reasons:

- Participant withdraws consent for any reason.
- Site PI, Safety Review Committee (SRC), or PATH medical officer decides that termination is in the best interest of the participant.
- Site PI, SRC, or PATH medical officer decides that termination is necessary to protect the integrity of the study or achieve the objectives of the study.
- Interruption of study schedule makes the participant's data unusable according to protocol requirements.

6.5 Participant Discontinuation/Withdrawal from the Study

Participants have the right to decline study treatment or procedures for any reason and at any time during the study. If a participant declines further vaccination or study procedures this will be recorded as a protocol deviation and the reason will be clearly documented in the source document. The participant will be encouraged to complete the remaining applicable safety related follow-ups and procedures. If the participant does not wish to remain in the study by declining any follow-ups or procedures, the participant can choose to withdraw consent and be withdrawn from the study. There will be no replacement for participants who withdraw from the study after randomization.

In the event of early termination of a subject before completing the study, the following information will be collected and procedures performed, as agreed to by the study subject:

- The subject's current health status since the last study visit will be reviewed
- A study memory aid will be completed if the early termination occurs within 7 days of study vaccination;
- A history of all concomitant medications will be documented if early termination occurs before Day 57;
- A 20 mL venous blood sample will be collected for ALS and ASCs if the early termination occurs within 10 days of study vaccination;

- A stool sample will be collected for viral shedding (and stool IgA if within a study-specified visit window) if early termination occurs after bOPV challenge;
- Information will be documented regarding AEs if the early termination occurs within 56 days of study vaccination. Any ongoing related AEs will be followed to resolution or until a stable chronic condition has been established;
- Continued follow-up of AEs and collection of scheduled blood samples, if possible, including a safety check 6 months after the study vaccination to assess for any serious medical concerns.

Reasonable efforts should be made to continue follow-up of participants and undertake protocol-specified safety follow-up procedures to capture AEs, SAEs, and unanticipated problems.

Discontinuation from further vaccination may be at the discretion of the PI if it is in the interest of the subject or based on the SRC safety review as per Section 9. In addition, participants will be discontinued from further vaccination for the following reasons:

- Pregnancy
- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event that requires discontinuation of study treatment or results in inability to continue to comply with trial procedures
- Intercurrent illness or diseases or medical treatment that occur during the trial and might influence the study results or ability to continue to comply with trial procedures

6.6 Lost to Follow-up

To prevent loss to follow-up, participants will be reminded by phone/text message/home visit one day before scheduled visit. In the event of a missed visit, participants will be contacted by phone/text message/home visit within one day. A participant who cannot be located after several contact attempts and who has missed two consecutive clinic visits will be considered lost to follow-up. Efforts to contact the participant will be documented in source documents. Any participant who failed to attend the final study visit will also be classified as lost to follow-up. There will be no replacement for participants who are lost to follow-up.

6.7 Use of Concomitant Vaccine(s) During the Study

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 in the Concomitant Medication CRF.

6.8 Pregnancy During the Study

If a female participant becomes pregnant following randomization, the bOPV challenge will not be administered and she will be encouraged to complete remaining visits and study procedures unless medically contraindicated. Any participant who becomes pregnant anytime during the study will continue to be followed for pregnancy outcome, if possible and agreed by the subject. The pregnancy and its outcome will be reported on the Pregnancy CRF.

6.9 Clinical Procedures

Vital Signs

- Temperature in degrees Celsius (recorded to the nearest 0.1 degree) will be measured by oral thermometer. Fever is defined as oral temperature ≥38.0°C.
- Respiratory rate in breaths per minute.
- Blood pressure and heart rate (in beats per minute) will be measured by automated device or manually.

Height/length and Weight

- Height/length is measured and recorded to the nearest cm.
- Weight is measured in kg and recorded to the nearest 0.1 kg.

Physical Examination

- Full physical examination will include assessment of vital signs, head, eyes, ears, nose, oropharynx, neck, chest (auscultation), lymph nodes (neck, supraclavicular, axillary, inguinal), abdomen (auscultation and palpation), musculoskeletal, skin (especially injection sites), and neurological.

Targeted Physical Examination

- A focused physical examination based on symptoms reported by the participant and the presence or absence of solicited AEs collected to assess local and systemic vaccine reactogenicity.

Medical History

- A comprehensive medical history will be collected including details of any previous vaccinations in the past 30 days and reaction to vaccinations, participation in clinical trials, surgery, previous hospitalization, allergy to food/drugs, prior/current medication and history of any chronic or recurrent medical conditions.
- An interval medical history will consist of inquiring regarding changes since the last medical history discussion (healthcare events, signs, symptoms and changes in use of prescription or nonprescription drugs or herbal preparations).

Injection Site Examination

- For Groups 1 and 2, injection site assessment will be done by trained study personnel and recorded in the corresponding CRF. Timing and severity grading of local reactions will be performed according to Section 8 (safety assessment) and toxicity grading scale in Appendix II respectively.
    - Erythema/redness and hyperpigmentation will be examined under standardized lighting conditions and measured. Severity of the reaction will be determined by staff on the basis of the severity grading table (Appendix II).
    - Swelling/induration will be examined by palpation and visual inspection under standardized lighting conditions; the examiner may temporarily mark skin at margins of visible swelling/induration, then must measure maximum diameter (as per Appendix II and as above).
    - Pain/tenderness will be self-reported by the subject.

- Draining lymph nodes will be palpated for the presence of lymphadenopathy.

