Evaluation of immunogenicity of fractional-doses of inactivated poliovirus vaccine (IPV): A randomized, controlled, non-inferiority trial

INTEGRATED CLINICAL
and
STATISTICAL REPORT

Overview

Sponsor	World Health Organization (WHO), Polio Eradication Initiative, Geneva, Switzerland
Implementing Agencies	Pedro Kourí Tropical Medicine Institute, Havana, Cuba Provincial Health Authority, Camaguey Province, Cuba Center for the state control of drug quality (CECMED), Havana, Cuba
Collaborating Agency	WHO, Geneva, Switzerland
Investigational Products	Full dose (0.5ml) inactivated poliovirus vaccine (IPV) formulated to contain 40-8-32 DU/dose Fractional-dose (1/5, 0.1ml) IPV
Vaccine Manufacturer	Bilthoven Biologicals (BBio), The Netherlands
Sponsoring Representative	Dr Roland Sutter, MD World Health Organization, Geneva, Switzerland
Clinical Coordinator	Dr Hiromasa Okayasu, MD World Health Organization, Geneva, Switzerland
Principal Investigator	Dr Sonia Resik, MD, PhD Pedro Kourí Tropical Medicine Institute, Havana, Cuba
Description of Clinical Trial	This was an open label randomized controlled interventional study. The goal was to compare immunogenicity against poliovirus types 1, 2, and 3 after one and two doses of vaccination, with either a fractional or a full- dose of IPV, in OPV-vaccinated adults. The study will generate data for IPVs produced by Bilthoven Biologicals (BBio). The specific objective was to demonstrate the non-inferiority of boosting immune responses induced by fractional- or full-dose IPV in healthy adults.
Clinical Trial Registration No.	ACTRN12615000305527
Clinical Trial Initiation Date	5 December 2014
Date of recruitment of last participant	8 January 2015
Date of Report	7 October 2015

This study was performed in compliance with Guidelines for Clinical Trials on Pharmaceutical Products in Cuba – Good Clinical Practice Guidelines, issued by the Government of Cuba

Investigator's agreement

I have read and agreed to this trial report which was carried out according to the procedures outlined in the protocols, and in accordance with local regulations and Good Clinical Practices (GCP).

Evaluation of immunogenicity of fractional-doses of inactivated poliovirus vaccine poliovirus vaccine (IPV): A randomized controlled non-inferiority trial.

Name	**Signature and date**
Dr Roland Sutter Coordinator, Research & Product Development World Health Organization Geneva, Switzerland	
Dr. Sonia Resik Principal Investigator Pedro Kouri Tropical medical Institute Havana, Cuba	
Dr. Alina Tejeda Investigator Provincial Health Office Camaguey, Cuba	

Synopsis

Sponsor	World Health Organization (WHO), Polio Eradication Initiative, Geneva, Switzerland
Collaborating Agency	Pedro Kouri Tropical Medicine Institute, Havana, Cuba
Investigational Product	WHO, Geneva, Switzerland
Vaccine Manufacturer	Bilthoven Biologicals (BBio), The Netherlands
Trial title	Evaluation of immunogenicity of fractional-doses of inactivated poliovirus vaccine poliovirus vaccine (IPV): A randomized, controlled, non-inferiority trial
Clinical Trial Registration No.	ACTRN12615000305527
Principal Investigator	Dr Sonia Resik, MD, PhD Pedro Kourí Tropical Medicine Institute, Havana, Cuba
Implementing Agencies	Pedro Kourí Tropical Medicine Institute, Havana, Cuba Provincial Health Office, Camaguey Province, Cuba Center for the State Control of Drug Quality (CECMED), Havana, Cuba
Additional Investigators (Corresponding Trial Site)	1. Oslayda Pérez Herrera (Centro) 2. Leonor Gorrin González (Norte) 3. Magaly González Hernández (Este)

	4. Rosa Batista de la Paz (Florida Norte) 5. Yanet Torres Carreño (Vertientes) 6. Katherine Carmenates Gutiérrez (Finlay) 7. Danmarys Rojas Gutiérrez (Nuevitas) 8. Maria del Carmen Mantecón (Minas) 9. Yaquelin Cruz Bárcenas (Mella) 10. Deivis Ruiz Quiñones (Previsora) 11. Mayra Pimentel Salt (Oeste) 12. Doris Mejias Reyes (Pirre) 13. Yusbisleidys Rodríguez Fernández (Agramonte) 14. Jani Méndez López (Florida Sur)
Trial Period	5 December 2014 – 8 March 2015
Primary Objective	To demonstrate non-inferiority of boosting immune response against poliovirus types 1, 2 and 3 after administration of one and two intradermal fractional IPV doses, compared with one and two intramuscular IPV doses in healthy male adults with a history of vaccination with at least one dose of OPV
Secondary Objective	Safety of intradermal fractional IPV as assessed by the number and intensity of local and systemic adverse reactions
Trial Design	Open label randomized controlled interventional study of IPV; participants were randomized into two study groups to receive 2 doses of IPV administered 28 days apart through 1 of 2 routes: intradermal fractional IPV or intramuscular full dose IPV
Planned Sample	600 healthy males aged 18-30 years
Inclusion Criteria	• Healthy males aged 18 to 30 inclusive • Received polio vaccination(s) with at least one OPV dose, in accordance with the Cuban National Immunization Program as a child • Gave verbal and written informed consent
Exclusion Criteria	• IPV or OPV booster dose after the age of 12 years • Known or suspected exposure to wild poliovirus • Known or suspected allergy against any of the vaccine components • History of unusual or severe reactions to any previous vaccination • Known or suspected disease or use of medication blood products that could influence the immune system • Known or suspected immune deficiency, and/or known HIV infection • Any vaccination within three months before screening and during the study until the last visit • History of any neurological disorder including epilepsy or febrile seizures • Participation in another clinical trial within three months before enrolment

Primary Evaluation Criteria	The proportion of participants with immune response defined as a change from seronegative to seropositive (reciprocal titer of <8 to ≥8) after vaccine administration, or ≥4-fold increase in reciprocal titer for participants with baseline reciprocal titer >8
Safety Evaluation Criteria	Number and percentage of infants with adverse events within 1hr and up to 7 days after receiving vaccine dose; systemic and potential adverse events occurring within 6 months of receiving vaccine dose; safety comparison between groups