7 LABORATORY EVALUATIONS /REQUIREMENTS

Table 3: Summary of Sample Collection and Analysis (by date and collaborating lab):

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

Other than stool for IgA, and neutralizing activity, serum for neutralizing antibody, viral shedding stool samples, PBMCs for multiplex cytokine assays, and fresh blood for ASC/ALS and ASC α4β7 and memory B and CD4+ T cells; all collected blood, stool and saliva samples will be shipped to the biorepository either for temporary storage until processing in the respective laboratories or long-term storage for potential future use. Some of the secondary and exploratory immunological assays may only be done contingent upon evidence of study intervention's impact on shedding. The algorithm that clarifies this step by step process can be found in the study Manual of Procedures.

Samples to evaluate clinical safety (safety blood specimens) will be obtained and processed at the clinical trial site and transported to AML-Riatol clinical laboratory for testing.

Whole blood research specimens collected for PBMC preparations will be processed and separated by the Cools' lab into multiple PBMC aliquots according to the study's manual of operating procedures (MOP) and stored in liquid nitrogen until further testing or shipment. One of these PBMC aliquots will be retained and used to perform a multiplex cytokine assay at the Cools' lab. The remaining PBMC aliquots will be shipped (in a liquid nitrogen dry shipper) to the Institute for Medical Immunology, Université libre de Bruxelles (ULB) for memory B and CD4+ T cells. After

completion of all protocol-specified laboratory assays at ULB, remaining aliquots of PBMCs will be shipped (in a liquid nitrogen dry shipper) from ULB to the Biorepository for long-term storage for potential future use.

ULB will also receive fresh whole blood (room temperature) for determination of ASC, ASC homing (a subset of samples) and production of ALS supernatants. ALS supernatants will be temporarily stored at -80°C or below until transported in dry ice to the Biorepository for storage until testing at the University of Maryland, Center for Vaccine Development (UMB CVD), for IgA and IgG ELISA. After completion of all protocol-specified laboratory assays at ULB and UMB CVD, remaining ALS supernatants will be shipped (in a liquid nitrogen dry shipper) to the Biorepository for long-term storage for potential future use.

Serum samples for Poliovirus Serum Neutralizing Antibody assay and stool samples to assess for presence of viral shedding and CCID50/g of shed virus will be processed and temporarily stored at the clinical trial site until transported in dry ice to the laboratory at the Centers for Disease Control and Prevention, Atlanta, GA, USA. Following testing, any remaining stool aliquots will be stored at -80°C or below.

Stool samples to assess stool IgA and stool for Poliovirus neutralizing activity will be processed and temporarily stored at the clinical trial site until transported in dry ice to the laboratory at Wright Lab, Dartmouth University, Geisel School of Medicine, Lebanon, NH, USA. Any remaining stool aliquots will be stored at -80°C or below.

Serum and saliva samples for IgA and IgG ELISA will be processed and temporarily stored at the clinical trial site until shipped in dry ice to the biorepository. These samples will be tested for IgA/IgG (by ELISA) at the University of Maryland, Center for Vaccine Development at a later timeline.

Backup generators are available for proper sample storage at all locations.

7.1 Clinical Laboratory Tests

Protocol-mandated clinical screening and safety laboratory tests will be conducted in real time by laboratories that are properly accredited and subscribed to a proficiency testing program. These tests include:

- Serum Chemistry: ALT, AST, Creatinine, Albumin, Total Bilirubin, CRP
- Hematology: Hemoglobin, WBC, ANC, Platelets
- Pregnancy test: Serum and urine (conducted on site) β-HCG
- HIV: HIV EIA
- Hepatitis B: HBsAg
- HCV: anti-HCV (if positive, by PCR)

Laboratory results will be reviewed promptly by the PI or designee. Participants will be notified of any clinically significant abnormalities. If clinically significant abnormalities are identified during screening, participants will be referred to their primary health provider or appropriate medical center. If identified during the study, participants may be asked to return to the study site for further evaluation, including clinical evaluation and repeat laboratory testing as medically warranted.

7.2 Immunological Laboratory Assays

- 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

7.3 Microbiological / Virological Endpoint Assays

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

7.4 Assays Qualification, Standardization, Validation

The serum neutralizing antibody assay is a serological test that is CLIA certified and validated, with the qualification of the laboratory to be recognized by the WHO.

Fecal IgA and neutralizing activity assays are standardized.

The ELISA assays for serum, saliva and ALS IgA and IgG antibodies employed to determine the study endpoints will be qualified. A qualification report will be available to append to the CSR.
Stool specimen for viral shedding assay has been validated against the gold-standard cell culture isolation procedure used by the Global Polio Laboratory Network (GPLN) for poliovirus isolation. Stool specimen for viral culture ($CCID_{50}$) is a standardized assay.

7.5 Future Use of Stored Samples

With the participant's consent and as approved by Committee for Medical Ethics Antwerp University Hospital/University of Antwerp, coded biological samples will be stored at a Biosample Repository at Precision for Medicine with the goal of using them for future polio research. Permission to transmit the samples to the Biosample Repository at Precision for Medicine will be included in the informed consent. These samples may be used to research the other polio eradication strategies, its complications and other conditions for which individuals who received polio vaccine are at increased risk, and to improve efficacy of the vaccine. Genetic testing will not be performed on these samples.

7.6 Biohazard Containment

All protocol specimens will be shipped using packing that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, biological

Substance, Category B, and Packing Instruction 650. Culture isolates, if obtained in this study, are to be shipped as specified for UN 2814 Category A Infectious Substances.

8 SAFETY ASSESSMENT AND REPORTING

8.1 Definitions

8.1.1 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 8.4.

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. Investigators will not be required to assess causality of solicited AEs if the onset is during the solicitation periods. 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 (oral temperature ≥38.0°C), chills, fatigue, headache, muscle aches/myalgia, joint ache/arthralgia, rash, nausea, vomiting, diarrhea

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.

8.1.2 Adverse Reaction

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 casual 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.

8.1.3 Serious Adverse Event (SAE)

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

1. Death
2. 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).
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital abnormality or birth defect
6. 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 subject 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.

8.2 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 subject to be stable. Abnormal laboratory values will be repeated and/or investigated as appropriate. Attempts will be made to follow the subject 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, inclusive (Day 57).

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.

8.3 Severity of Adverse Events

The severity of all AEs will be assessed by the investigator and participant (as applicable) based on the severity grading criteria provided in Appendix II 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".

8.4 Causality of Adverse Event

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.

8.5 Follow-up of Adverse Event

All reported unsolicited AEs should be followed until resolution or stabilization, or until the participant's participation in the study ends. Participants who have an ongoing study product-related SAE at study completion or at discontinuation from the study will be followed by the PI or his designee until the event is resolved or determined to be irreversible, chronic, or stable by the PI. If the event is ongoing at the time of study termination, the subject's permission will be requested for the follow-up.

The outcome of the adverse event will be assessed at the time of last observation according to the following categories:

- Recovered/resolved without sequelae
- Recovered/resolved with sequelae
- Recovering/resolving
- Not recovered/Not resolved
- Fatal
- Unknown (The outcome of the AE is not known)

8.6 General Guidance on Recording Adverse Events

To improve the quality and precision of acquired AE data, the PI should observe the following guidelines:

- Whenever possible, use recognized medical terms when recording AEs on the AE CRF. Do not use colloquialisms and/or abbreviations.
- If known, record the diagnosis (i.e., disease or syndrome) rather than component signs, symptoms and laboratory values (e.g., record congestive heart failure rather than dyspnea, rales, and cyanosis). However, signs and symptoms that are considered unrelated to an encountered syndrome or disease should be recorded as individual AEs (e.g., if congestive heart failure and severe headache are observed at the same time, each event should be recorded as an individual AE).
- AEs occurring secondary to other events (e.g., sequelae) should be identified by the primary cause. A "primary" AE, if clearly identifiable, generally represents the most accurate clinical term to record. If a primary SAE is recorded, events occurring secondary to the primary event should be described in the narrative description of the case.
- Death is an outcome of an event. The event that resulted in the death should be recorded and reported on the SAE CRF.
- For hospitalizations for surgical or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself. The procedure should be captured in the case narrative as part of the action taken in response to the illness.
- Pregnancies that occur in study participants are not considered AEs and will be recorded on a separate Pregnancy CRF. Pregnancy outcomes that include stillbirth and any congenital anomalies must be reported as SAEs.

8.7 Reporting of SAE

8.7.1 Investigator Reporting to Sponsor

The sponsor has designated The Emmes Company, LLC (Emmes) as the study coordination center with authority to coordinate SAE reporting activities. Within 24 hours of an investigator's awareness of a SAE as defined in the protocol, an AE/SAE form must be completed and submitted to the Coordinating Center through the Advantage eClinical electronic data capture system.

The investigator must not wait to collect additional information to fully document the event before submitting the AE/SAE form. When additional information becomes available, follow-up submission must be completed. The initial AE/SAE form should be completed with all information known at the time and should include as much of the following as possible:

- Name and contact of the investigator submitting the AE/SAE report
- Participant ID number
- Date participant received study vaccine/s, including cohort group if applicable
- Description of the SAE and date of event onset
- Investigator's assessment of severity and causality
- Action taken and current status
- If available, any diagnostic test reports or hospital records that may help the sponsor to evaluate the SAE (All reports and records should have PHI/PII removed prior to providing it to the sponsor/other authorized parties)

The investigator will be responsible for notifying the Ethics Committee of the University Hospital of Antwerp/University of Antwerp and the Sponsor for notifying PATH REC as per the respective EC/IRB's reporting requirements.

8.7.2 Notification and Review of SAEs

Emmes is responsible, on behalf of the Sponsor, for evaluating SAEs submitted by the investigator and for notifying the SRC within 24 hours of their awareness to convene a safety review if the investigator reported the SAE as fatal or life threatening or suspected to be related to study vaccine. The SAE outcomes will be reported to the sponsor's representative (see below) using the Supplemental SAE Report Forms.

PATH MEDICAL OFFICER:	*[redacted]*

Medical Officers from PATH serving as technical consultants will provide technical guidance regarding SAE management including classification and reporting.

The Emmes medical monitor will review all unanticipated events involving risk to participants or others, SAEs and all participant deaths associated with the protocol and will provide a written report. At a minimum, the Emmes medical monitor must comment on the outcomes of the event or problem and, in case of an SAE or death, comment on the relationship to participation in the study. The Emmes

medical monitor must also indicate whether he/she concurs with the details of the report provided by the site PI.

8.7.3 Sponsor Reporting to Regulatory Agency

The Sponsor has authorized a local Belgium third-party CRO to execute its responsibility for safety reporting to the appropriate regulatory authorities within specified time period of notification.

This Belgium third-party CRO will be responsible for reporting to the Belgian Federal Agency for Medicines and Health Products (FAMHP) of:

- All fatal and life-threatening, suspected unexpected adverse drug reactions should be reported within 7 calendar days after first knowledge by the applicant. The initial notification must be followed by as complete a report as possible, within an additional 8 calendar days.
- SUSARs that are not fatal or life-threatening must be reported as soon as possible, and not later than 15 calendar days after first knowledge by the applicant.
- The FAMHP must be notified, within 15 calendar days after first knowledge by the applicant, when there is a suggestion of a change in the nature, severity or frequency of expected adverse drug reactions or when new risk factors are identified. The basis on which these assessments are made should be included.
- Any information, that may in any way influence the benefit-risk assessment of a medicine or that would be sufficient to consider changes in the administration of the medicine or in the overall conduct of a clinical trial, must be reported to the FAMHP. The applicant must submit this information to the FAMHP within three calendar days of first knowledge by the applicant.