RESULTS (per protocol analysis)

Baseline Seroprevalence

	Intradermal (ID) fractional IPV (fIPV)		Intramuscular (IM) IPV		
Variable	**N**	**Mean (95% CI)**	**N**	**Mean (95% CI)**	**p-value**
Age		23.9 (23.5-24.4)		24.1 (23.7-24.6)	0.538
	n/N	**% (95% CI)**	**n/N**	**% (95% CI)**	
P1 Seroprevalence	244/268	91.0 (87.0-93.9)	233/266	87.6 (83.1-91.0)	0.575
P2 Seroprevalence	237/268	88.8 (84.1-91.7)	232/266	87.2 (82.7-90.7)	0.964
P3 Seroprevalence	188/268	70.2 (64.4-75.3)	178/266	66.9 (61.1-72.3)	0.734

Seroconversion Rates

		Day 0 to 7			Day 0 to 28			Day 0 to 56		
		n/N	**% (95%CI)**	**p-value**	**n/N**	**% (95%CI)**	**p-value**	**n/N**	**% (95%CI)**	**p-value**
P1	ID IPV	22/22	100.0 (85.1-100.0)	NA	22/22	100.0 (85.1-100.0)	NA	21/21	100.0 (84.5-100.0)	NA
	IM IPV	33/33	100.0 (89.6-100.0)		33/33	100.0 (89.6-100.0)		31/31	100.0 (89.0-100.0)	
P2	ID IPV	31/31	100.0 (89.0-100.0)	NA	31/31	100.0 (89.0-100.0)	NA	31/31	100.0 (89.0-100.0)	NA
	IM IPV	34/34	100.0 (89.9-100.0)		34/34	100.0 (89.9-100.0)		33/33	100.0 (89.6-100.0)	
P3	ID IPV	77/78	98.7 (93.1-99.8)	0.476	76/77	98.7 (93.0-99.8)	0.470	75/76	98.7 (92.9-99.8)	0.469
	IM IPV	86/86	100.0 (95.6-100.0)		87/87	100.0 (95.8-100.0)		86/86	100.0 (95.7-100.0)	

Immune Response Rates

		Day 0 to 7			Day 0 to 28			Day 0 to 56		
		n/N	**% (95%CI)**	**p-value**	**n/N**	**% (95%CI)**	**p-value**	**n/N**	**% (95%CI)**	**p-value**
P1	**ID IPV**	238/257	92.6 (88.7-95.2)	0.002	239/252	94.8 (91.4-97.0)	0.058	234/249	94.0 (90.3-96.3)	0.072
	IM IPV	246/250	98.4 (96.0-99.4)		250/255	98.0 (95.5-99.2)		241/247	97.6 (94.8-98.9)	
P2	**ID IPV**	239/258	92.6 (88.8-95.2)	0.012	248/253	98.0 (95.5-99.2)	1.000	245/250	98.0 (95.4-99.1)	0.751

		IM IPV	248/254	97.6 (94.9-98.9)		254/259	98.1 (95.6-99.2)		247/251	98.4 (96.0-99.4)	
	P3	ID IPV	250/257	97.3 (94.5-98.7)	0.545	248/252	98.4 (96.0-99.4)	0.445	247/249	99.2 (97.1-99.8)	1.000
		IM IPV	249/253	98.4 (96.0-99.4)		256/258	99.2 (97.2-99.8)		248/250	99.2 (97.1-99.8)	

Median Antibody Titers

		Day 0		Day 7		Day 28		Day 56	
		Median (95% CI)	p-value	Median (95% CI)	p-value	Median (95% CI)	p-value	Median (95% CI)	p-value
P1	ID IPV	45 (36-57)	0.895	3573 (2839-4499)	<0.001	4499 (3573-4499)	0.040	2839 (2839-3573)	0.023
	IM IPV	45 (36-57)		4499 (4499-≥5664)		4499 (3573-4499)		3573 (2839-3573)	
P2	ID IPV	36 (28-45)	0.839	2839 (1791-3573)	<0.001	2839 (2839-3573)	0.006	2839 (2255-2839)	0.024
	IM IPV	36 (28-45)		4499 (4499-≥5664)		4499 (3573-4499)		2839 (2839-3573)	
P3	ID IPV	14 (11-18)	0.745	≥5664 (4499-≥5664)	<0.001	≥5664 (4499-≥5664)	0.035	3573 (3573-4499)	0.384
	IM IPV	14 (11-18)		≥5664 (≥5664-≥5664)		≥5664 (≥5664-≥5664)		3573 (3573-4499)	

Non-Inferiority Assessment

	Day 0 to 7 % Difference (95% CI)	Day 0 to 28 % Difference (95% CI)	Day 0 to 56 % Difference (95% CI)
P1	5.8 (2.2-9.4)	3.2 (-0.02-6.4)	3.6 (0.1-7.1)
P2	5.0 (1.3-8.7)	0.1 (-2.4-2.4)	0.4 (-1.9-2.7)
P3	1.1 (-1.4-3.7)	0.8 (-1.1-2.7)	0.0 (-1.6-1.6)

Safety Assessment

ID or IM IPV	n/N	Any adverse events % (95% CI)	p-value
ID	28/268	10.5 (7.3-14.7)	0.564
IM	19/266	7.1 (4.6-10.9)	

- No serious adverse events or sudden unexpected serious adverse reactions were observed during the study period (at 1hr, 24hr, 48hr, 72hr, 7 days post vaccination)
- There were no significant differences in the number of any adverse events reported by a participant (at 1hr, 24hr, 48hr, 72hr, 7 days post vaccination) between the study groups

Abstract

Background

The current tight supply in global inactivated poliovirus vaccine (IPV) supply is forecast into late 2016. Dose-sparing strategy delivering 1/5 fractional IPV (fIPV) dose administered intradermally (ID) would stretch IPV supply by 5-fold. We assessed the non-inferiority of immune response elicited by ID fIPV compared to intramuscular (IM) full dose IPV.

Methods

We conducted a randomized controlled trial to assess the non-inferiority of boosting dose of ID fIPV compared to IM full dose IPV. The primary outcome was immune response after one and two boosting doses administered 28 days apart, following ID fIPV administration compared to IM IPV. The secondary endpoints were safety assessed by the number and intensity of local and systemic adverse reactions.

Immune response was defined as change from seronegative to seropositive (reciprocal antibody titer of <8 to ≥8) after vaccine administration, or a four-fold or more increase in reciprocal titer for participants with a baseline titer >8. The non-inferiority limit was defined as 10%. The study is registered with the Australian New Zealand Clinical Trials Registry (ANZCTR), trial ID ACTRN=12615000305527.

Results

The study was conducted at 14 health facilities in Cuba between 5 December 2014 and 8 March 2015; 534 healthy adults were randomized to receive boosting dose of IPV through one of 2 study groups: fIPV ID (n=268) or full dose IM (n=266); 504 (94%) participants were included in the per protocol analysis. Non-inferiority in immune response (<10% margin) was demonstrated at day 7 and maintained at day 28 and 56 after 1st and 2nd IPV dose for all poliovirus types.

The non-inferiority limit of ID fIPV compared with IM full dose IPV for immune response 28 days after 1st boosting dose was 3.2 (95% CI -0.02-6.6), 0.1 (95% CI -2.4-2.4), 0.8 (-1.1-2.7); and at day 56, 28 days after 2nd boosting dose was 3.6 (95% CI 0.1-7.1), 0.4 (95% CI -1.9-2,7), 0.0 (-1.6-1.6), for poliovirus types 1, 2 and 3, respectively.

The proportions of participants with immune response for fIPV ID and full dose IM IPV groups 28 days after 1st booster dose were: 239/253 94.8%, (95% CI 91.4-97.0), 250/255 98.0% (95% CI 95.5-99.2); 248/253 98.0% (95% CI 95.5-99.2), 254/259 98.1% (95% CI 95.6-99.2); 248/252 98.0% (95% CI 98.4-99.4), 256/258 99.2% (95% CI 97.2-99.8), for poliovirus types 1, 2 and 3 respectively. No serious adverse events were reported; between 7.1%-10.5% participants reported any adverse event (for ID and IM study groups respectively; p=0.564).

Conclusion

The study demonstrated non-inferiority of fIPV ID compared with full dose IPV IM, to elicit immune response to poliovirus types 1, 2 at day 7, 28 and 56 days after 1st or 2nd boosting doses. Implementing fIPV ID as a dose-sparing strategy in SIA outbreak response can have a critical role in stretching global IPV supply 5-fold.

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Abbreviations

AE	Adverse event
AR	Adverse reaction
CA	Competent authority
CV	Curriculum vitae
cVDPV	Circulating vaccine derived poliovirus
DSMB	Data safety monitoring board
DU	D-antigen units
GCP	Good clinical practice
GPEI	Global polio eradication initiative
GMT	Geometric mean titer
IEC	Independent ethics committee
IC	Informed consent
IMP	Investigational medicinal product
IMPD	Investigational medicinal product dossier
IPV	Inactivated poliomyelitis vaccine
NVI	Netherlands Vaccine Institute, now Bilthoven Biologicals (since January 2011)
OPV	Oral poliovirus vaccine
RIVM	National institute for public health and environment (Dutch: Rijksinstituut voor Volksgezondheid en Milieu)
(S)AE	(Serious) adverse event
SAGE	Strategic Advisory Group of Experts
Salk-IPV	IPV based on wild poliovirus strains
SPC	Summary of product characteristics
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidizing party.
SUSAR	Suspected unexpected serious adverse reaction
VAPP	Vaccine associated paralytic poliomyelitis
VDPV	Vaccine derived poliovirus
WHA	World health assembly
WHO	World Health Organization
WPV	Wild poliovirus

1. Objectives

I. PRIMARY OBJECTIVE

To demonstrate the non-inferiority of boosting immune response against poliovirus types 1, 2 and 3, after administration of one and two intradermal IPV doses compared with one and two intramuscular IPV doses in healthy adults with a history of vaccination with at least one dose of OPV.

SPECIFIC OBJECTIVE

To demonstrate for IPV produced by BBio the non-inferiority of boosting immune response against poliovirus types 1, 2, and 3.

Immune response was defined as proportion of participants with a change from seronegative to seropositive (reciprocal titer of <8 to ⩾8) after vaccine administration), or a four-fold or more increase in reciprocal titer for participants with a baseline reciprocal titer >8, after one or two ID IPV doses, compared to one or two IM IPV doses in healthy adults.

II. SECONDARY OBJECTIVE

To demonstrate the safety of intradermally administered fractional IPV, as assessed by the number and intensity of local and systemic adverse reactions after vaccination.

2. Introduction

I. BACKGROUND

Established in 1988, the GPEI is committed to eradicating poliomyelitis worldwide and has overseen strong progress. Between 1988 and 2014, the number of endemic countries decreased from 125 to 3; wild poliovirus (WPV) cases decreased from 350,000 to 359; the last WPV1 and WPV3 cases were reported in October 1999 and November 2012, respectively [1]. The GPEI's Eradication and Endgame Strategic Plan 2013-2018, outlines the remaining steps towards achieving eradication and entering the post-eradication era. Key to this plan is OPV cessation; this is because OPV contains live attenuated poliovirus which can revert to vaccine derived poliovirus (VDPV) with wild-type characteristics of increased neuro-virulence and transmissibility with potential to circulate (cVDPV) and re-establish transmission [2-4]. Furthermore although extremely rare, OPV can cause vaccine associated paralytic poliomyelitis (VAPP) estimated at 2-4 million cases per birth cohort per year in countries using OPV [5].

Transitioning from OPV to IPV

In 2004 the Advisory Committee on Polio Eradication (ACPE) endorsed the need for OPV cessation and identified inactivated poliovirus vaccine (IPV) as an alternate vaccine option [6]. IPV does not contain live virus and its use addresses the risk of VDPVs and VAPP. IPV is already widely used in industrialised countries however its use in developing countries is largely inhibited by cost. In 2007 the ACPE recommended pursuing strategies to develop affordable IPV for widespread use in developing countries and subsequently it was recommended at the 2008 World Health Assembly (WHA) that WHO take the lead in spearheading this task [7, 8].