8.8 Other Events Requiring Expedited Reporting

8.8.1 Pregnancy

Reproductive animal toxicity studies have not been conducted with dmLT. Therefore, the risk to the unborn fetus or pregnant mother is unknown. Consequently, women of childbearing potential will be required to use appropriate contraceptive methods to avoid pregnancy or will be excluded from the study. Highly effective contraception should be maintained for three months after the administration of bOPV challenge on Day 29 of the study.

If pregnancy occurs, each pregnancy must be reported immediately (within 72 hours of identification) by the Advantage eClinical electronic data capture system to the sponsor's representative.

Should a female subject become pregnant subsequent to study vaccination, the Investigator will obtain permission from the subject to follow the subject's pregnancy. If permission is obtained, subjects who become pregnant within three months after the administration of bOPV will be followed to the end of their pregnancy, and information will be gathered for outcome, date of delivery, health status of the mother and child including the child's gender, height and weight. Complications and/or abnormalities should be reported, including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale, or a birth defect in the baby.

8.8.2 AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported immediately (within 72 hours of identification) by email to the sponsor's representative.

9 SAFETY OVERSIGHT

The study site Investigators will be responsible for continuous close safety monitoring of all study participants, and for alerting the protocol team if unexpected concerns arise. In addition, a monitoring team, the Safety Review Committee (SRC), will be closely involved in safety monitoring.

9.1 Safety Review Committee (SRC) Reviews

The SRC, composed of the PI, the PATH Medical Officer, and two 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.

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 FAMHP and PATH REC. The site investigator of record will notify the responsible local Institutional Review Boards/ Ethics Committees (IRB/EC) expeditiously.

9.2 Stopping/Pause Rule

9.2.1 Study Stopping Rules

The following study stopping rules will automatically pause or halt further vaccinations. However, participants already enrolled will continue to be followed for safety during the pause. These stopping rules refer to suspected adverse reactions and will be triggered automatically if any of the events described below are met during the conduct of the study:

- The occurrence of one or more serious adverse reactions to the investigational vaccine within the three-day period post vaccination
- Two or more participants with the same severe (Grade 3) systemic reactogenicity signs or symptoms, within seven days following vaccination
- Two or more participants experience the same vaccine-related severe (Grade 3) reaction of either clinical or laboratory abnormality, within seven days following vaccination
- One serious and unexpected, solicited or unsolicited, suspected or confirmed adverse reaction evaluated by the PI, Medical Monitor, and PATH Medical Officer and determined to be an unacceptable risk to the health and safety of other investigational product recipients

9.2.2 Individual Subject Stopping Rules

Subjects who meet any of the following criteria must be assessed by the PI to determine whether or not to cease the individual study procedures:

- Participant's non-compliance
- Development of a significant medical condition and/or participation in the study is no longer in the best interest of the subject

A subject can withdraw consent at any point during the study.

9.3 Study Stopping/Pause Procedure

The SRC will be notified by the Emmes Coordinating Center and convene an ad hoc review if stopping criteria may have been met.

At scheduled study review, the SRC may also identify AEs that could potentially qualify as stopping rules.

If any member of the SRC identifies that a stopping criterion may have been met or proposes the study be paused on a discretionary basis, all vaccinations and enrollment will be suspended. The SRC may request review of unblinded safety data by independent experts in deliberating whether an event poses undue risk to study participants and the study should be paused or stopped. If study stopping rules are met, the SRC is to be notified and convened, as described in Section 9.2.1. The SRC reviews will be summarized with consensus recommendations to the study Sponsor as to whether there are safety concerns and whether the study should continue without change, be modified, or be stopped.

If at any time, a decision is made to discontinue administration of study product in all participants, expeditious notification will be provided by a local Belgium third-party CRO to the FAMHP and by the PI to the local EC within 48 hours. The Sponsor will notify PATH REC.

If the Sponsor re-starts the study after SRC review and recommendation, enrollment and vaccination may resume.

Although the study Sponsor has every intention of completing this study, the Sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to SRC review and recommendation and at the discretion of the Sponsor.

10 DATA HANDLING and RECORDKEEPING

The PI is responsible for assuring that the data collected are complete, legible, attributable, accurate, and recorded in a timely manner. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. When making a change or correction, the original entry will be crossed out with a single line. The initials of the person making the change and the date of the change will be recorded next to the change. Corrections will NOT be made by obliterating, erasing, overwriting, or using correction fluid or tape on the original. Dates reported in the eCRF derived from source documents will be consistent with the source documents or the discrepancies will be explained in the data system. All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs must be graded, assessed for severity and causality, and reviewed by the site PI or designee. After data are quality controlled by the clinical and/or laboratory staff, they will be entered into the Emmes Advantage eClinical database.

Emmes is responsible for data management activities, including quality review, analysis, and reporting of the study data according to SOPs.

10.1 Definitions

10.1.1 Source Data

Source data include all information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

10.1.2 Source Documents

Source documents are the original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries of evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial). Note that, for this study, the participant data collection tool (i.e., the Memory Aid) will be transcribed to the Reactogenicity Record data collection form. In addition, CRFs, if used as source documents, will be pre-specified in the Study Manual of Procedures.