The global cessation of OPV will commence with the withdrawal and replacement of OPV2 (in tOPV) with bOPV ("the switch") forecast for April 2016. This strategy was endorsed by the WHA in May 2015 [7]; subsequently all OPV will be withdrawn globally between 2018 and 2019. The rationale for this step-wise process is that 95% of cVDPV outbreaks reported between 2000 and 2015 were due to circulating type 2 VDPV (cVDPV2) [1]. In parallel with tOPV withdrawal and as a risk mitigation strategy to address the risk of type 2 poliovirus re-emergence, the Strategic Advisory Group of Experts (SAGE) recommended in 2012 that all countries using tOPV introduce at least 1 dose of IPV into their routine immunization schedules prior to its withdrawal [9]. Using more immunogenic bOPV (compared to tOPV, for types 1 and 3) will accelerate the path to eradication while the addition of IPV dose will close existing immunity gaps. This strategy is supported by multiple studies demonstrating that administering one IPV dose with OPV boosts immunity to poliovirus types 1 and 3 while closing existing immunity gaps to poliovirus type 2[1] [10].

[1] Clinical trial reports 2015: Chile; India; Bangladesh; Multiple Central and Latin American countries (Panama, Colombia, Dominican Republic, Guatemala)

This led to the 2014 IMB recommendation that IPV be added to selected supplementary immunization activities (SIAs) in high risk areas [11]. In 2015, the 68th World Health Assembly (WHA) adopted resolution 3.4 to: *implement measures to fill such [population immunity] gaps and further boost population immunity through timely and complete routine immunization and, high-quality supplementary immunization activities [7]*. Consequently the GPEI will conduct multiple SIAs with the addition of IPV in high risk areas, and it is forecast that 5M doses of IPV will be required for SIAs between April and December 2016[2].

Global shortage of IPV

The increased demand for IPV for introduction into routine schedule prior to the switch, the need to maintain IPV supply in countries which have introduced IPV, the targeted use of IPV in high-risk areas during SIAs as well as the need to maintain an IPV supply for SIAs in the event of an outbreak after the switch has placed a significant demand on global IPV. There are currently only 2 IPV manufacturers supplying the global market and there already exists a significant deficit in IPV vaccine commitment; this combined with increased IPV demand, will result in extremely tight IPV supply until 2017 [6].

II. RATIONALE for LICENSURE of ID fIPV use as BOOSTER DOSE

Intradermal (ID) IPV administration delivers 1/5 vaccine dose (0.5ml) compared to conventional full intramuscular (IM) dose and its use could therefore potentially stretch IPV supply five-fold. Consequently in the quest to stretch IPV supply through dose (antigen)-sparing strategies the WHO is assessing ID fractional dosing (fIPV dose) administration. Specifically, in an outbreak setting, the use of ID fIPV as a strategy could significantly increase the number of children reached during SIAs.

Several studies have demonstrated the immune response (seroconversion and boosting) as well as priming response following ID fIPV administration [12-14]. In the event of an outbreak, IPV would be introduced as a booster dose, therefore studies assessing the immunogenicity of fIPV administered ID as a booster dose in addition to the multiple OPV doses given as part of a routine schedule are critical in order to obtain regulatory approval for ID fIPV use as booster dose.

In 2012, the WHO and the Nationale de Securite du Medicament et des Produits de Sante (ANSM), France, and the Federal Agency for Medicinal and Health Products (FAGG), Belgium, met to discuss IPV use in the endgame strategy. After further discussions with Belgian and Dutch National Regulatory Agencies (NRAs) in 2013, it was proposed that WHO conduct a non-inferiority trial of ID fIPV dose in adults with a history of OPV vaccination. If non-inferiority to full IM IPV dose were demonstrated, this would provide the scientific evidence for the label change for ID fIPV use as booster dose. The submission of the variation for label change for ID fIPV administration however remains the responsibility of BBio, the marketing authorization holder for the IPV vaccine used in the clinical trials. Once approval for label change for ID fIPV use as booster dose occurs in the Netherlands the country of manufacture, this will pave the way for subsequent WHO PQ.

[2] Internal communication WHO, HQ, Geneva

3. Study administration

I. STUDY PERSONNEL

National collaborators

Function	Name
Principal Investigator	Dr. Sonia Resik Pedro Kourí Tropical Medicine Institute, Havana, Cuba
Location of Study Sites/Health centers	Este, Norte, Florida Sur, Vertientes, Centro, Finlay, Nuevitas, Minas, Mella, Previsora, Oeste, Pirre, Agramonte, Florida Norte, Camaguey Province, Cuba
Study Coordination	Pedro Kourí Tropical Medicine Institute, Havana, Cuba Provincial Health Office, Camaguey Province, Cuba
Laboratory Processing	Pedro Kourí Tropical Medicine Institute, Havana, Cuba
Study Sponsor	World Health Organization Geneva, Switzerland

International collaborators

Function	Name
Technical assistance and Funding Support	World Health Organization, Geneva, Switzerland
Statistician	Natalie Molodecky, Imperial College, London, UK
Vaccine supplier	Bilthoven Biologicals (BBio) The Netherlands

4. Research Ethics

I. ETHICAL CONSIDERATIONS

Ethical approval - International

The study protocol was submitted for review by the World Health Organization's (WHO) Ethical Review Committee (ERC) on 29 November 2013, with approval granted on 7 April 2014, in Geneva, Switzerland. The clinical study was conducted in accordance with the guidelines for Good Clinical Practice (GCP) as described by the Committee for Proprietary Medical Products (CPMP) of the European Union and the International Committee on Harmonisation (ICH) in Note for Guidance on Good Clinical Practice, document CPMP/ICH/135/95, effective since 17 January 1997, Clinical Trial Directive 2001/20/EC. The study was also conducted according to the principles of the Declaration of Helsinki (dated 22 October 2008).

Ethical approval - National

The study was approved by the Independent Ethics Committee (IEC) and conducted within Cuban national legal and regulatory requirements. The study protocol was reviewed and approved at the national level by the Center for State Control of Drug Quality (CECMED) and the Pedro Kouri Tropical Medicine Institute Ethics Committee; and at provincial level, by the Provincial Ethics Committee, at the Provincial Health Office in Camaguey Province.