10.2 Data Capture Methods (Case Report Form Development and Completion)

The clinical data in source documents will be entered directly into the Emmes Advantage eClinical Electronic Data Capture (EDC) system by trained and qualified study staff. The electronic Case Report Form (eCRF) for the EDC system will be developed by the Emmes data management with input from study staff and approval of the Sponsor. Any post-initiation changes to the eCRF or EDC system will be subject to Sponsor approval. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data for each participant will be entered directly to the eCRF from the source documents.

The site PI/institution will maintain all information in the eCRFs and all source documents that support the data collected from each participant in a secure area and treated as confidential material.

10.3 Data Management

Emmes will develop a Data Management Plan (DMP) for approval by PATH before implementation. The DMP will describe roles of stakeholders and specific procedures to ensure appropriate handling of data at all steps of the data management process to assure valid and high-quality database at the end of the study, ready for analysis.

10.4 Retention of Study Records

The site PIs are responsible for retaining study records for a period of twenty five years following the date that a marketing application is approved for the product or, if no application is to be filed or, if a filed application is not approved, until two years after the investigation is discontinued and the FAMHP is notified. The Sponsor will be responsible for providing the site with date of vaccine approval or regulatory withdrawal. No records will be destroyed without the written consent of the Sponsor.

These records are also to be maintained in compliance with local IRB/EC and local authority medical records retention requirements, whichever is longest. Storage of all trial-related documents will be such that confidentiality will be strictly maintained to the extent provided by local law.

11 STATISTICAL CONSIDERATIONS

11.1 Overview and General Design

This is a single center, three group, 3:3:2 randomized, partially double-blind, controlled trial to test the hypotheses that IM administration of IPV with dmLT adjuvant is safe, well-tolerated, and increases mucosal immune responses to polioviruses types 1, 2, and 3 in comparison with administration of IPV alone. A positive control group is included, composed of 20 subjects receiving bOPV. All statistical analyses will be conducted by Emmes, under the guidance and supervision of the Sponsor.

Primary objectives are:

- Safety, reactogenicity, and tolerability:
  - To evaluate and compare the safety and tolerability of a single dose of IPV + dmLT to IPV alone, administered IM in healthy adults
- Viral Shedding:
  - To evaluate and compare stool viral shedding following bOPV challenge 28 days after IM administration of IPV + dmLT to IPV alone

A key secondary objective is to evaluate and compare other mucosal and systemic immune responses to IPV + dmLT to IPV alone, administered IM.

11.2 Randomization Procedures

Randomization is defined as the process of assigning a participant to a study arm. Randomization of participants will be done online using the enrollment module of Advantage eClinical®. Coded assignments of participants are made using the enrollment module. Listings of coded treatment assignments are prepared by unblinded statisticians at Emmes. The dosing of study product must occur within 24 hours of randomization. The day of administration of the study product is designated Day 1. The unblinded research pharmacist will be provided with the treatment assignment codes for the preparation of vaccine product to be given to each participant. The unblinded research pharmacist will not reveal the randomization code to any other study staff member or subject. All code breaks will be reported to the sponsor, including accidental. The most recent evaluation of a given type obtained prior to the first vaccination is the baseline for subsequent safety and immunogenicity assessments.

Participants will be block-randomized using a 3:3:2 ratio to one of the three groups. The randomization scheme will be generated and maintained by Emmes.

11.3 Sample Size

It is anticipated that almost all participants enrolled will receive vaccination, and will provide at least some data for safety analysis, and at least 90 percent of enrolled subjects will be evaluable for virologic and immunogenicity assessments.

Safety

Rates of solicited and unsolicited AEs, including SAEs will be determined for each experimental group. With 30 subjects per treatment group, this study will have 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%.

Virologic Assessment

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%.

Table 3: Power for Relative Reduction in Shedding Rate with 27 Evaluable Subjects per Group

Shedding Rate				
IPV Alone	**IPV+dmLT**	**Relative Reduction**	**Power* (one type)**	**Power* (types 1 and 3 simultaneously)**
0.6	0.06	90%	>99%	>99%
	0.12	80%	98%	95%
	0.18	70%	91%	82%
	0.24	60%	77%	59%
	0.3	50%	59%	34%

0.7	0.07	90%	>99%	>99%
	0.14	80%	99%	99%
	0.21	70%	96%	93%
	0.28	60%	88%	77%
	0.35	50%	73%	52%
0.8	0.08	90%	>99%	>99%
	0.16	80%	>99%	>99%
	0.24	70%	99%	98%
	0.32	60%	96%	92%
	0.4	50%	87%	75%
0.9	0.09	90%	>99%	>99%
	0.18	80%	>99%	>99%
	0.27	70%	>99%	>99%
	0.36	60%	99%	99%
	0.45	50%	97%	94%
* Based on the Score test (Farrington and Manning) at 2-sided alpha of 0.05.				

Secondary Viral Shedding Endpoint:
Sample size calculations were based on a combination of prior experience of OPV shedding from IPV-vaccinated participants. In order to parameterize the comparisons with expected data, data from mOPV1-challenged subjects were obtained from Dr. Marc Collett [12] and were aggregated to provide a reasonable expectation for the absolute level of AUC of shed virus in the previously IPV-vaccinated adults to be enrolled in the IPV-only arm. In addition, data from that study as well as from Asturias, et. al. [12] were used to estimate the relationship between mean AUC and variability in AUC, to further inform model parameterization of the expectation for the IPV + dmLT arm. In this aggregation of data, it is assumed that the mean-variance relationship of $AUC_{(0\text{-}28)}$ (infants, OPV2) and $AUC_{(0\text{-}21)}$ (adults, OPV1) is similar. This leads to a somewhat more conservative (larger) sample size requirement than simply using the coefficient of variation of data from Collett, et. al[12] (and personal communication). These data were used 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).