The organizational level of ethical approval and the date of approval are presented in **Table 4.1.**

Table 4.1 National Ethical approval and dates

Organization	Date of approval
Center for the state control of drug quality (CECMED)	28 November 2014
Pedro Kourí Tropical Medicine Institute Ethics Committee	14 January 2014
Provincial Health Office in Camaguey Province Ethics Committee	20 November 2014

II. TRIAL REGISTRATION

The study is registered with the Australian New Zealand Clinical Trials Registry (ANZCTR), trial ID ACTRN=12615000305527.

III. PARTICIPANT INFORMED CONSENT

The trial was conducted by the Provincial Health Office in Camaguey Province, Cuba, which is an organization accredited by the Cuban National Regulatory Authority (CECMED) to conduct vaccine related clinical trials. The study enrolled university students who lived in the urban areas of Camaguey Province who were recruited by the provincial health clinic through an advertisement and internal site database. Before obtaining informed consent, the investigator provided potential participants sufficient time and opportunity to enquire about

details of the trial, in order to decide whether or not to participate. Prior to study participation the participant signed and dated the informed consent form, the original of which was retained by the investigator and a copy made for the participant's record.

IV. CONFIDENTIALITY

Personal identifiers were not recorded on study materials and a unique participant number was used to label all study materials. All collected information associated with the study protocol was kept under locked storage with the key/code accessible only to the investigator. The handling of personal data complied with International Conference on Harmonization (ICH) guidelines on the protection of personal data. Personal data were recorded by the investigator or a designated representative using checklists, or by the participant in diaries. Study findings stored on a computer were stored in accordance with local data protection laws and study materials were legally deemed to be the property of the sponsor of the trial. Essential documents and source documents will be retained at the study site for 15 years after the completion date of the last participant in accordance with GCP.

V. BENEFITS and POTENTIAL RISKS of PARTICIPATION in STUDY

The benefits of the study were to:

- Provide data on immune response[3] to polioviruses
    - Seroconversion from seronegative to seropostive, in seronegative participants
    - Boosting of antibody titers, in sero-negative participants
- Inform public health policy-makers on the adoption of fractional IPV dose as a potential option, to increase the availability and affordability of IPV in resource limited settings, so as to facilitate the wide-spread scalable use of IPV, in Cuba, as well as globally

The expected risks associated with study participation were expected to be low. IPV has been used extensively since its licensure in the USA in 1955; an enhanced potency IPV formulation developed in the 1980s, is still used today, with approximately 80 million doses administered annually, primarily in industrialized countries. IPV has a well characterized safety profile, and reported adverse events (AE) include local erythema, induration and tenderness; therefore the local and systemic AE associated with IPV administration in this study were expected to be mild and of short duration [5]. The potential risks of venepuncture such as mild pain, haematoma were also considered to be low.

VI. COMPENSATION for INJURY

In line with previous clinical trials conducted in Cuba, the Government of Cuba declined assumed liability for the trial. The Government of Cuba provides free health care which covers any illness or adverse health condition, including any AE related to clinical trials.

[3] See Section 5: Trial design and methodology

VII. AMENDMENTS

Amendments were defined as any changes made to the study protocol after an opinion was sought and approval granted by the accredited Independent Ethics Committee (IEC). A "substantial amendment" was defined as an amendment to the terms of the IEC application, or to the protocol, or any other supporting documentation, which was likely to affect to a significant degree, any of the following:

- The safety or physical or mental integrity of the participants of the trial
- The scientific value of the trial
- The conduct or management of the trial
- The quality or safety of any intervention used in the trial.

Substantial amendments were required to be approved by the IEC and the competent authority. Although non-substantial amendments were not required to be notified to the accredited IEC and the competent authority, any non-substantial amendments were nevertheless recorded and filed by the sponsor.

The history of amendments to the protocol, and dates of ERC approval or notification are:

- ERC approval, 7 April 2014: Protocol version 2.3 updated to incorporate ERC comments
- ERC notified in memo, 28 July 2014: Protocol version 3.0 updated to incorporate 2nd IPV dose at day 28 and blood collection at day 56
- ERC approval, 16 July 2014: Protocol version 4.0 updated to remove GSK IPV vaccine

Evidence of WHO ERC approval for amendments (protocol Versions 2.3 and 4.0) are attached in **Annexe 3**.

VIII. END of STUDY REPORT

The investigator notified the accredited IEC of the end of the study within a period of 8 weeks, with the end of the study defined as the last participant's last visit. In the case of premature termination of the study, the investigator notified the accredited IEC within 15 days of study termination and provided reasons for the premature termination. Within one year after the end of the study, the final study report including results and publications/abstracts will be submitted by the investigator/sponsor to the accredited IEC.

5. Trial design and methodology

I. TRIAL DESIGN and CALENDAR

This was a two-arm randomized controlled open label interventional study. Healthy male adults aged 18-30 years with a vaccination history of at least one OPV dose during childhood administered before 12 years of age, were recruited and randomized to one of two intervention groups: one group received two doses of ID fIPV (1/5 dose); the other group received two doses of IM IPV (full dose). Vaccines were administered at days 0 and 28. The total study duration was 10 months (including recruitment, screening, follow up, data analysis and completion of the clinical trial report).

Table 5.1: Trial design

Event	Date
Recruitment, screening, enrolment	5 December 2014
Last participant recruited for study	8 January 2015
Immunogenicity and safety analysis	17 September 2015
Report	7 October 2015
Total duration	10 months

Table 5.2: Trial calendar

Study Arm	**Control group** **Full dose IPV** **IM**	**Intervention group** **1/5 dose IPV (fIPV)** **ID**
Timing	**Samples collected** **Vaccines Administered**	
Day 0	Blood IM IPV	Blood ID fIPV
Day 7	Blood	Blood
Day 28 (+/-3)	Blood IM IPV	Blood ID fIPV
Day 56 (+/-3)	Blood	Blood

a) Trial endpoints

The primary endpoint was immune response defined as the proportion (%) of participants with a change from seronegative (reciprocal titer <1:8 dilution) to seropositive (seroconversion) after vaccine administration; or, an increase of ≥four times in reciprocal titer for seropositive participants (participants with baseline reciprocal titer >1:8) (boosting) at day 28 after administration of single vaccine dose, and day 56 after administration of two vaccine doses.

The secondary endpoint was safety, as assessed by the number and severity of local and systemic adverse reactions. A diary listing solicited AE was given to each participant to record any AE in the first four days following vaccination[4]. The size of injection site reaction was measured using the provided measuring device[4]

b) Trial procedures

The study required four visits to the health center. On days 0, 7, 28, and 56, 2ml of blood were collected from the participant; on days 0 and 28 blood was collected after which the study vaccine (fIPV or full dose IPV) was administered. Regarding the third and fourth visits, blood was taken within a window period of three days before or after day 28 (day 25-31) or day 56 (day 53-59). On day 56 after study completion the participant received a small gift as an appreciation of their participation.

c) Choice of control group

The control group was the full dose IM IPV study group.

II. STUDY SITES/HEALTH CENTERS

There were 14 participating health centers in Camaguey Province: Este, Norte, Nuevitas, Florida Sur, Vertientes, Centro, Finlay, Minas, Mella, Prevsora, Oeste, Pirre, Agramonte and Florida Norte.

III. MONITORING and AUDITING

The study was monitored by the principal investigator and sponsor WHO Headquarters, Geneva. The Data Safety and Monitoring Board (DSMB)[4] monitored all AE and data quality. Training was provided at the study site prior to study implementation to clarify staff on their roles, study procedures including ongoing monitoring and logistics. The principal investigator was in regular contact with the study site investigators at the health centers in order to assess the progress of the study through regular on-site monitoring visits as well as regular phone contact.

The study monitor visited the study sites and the laboratory on three occasions throughout the study; and regularly discussed progress with the study site investigators as well as with the principal investigator who set aside suitable time for the monitoring visits. During each visit, the study monitor reviewed all case report forms[5] (CRF) with regard to completeness, thoroughness and compliance with the study protocol; the original participant data were reviewed to ensure informed consent had taken place and that inclusion/exclusion criteria had been followed; data were matched with the original questionnaires; completeness and accuracy of clinical history including concomitant medication and laboratory findings were validated; it was checked and confirmed that correct quantity and dosing vaccine schedule had been administered, and that all relevant information including AEs had been recorded appropriately.

The study monitor also ensured that the investigational products were stored correctly and that inventories were accounted for. Lastly the study monitor ensured that collected samples were handled, stored, shipped as per protocol following standard operating procedures. During each monitoring visit, the study monitor submitted any incomplete or incorrect CRFs to the study site investigator for clarification and correction.

[4] See section 7: Adverse events
[5] See Section 5: Trial design and methodology

BBio and the relevant regulatory authorities had the jurisdiction to undertake an audit of the study clinic at any stage of the study in order to review data handling and quality and to determine if the performance of the study complied with study protocols, SOPs and other relevant guidelines. In the event that an audit was requested by a regulatory authority, BBio immediately informed the principal and study investigators.

IV. TRIAL POPULATION

The study population were healthy adult males, aged 18-30 years.

a) Inclusion criteria

Participants fulfilled all of the following criteria:

- Males aged 18 to 30 inclusive, at the time of enrolment
- Were in good health as determined by their medical history, physical examination and the clinical judgment of the investigator
- Received polio vaccination(s), with at least one OPV dose, according to the Cuban National Immunization Program as a child
- Gave verbal and written informed consent

b) Exclusion criteria

The participant was temporarily excluded from vaccination if their body temperature was ≥ 38.5°C. In this case trial participation and vaccination were postponed.

Participants fulfilling one or more of the following criteria were excluded from participating in the trial:

- IPV or OPV booster dose after the age of 12 years
- Known or suspected exposure to wild poliovirus
- Known or suspected allergy against any of the vaccine components
- History of unusual or severe reactions to any previous vaccination
- Known or suspected disease or use of medication that could influence the immune system
- Known or suspected immune deficiency, and/or known HIV infection
- Systemic treatment with corticosteroids or immunosuppressants within one month before screening or during the study
- Administration of plasma (including immunoglobulins) or blood products three months prior or during the study
- Blood donation within one month before screening
- Any vaccination within three months before screening and during the study until the last visit
- History of any neurological disorder including epilepsy or febrile seizures
- Evidence of excessive alcohol or drug use
- Any infectious disease
- Participation in another clinical trial within three months before enrolment
- History of bleeding disorders or anticoagulant therapy

c) Withdrawal criteria

Participants were able to leave the study at any time for any reason if they wished to do so without any repercussions. The principal clinical investigator had the jurisdiction to withdraw participants from the study in the event of:

- Illness or diseases that occurred during the trial which could influence the trial results. In this instance, cases were reviewed individually by the principal clinical investigator
- Participant was lost to follow-up (3 failed attempts to contact on different days)
- Protocol violations (in consultation with the sponsor)

d) Replacement of participants after withdrawal, and loss to follow up

Participants were included until 300 eligible participants meeting essential criteria completed the study, were enrolled per group, and any participants who withdrew before study completion were replaced. Participants were treated as lost to follow up when three attempts to contact them on three different days had failed.

e) Premature termination of the study

The study would be terminated in the event that:

- The sponsor was aware of new data regarding the safety or efficacy of the study vaccine. In this circumstance the IEC would be informed of the decision
- The investigator was not able to conduct the trial at the study site
- Repeated unacceptable protocol violations occurred

Furthermore, in accordance with national regulations the investigator would inform the participants and the reviewing accredited IEC, should it be apparent that the disadvantage of participation was significantly greater than was foreseen in the research proposal. In this scenario the study would be suspended pending further review by the accredited IEC and the regulatory authorities except insofar as suspension would jeopardize the participants' health. In this case, the investigator would inform the participants.

f) Prior and concomitant therapy

Any additional treatment(s) taken by the participants on entry to the study or at any time during the study were regarded as concomitant treatments and documented. Any changes to the dosage or cessation of concomitant treatment(s) were documented. Concomitant medication(s) were kept to a minimum during the study. However, if considered necessary for the participant's welfare and unlikely to interfere with the study medication, they were given at the discretion of the investigator. The following concomitant treatments were not permitted until after visit 4 of the study:

- Any treatment influencing the immune system, except local or inhaled corticosteroids
- Plasma products

g) Treatment compliance

The only compliance required was the timely return for all scheduled visits and study completion.