The minimum 50% reduction selected for comparison of the IPV+dmLT to the IPV only arm is is 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 is assumed that AUC ratio $AUC(\mu_d)/AUC(\mu_I)$ will be $\leq$0.35. Estimated variances of the AUC of each group are obtained from the mean-variance relationship described above, and power of the comparison, facilitated by a Wilcoxon rank-sum test on the log-transformed AUC values, is estimated via repeated simulation and testing.

For evaluation 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 29 shedding with 18 evaluable subjects in the bOPV arm is displayed under varying assumptions of the shedding rate.

Rate of Day 29 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%

Among the subset of bOPV-vaccinated subjects who cease viral shedding prior to challenge, the post-challenge shedding will be described in detail; these details will be further described in the SAP.

Resultant calculations

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

11.4 Definitions of Populations to be Analyzed

11.4.1 Enrolled Population

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

11.4.2 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.

11.4.3 Per Protocol Population

All participants in the total vaccinated population who have no major protocol violations that are determined to potentially interfere with the immunogenicity assessment of the study vaccine. This population will serve as the primary analysis population for the immunogenicity 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 CRO representatives.

11.4.4 Statistical Methodology

All analyses will be performed by Emmes, under the supervision and responsibility of the sponsor. All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalized prior to database lock.

SAS version 9.4 or greater will be used for analysis. Except where otherwise indicated in this document or the SAP, summary statistics will be composed of the mean, standard deviation, 1st, 2nd, and 3rd quartile, and the minimum and maximum for continuous variables. For categorical variables, the count and proportion (using one digit beyond the decimal point) will be presented. All study data will be presented in listings. In general, summary tables will be presented by group, including a column for the total across all participants.

For all immunological and viral shedding assays with predefined limits of quantitation, percentiles and bootstrap-based confidence intervals (CIs) which achieve the LLOQ or ULOQ will be replaced with "<LLOQ" or ">ULOQ", as appropriate. Analysis of immunological and viral shedding assays may be supplemented by reverse cumulative distribution (RCD) curves with each group for a given time point displayed on the same plot, and different panels (preferred) or figures for different time points.

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 and per protocol populations. 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. For the total vaccinated population, medical history will be listed and summarized by category. Prior and concomitant medications will be coded according to WHO Drug version 2018, listed, and summarized by Anatomic Therapeutic Chemical (ATC) classification level 1 and level 4 within level 1, where each subject only contributes once per drug category. 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 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).

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

11.4.5 Analysis of Primary Objectives

Safety

All safety assessments will take place in the total vaccinated population, according to the treatment received. 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.

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 PT within SOC, where again each subject only contributes once per SOC/PT combination, and once per SOC. An additional table will include preferred terms 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.

Reactogenicity

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.

Safety Laboratory Assessments

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 the requirements of Appendix II. Grade 2 or higher will automatically be considered as

clinically significant (with a corresponding AE entered), while Grade 1 abnormalities will be 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.

Vital Signs

Vital signs, including change from pre-vaccination baseline for post-vaccination visits/time points, will be summarized by parameter and time point. A listing of all vital signs will be produced, which includes the study day, value, units, and change from baseline.

Physical Exam

Baseline (full) physical examination results will be listed, as well as summarized as categorical descriptive statistics, where each subject is only counted once per category. Post-baseline physical examination abnormalities will be summarized by category, and a separate listing will be provided.

Viral Shedding

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. The denominator for each of these percentage calculations will reflect the number of subjects providing a sample to be assayed, per time point.

The percent reduction in proportion of subjects shedding challenge virus on Day 7 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.

Additionally, viral shedding (log_{10} $CCID_{50}$/g, not type-specific) will be summarized descriptively 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).

11.4.6 Analysis of secondary objective/s

Viral Shedding

In addition to the primary viral shedding endpoint and associated descriptive statistics, the area-under-the-curve (AUC) of log_{10} $CCID_{50}$/g will be computed and summarized. 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.
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; 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 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 AUCs (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 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.

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.

Fecal IgA and Neutralization

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 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, 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 geometric mean fold rise (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) at the Day 29 visit will also be computed, 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.

Serum Neutralizing Antibody Response (NAbs)

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.

Antibody-Secreting Cells (non-homing)

An antibody-secreting cell (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 addition, these frequencies will be summarized for ASCs as a continuous variable across type, group and time point. 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.

11.4.7 Analysis of exploratory objective/s

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 restimulation 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.

Serum IgA and IgG

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.

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.

Saliva

The type-specific IgA and IgG immune response in saliva will be summarized as for serum IgA and IgG, described above, before and after vaccination as well as bOPV challenge, using assay-specific LLOQ/ULOQ as necessary; however, the antibody levels analyzed will instead be the ratio of specific/total antibody, separately for IgG and IgA. The UMB lab will provide a memo with assay LLQ to assist with data analysis.

Antibody-Secreting Cells (Homing Markers)

The proportion of subjects with type/antibody-specific mucosal ASC (those expressing gut-homing marker, $\alpha 4\beta 7$) 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.

Other

Additional exploratory analyses may be performed on collected endpoints, for example to determine the nature of any relationships between variables. Additional exploratory analyses will be defined in the SAP and will be labeled *exploratory* or will be performed as post hoc analyses and labelled accordingly, if agreed by the participating institutions.

11.5 Handling of Dropouts and Missing Data

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.

Some analyses defined herein rely on all values from repeated sampling to be available for a given subject (e.g., shedding 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.

11.6 Timing of Analysis

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 should be through 28 days post-challenge. Only the sponsor will receive the report, and will receive tables only, without listings, and will receive no individual treatment assignments. Immunogenicity and viral shedding results will be unblinded to group, and safety results will be presented in aggregate only (blinded to IPV vs IPV+dmLT group).