V. RANDOMIZATION, TREATMENT ALLOCATION and BLINDING

Participants fulfilling the parameters for enrolment were examined by a physician to determine eligibility for the study. The randomization list containing participant numbers and their corresponding vaccination groups was produced by the biostatistician and distributed to the health centers. Eligible participants drew a sealed

envelope from those available just prior to the administration of the vaccine; the envelope contained their assigned participant number which in turn corresponded to a vaccination group on the randomization list. Participants were randomly assigned in a 1:1 ratio to one of the two study arms to receive either full IM or fIPV ID vaccination.

The biostatistician prepared the study randomization list independently of the study investigators. During the study period there was no contact between the biostatistician and the study site investigators.

VI. TRIAL PLAN: VISIT PROCEDURES

a) Day 0 (Visit 1): 1st vaccination visit

The purpose of visit 1 was to:

- Explain purpose of the study and provide the information sheet
- Answer any questions regarding the study
- Check inclusion and exclusion criteria
- Obtain informed consent
- Record medical history and any concomitant medication
- Assign participant number and randomization to study group
- Record demographic data
- Measure oral body temperature, blood pressure and heart rate
- Perform a general physical exam
- Collect 2ml blood to test for polio immunogenicity
- Administer vaccine (1st)
- Record treatment number in CRF
- Observe participants for 30 minutes immediately after vaccination
- Record AE
- Hand out participant diary and measuring device to measure size of local site reaction
- Inform participant that telephone contact would be made 24 hours after vaccination

In addition, 4-6 hours after vaccination, participants were asked to:

- Measure their oral body temperature and record the result in the provided participant diary
- Inspect and measure any local reaction (redness, swelling, ecchymosis and induration) using the provided measuring device and record the result in the provided diary
- Record any other (systemic) AE in participant diary

Contact was made 24 hours after vaccination on Days 1 and 3, to:

- Assess and discuss any local and systemic AE
- Complete the questionnaire
- Record any concomitant medication usage
- Answer any further questions related to the study

b) Day 7 (Visit 2): Follow up visit

The purpose of visit 2 was to:

- Collect 2ml blood to test for polio immunogenicity
- Record any concomitant medication
- Measure oral temperature, heart rate and blood pressure
- Perform a general physical exam
- Record any AE
- Follow-up on any previous AE

c) Day 28 (Visit 3): Follow up + 2nd vaccination visit

- Collect 2ml blood for polio immunogenicity
- Record concomitant medication
- Measure oral temperature, heart rate and blood pressure
- Perform physical exam
- Record AE
- Follow-up on any previous AE
- Collect participant diary and check completeness
- Collect measuring device
- Administer vaccine

In addition, 4-6 hours after vaccination, participants were asked to:

- Measure their oral body temperature and record the result in the provided participant diary
- Inspect and measure any local reaction (redness, swelling, ecchymosis and induration) using the provided measuring device and record the result in the provided participant diary

d) Day 56 (Visit 4): Follow up visit

- Collect 2ml blood to test for polio immunogenicity
- Record any concomitant medication
- Measure oral temperature, heart rate and blood pressure
- Perform physical exam
- Record AE
- Follow-up on AE
- Collect participant diary and check completeness
- Collect measuring device
- Provide small gift to appreciate participation (<USD10 value)

Table 5.3. Visit schedule

Timing (days) Procedure	Day 0 Visit 1	Day 7 (4-10) Visit 2	Day 28 (25-31) Visit 3	Day 56 (53-59) Visit 4
	Blood Vaccination	Blood	Blood Vaccination	Blood
Study information				
Informed consent				
Check of in-/ exclusion criteria				
Recording demography				
Recording medical history				
Vital signs				
Physical exam				
Check co-medication				
Randomization				
Blood sampling: polio immunogenicity				
Recording oral body temperature				
Vaccination				
Recording adverse events				
Recording concomitant medication				
Adverse events evaluation	0-7 days after Visit 1		0-7 days after Visit 3	
Serious Adverse Events assessment				(up to 6 months)

VII. PROCEDURE for OBTAINING and HANDLING SAMPLES

a) Methods of measurement

The primary endpoint for the study was immune responses to one and two doses of IM or ID IPV. All sera were assayed for virus neutralizing antibody against all three poliovirus serotypes.

Two ml of blood were collected during all visits, 1, 3 (vaccination visit) and 2, 4 (follow-up visits). Pre- and post-vaccination sera were processed simultaneously.

Immune response rate was defined as the proportion of participants with a change from seronegative (reciprocal titer ≤1:8) to seropositive after vaccine administration or an increase of four times or more in reciprocal titers for participants with baseline reciprocal titers ≥1:8. For example, if a participant with

reciprocal titres of 1:16 before vaccination had reciprocal titres of 1:64 or higher after vaccination, they were considered to have immune response.

All serological poliovirus neutralization assays were conducted using validated and standardized micro-neutralization assay tests at the Pedro Kouri Tropical medicine Institute (IPK), national polio laboratory network, Cuba and were tested at doubling titration of 1:8 up to 1:4096.

b) Blinding and randomization of sample testing

Given the different routes of vaccine delivery, it was not possible to blind the health workers administering the vaccine or the participant to the study group assigned. However the laboratory personnel were blinded to the study group from whence the blood samples came.

VIII. CASE REPORT FORM, DATA MANAGEMENT and QUALITY ASSURANCE

a) Case report form (CRF)

All data obtained during the study were promptly recorded on the data collection tool, the paper-based CRF. All source documents from which CRF entries were derived were placed in the participant's personal records, and participant medical records were handled confidentially at all times to ensure anonymity.

b) Data management

The principal investigator was responsible for all data management and analysis. A data review and handling document was developed prior to the initiation of the clinical study which included specifications for consistency and plausibility checks on data, and data-handling rules for data errors. Any unresolved queries were sent to the study site investigators for resolution, and all corrections were counter-signed. The study data base was updated accordingly and regularly with this input.

c) Quality assurance

The original CRF entries for each patient were verified against source documents by the study monitor and any missing, uninterpretable or non-aligned data were discussed with the study investigator for timely resolution. During the clinical study, the study monitor visited the study center on three occasions, to review protocol compliance, compare CRFs to participant's personal records, assess investigational medicinal product accountability, and to ensure that the clinical study was being conducted according to required regulatory standards; the study monitor also validated all CRF for completeness and consistency with source documents, in compliance with GCP and other required regulations.