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 a final clinical study report (CSR).

12 QUALITY ASSURANCE AND QUALITY CONTROL

12.1 General Considerations

The study will be conducted in full compliance with the protocol and ICH GCP to provide public assurance that the rights, safety, and well-being of trial participants are protected, and that the clinical trial data are credible. To ensure quality and standardization, the site will develop Standard Operation Procedures (SOPs) for key protocol procedures and conduct the study guided by the study Manual of Procedures or other written guidelines. The site will also develop routine operational checks to verify that critical protocol requirements and procedures are executed correctly and completely at the time the work is being performed. Prior to the initiation of the study, the Sponsor and/or Emmes will conduct protocol training, including applicable SOPs, for study staff.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.2 External Monitoring

PATH, the sponsor of this study, is responsible for ensuring that the study is conducted in accordance with ICH GCP and regulatory requirements. For this purpose, Emmes will provide external monitoring for this study. A site initiation visit will be conducted prior to beginning the study, and monitoring will be conducted at initiation, during, and at closeout of the study. During the course of the study, monitors will visit the clinical site at intervals to verify compliance to the protocol; completeness, accuracy, and consistency of the data and study product accountability; and adherence to ICH GCP and applicable local regulations. As needed and when appropriate, the monitors will also provide clarifications and additional training to help the site resolve issues identified during the monitoring visit. As appropriate and informed by risk assessment, remote centralized monitoring activities may be considered in place of or to supplement onsite monitoring. These may include analysis of data quality (e.g. missing or inconsistent data, outlier data) and identifying data trends not

easily detected by onsite monitoring and performance metrics (e.g., screening or withdrawal rates, eligibility violations, timeliness and accuracy of data submission).

The extent and frequencies of the monitoring visits will be described in a separate Study Monitoring Plan developed prior to study initiation. The investigator will be notified in advance of the scheduled monitoring visit. The monitor should have access to all trial-related sites, participant medical records, study product accountability, and other study-related records needed to conduct monitoring activities. Emmes will share the findings of the monitoring visit, including any corrective actions, with the site investigator. The site PI and the monitor must agree to cooperate to ensure that any problems detected in the course of these monitoring visits are resolved in a predefined timeframe.

12.3 Independent Auditing

PATH or its designee may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs at the clinical site and that data are correct and complete. The site PIs will permit auditors (employees of the Sponsor or employee of a company designated by the Sponsor) to verify source data validation of the regularly monitored clinical study. The auditors will compare the entries in the eCRFs with the source data and evaluate the study site for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements.

12.4 Regulatory Agency Auditing

The site PIs must be aware that regulatory authorities, including EC/IRB, may wish to inspect the site to verify the validity and integrity of the study data and protection of human research participants. The site PIs will notify the Sponsor within 24 hours following contact by a regulatory authority. The site PIs must make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The site PIs will provide the Sponsor with copies of all correspondence that may affect the review of the current study or his/her qualification as an investigator in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance in responding to regulatory audits or correspondence.

13 ETHICAL CONSIDERATIONS (AND INFORMED CONSENT)

13.1 Ethical Standards

This study will be conducted in accordance with the ethical principles set forth in the World Medical Association Declaration of Helsinki/The Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines for Biomedical Research Involving Human Subject and in conformity with ICH GCP and FAMHP, Belgium.

13.2 Ethical Review

This study will be conducted under the auspices of the following Institutional Review Board (IRB)/ Ethics Committee (EC): Commissie Voor Medische Ethiek of the University Hospital Antwerp/Antwerp University.

The investigator is responsible for obtaining approval from the Commissie Voor Medische Ethiek of the University Hospital Antwerp/Antwerp University. This committee will review and approve the

protocol, informed consent form, and any recruitment materials (advertising or informational material)—including any modifications to these documents prior to or during the study. All changes to the protocol or informed consent form must be reviewed and approved prior to implementation, except where necessary to eliminate apparent immediate hazard to study participants. The investigator is also responsible for obtaining continuing review throughout the duration of the study in accordance with existing regulations.

13.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Before any study-related activities and in agreement with applicable regulatory requirements, the site PI must ensure that the participant is fully informed about the aims, procedures, potential risks, and potential benefits of the study. The participant will be given the written, IRB/EC approved ICF, allowed ample time to read the consent form, encouraged to ask questions about the study, have the questions answered and then be given time to decide if s/he would like to participate in the study. It will be emphasized that participation is voluntary, and that the participant has the right to decline to participate or subsequently withdraw from the study at any time without prejudice.
The site PIs or designees must obtain the participant's voluntary, signed and dated ICF (or, if the participant is unable to sign due to impaired vision, physical impairments, or illiteracy, impartially witnessed and documented consent) before any study-related procedures are performed. Impartial witness is a person, who is independent of the trial, who cannot be unfairly influenced by people involved with the trial, who attends the informed consent process if the participant or the participant's legally acceptable representative cannot read, and who reads the informed consent form and any other written information supplied to the participant. Study staff must document the informed consent process. The original, signed ICF must be kept in the site study file. A copy of the informed consent document will be given to each participant for their records.

13.4 Participant Confidentiality

The investigators, Sponsor and all staff from organizations involved with the implementation of the trial must ensure that the participants' confidentiality is maintained. Personal identifiers will not be included in any study report. All study records will be kept confidential to the extent provided by national and local laws. Medical records containing identifying information may be made available for review when the study is monitored by the sponsor or an authorized regulatory agency. Direct access may include examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study.

When appropriate and to the extent possible, study procedures will be conducted to protect participant privacy and confidentiality.

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens, and other reports will be identified by a coded number only to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant

ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Participants' study information will not be released without their written permission, except as necessary for monitoring.

All clinical trial data will be handled in accordance with the EU Directive 95/46/EC and as of 25 May 2018, in accordance with the GDPR.

13.5 Reimbursement

Pending IRB/EC approvals, participants will be compensated for their time and effort in this study and be reimbursed for travel to study visits. The study ICF will state the plan for reimbursement. Participants will not be charged for study injections, research clinic visits, research-related examinations, or research-related laboratory tests.

13.6 Risk and Benefits

No benefits can be guaranteed to participants for their participation in this research study.

Since we don't know what the risks are with the combination of IMOVAX® Polio and dmLT, the following risks those are listed attributed to one or the other component.

For the polio component most frequently reported injection site reactions among adults following vaccination are: erythema, induration and pain within 48 hours post-vaccination. Some individuals may also experience fever, fatigue, myalgia and arthralgia. Even with licensed vaccine, there is the theoretical risk of immediate hypersensitivity reaction. As such, monitoring for sensitization and/or allergic reactions will be conducted routinely during the study, including direct clinical evaluation and observation at least 30 minutes post immunization and will be followed up for 1 week after the vaccination.

For dmLT, mild to moderate, self-limiting local site reactogenicity with slight to well-defined erythema and slight to moderate edema was observed in the previous human studies. In all cases, these dose site reactions were short lived and appeared to resolve completely within approximately one week. Symptoms of moderate severity were reported for induration/swelling, pain and tenderness at the injection site. In the ID dmLT study, there were more events of hyperpigmentation and pruritis than anticipated and these were more severe and lasted longer than would be typically observed; none have been observed as necrotic.

Drawing peripheral blood can cause discomfort; it might cause minor bleeding and/or bruising where the needle enters the skin, and very rarely might cause infection. This risk will be mitigated by ensuring that only study staff members who are adequately trained in safe drawing of blood conduct this portion of the study.

The bOPV is very rarely associated with vaccine-associated paralysis (less than one case per one million doses administered). The use of bOPV in persons who have already been vaccinated against polio is not known to increase the risk vaccine-associated paralysis. Persons in close contact with a recently vaccinated person may very rarely be at risk of vaccine-derived paralytic poliomyelitis. Common adverse reactions to bOPV vaccine include mild fever, nausea, vomiting, diarrhea and rash. Supportive care will be available for these symptoms and findings if necessary; evaluation for other

etiologies of illness will be explored if symptoms are not mild. If a case of acute flaccid paralysis (AFP) occurs in a study participant, a full medical evaluation will be conducted to determine the etiology (meningitis, malaria, wild-type polio, VAPP, or other viral causes of AFP) and medical treatment and supportive care will be provided in accordance with the local guidelines.

There may be additional risks associated with disclosure of person's HIV status as well as disclosure of any abnormal lab tests found during screening. In the case of HIV, subjects who are tested will undergo pre- and post-test counseling in accordance with local guidelines. There is risk of disclosure of the subjects' HIV status, and careful measures will be taken to ensure confidentiality is maintained. Standard pre-testing and post-testing counseling and linking to care will be followed. To the greatest extent possible, abnormal tests will be communicated to participants and screen failures in simplified language and referrals made to their regular health care provider as appropriate.

13.7 Reporting of Communicable Disease

Since HBV, HCV and HIV are not mandatory reported communicable diseases in Belgium, if a study subject is identified positive for HBV, HCV or HIV by the screening labs the PI will inform the subject and the subject will receive a letter for his or her treating physician. But neither the study site nor the subject is required to report the results to the local authorities.

13.8 Compensation for Research-Related Injury

Participants will be insured against injury caused by the study according to legal requirements and compensation for research-related injury (to include costs of long term and future medical care needs) is available, should it occur. In the event that a participant suffers injury directly attributable to participation in this study, the participant is asked to contact the PIs using the emergency contacts that will be provided on the consent forms and emergency number in the participant ID card. Appropriate treatment during the trial will be provided by the site personnel and paid by PATH. Additional details on compensation for research-related injury will be provided in the informed consent form.

14 FINANCING AND INSURANCE

The trial is supported by a grant from The Bill and Melinda Gates Foundation (BMGF) to PATH.

PATH will provide locally admitted clinical trial insurance for CVIA 065 Clinical Trial in Belgium for the payment of any expenses related to the treatment of injuries directly attributable to participation in this study.

15 PUBLICATION POLICY

It is understood by the investigators that the information generated in this study will be used by the sponsor in connection with the development of the product and therefore may be disclosed to government regulatory agencies in various countries. PATH also recognizes the importance of communicating study findings and therefore encourages their publication in reputable scientific journals and presentation at seminars or conferences, while protecting the integrity of the ongoing trial. Any publication, lecture, manuscripts of the findings of this study by any individual involved with the study will be governed by the procedure outlined in the Clinical Trial Agreement. Within

any presentation or publication, confidentiality of individual subjects will be maintained, with identification by subject code number and initials, if applicable.

Following completion of the study, the Investigator may publish the results of this research in a scientific journal. According to the policy of the International Committee of Medical Journal Editors (ICMJE) member journals, this clinical trial will be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM), in accordance with the new NLM requirements under the Food and Drug Administration Amendments Act (FDAAA).

16 LITERATURE CITED

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17 APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

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	±1	±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]		√						
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	110 mL [E]	68.5 mL	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.

18 APPENDIX II: GRADING REACTOGENICITY, MEMORY AID SYMPTOMS AND AEs (TOXICITY TABLES)

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	Any use of narcotic pain reliever or prevents daily activity

		with 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.