Furthermore, competent authorities such as the Institutional Review Boards (IRBs), IECs, independent auditors, and a representative from the sponsor's Clinical Quality Assurance Group, had the jurisdiction to carry out source data inspections on-site, and audits as requested. These reviews could occur at any time during the course of the study. Direct access to source data were provided for these inspections and audits with due consideration given for data protection and patient confidentiality. The investigator ensured that any required support was provided to the sponsor as necessary at all times.

The study monitor verified the following:

- The study facility and procedures undertaken at the site
- Presence of documented approval of the study by the IEC

- Adherence to the study protocol
- Presence of signed and dated informed consent forms
- Accuracy and completeness of trial data
- Adherence to the study schedule
- Presence of up-to-date database

IX. SUPPLY of MATERIALS

All materials required for the collection of samples and data including questionnaires, training materials, protocols, tally sheets and CRFs were provided prior to study initiation. Comprehensive training on the study protocols was provided to all study staff prior to study implementation.

6. Products

I. DESCRIPTION and COMPOSITION of INVESTIGATIONAL PRODUCTS

IPV was supplied in 3ml glass vials with a rubber septum, sealed with an aluminium capsule. Each vial contained a single dose of 0.5ml vaccine and was packaged in an outer box. Opened vials were used within 15 minutes, and were not stored for later use. Participants received 0.5ml IM or 0.1ml ID of the investigational vaccine. The formulation was detailed on the vaccine vial label:

Label on vial:

Protocol No. BB-256A

Participant no.: **XXX**

Site : 01

Inv : S Resik

Suspension for intramuscular/intradermal injection

Shake vial gently before use

Dose : 0.5 ml/0.1ml

Expiry date : dd-mm-yyyy

Storage condition : 2-8°C

For clinical trial use only

Bilthoven Biologicals, Bilthoven, the Netherlands

Label on outer packaging:

Protocol No. BB-256A

Investigational vaccine

Participant no: **xx**

For clinical trial use only

Site : 01

Inv : S. Resik

Expiration date : dd-mm-yyyy

Storage condition : 2-8°C

Content : 1 single dose vial

Suspension for intramuscular/intradermal injection

Shake vial gently before use

Bilthoven Biologicals

A. van Leeuwenhoeklaan 11,

Bilthoven, The Netherlands

Tel: + 31 30 2742755

II. PRODUCT ADMINISTRATION

The vaccine was administered in the left deltoid for both study groups using needle and syringe for IM full dose IPV, and tuberculin (BCG) syringe and needle for ID fIPV.

III. STORAGE of INVESTIGATIONAL PRODUCT

The study product was distributed to the investigator by the sponsor through an authorized pharmacist maintaining the cold chain to ensure vaccine storage at +2 to +8°C. The study product was transported in cold boxes with continuous temperature monitoring to ensure the cold chain was maintained. The investigator was responsible for correct storage of the vaccine in a cool dark storage space at a temperature of +2 to +8°C, and the temperature was monitored daily. Non-usable products such as expired or damaged vials, frozen vaccines, vaccines stored in temperatures ≥+8°C were either returned to the BBio or destroyed, and if necessary were replaced.

IV. SHIPPING CONDITIONS and ACCOUNTABILITY of INVESTIGATIONAL PRODUCT

Each vaccine shipment was accompanied by acknowledgement of receipt specifying quantity, expiry date and batch number of the transported vaccine. The form was dated and signed by the person shipping the vaccines as well as the person receiving the product. The investigator was responsible for vaccine accountability on-site. The investigator had to provide a written explanation in the case of discrepancies between delivered and used and un-used vaccines at the end of the study. At the end of the study, all unused vaccines were handled in accordance with the guidelines of BBio.

7. Adverse events

I. TYPES of ADVERSE EVENTS (AE)

An adverse event was defined as any undesirable experience occurring to a participant during the study, whether or not considered related to the investigational product or the experimental treatment. AE were classified into the following categories:

a) Adverse events following immunization (AEFI)

This was defined as any event that is either one of the following:

- Vaccine reaction – an event caused or precipitated by the vaccine when given correctly, or caused by inherent properties of the vaccine
- Injection reaction – an event from anxiety about, or pain from the injection itself rather than the vaccine
- Executional error – an event caused by an error in vaccine preparation, handling or administration
- Coincidental event – an event that occurs after immunization but is not caused by the vaccine i.e. a chance association

b) Serious adverse events (SAE)

This was defined as any untoward medical occurrence or effect that at any dose resulted in:

- Death
- Life threatening outcome (at the time of the event)
- Hospitalization or prolongation of existing participant's inpatient hospitalization
- Persistent or significant disability or incapacity
- A new event which occurred during the duration of the trial, which was likely to affect the safety of participants, including: unexpected outcome of an adverse reaction, lack of efficacy of an investigational medicinal product (IMP) used for the treatment of a life threatening disease, and major safety finding from a newly completed animal study

All SAEs were reported to the sponsor as soon as possible and within 24 hours of the knowledge of the event, by e-mail, fax or phone. Details on the SAE were recorded on SAE forms and scanned and emailed or faxed to the sponsor. Follow-up information was reported as soon as it became available. SAEs were reported in a line-list format bi-annually to the accredited IEC and regulatory authority that approved the protocol, as per the ICH GCP.

c) Suspected unexpected serious adverse events (SUSAR)

While adverse reactions were defined as all untoward and unintended responses to an investigational product related to any dose administered, unexpected adverse reactions were defined as adverse reactions, of which the nature, or severity, were not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorized medicinal

product). Prior to reporting the SUSAR the randomization code was broken by the sponsor, if not already done so by the investigator[6].

All SUSARs were reported to the accredited IEC and competent regulatory authority that approved the protocol within 15 days after the sponsor first had knowledge of the event. SUSARs resulting in death or life threatening outcomes were reported in an expedited manner within 7 days after the responsible investigator first had knowledge of the adverse reaction, in a preliminary report which was completed within a further 8 days. All SUSARs were recorded in an overview line-list which was submitted bi-annually to the IEC/competent regulatory authority accompanied by a brief report highlighting the main points of concern, to provide an overview of all SUSARs arising from the study medicine.

The sponsor reported in an expedited manner to the IEC/competent regulatory authority the following:

- SUSARs arising during the clinical trial which were assessed by the IEC/competent regulatory authority
- SUSARs arising in other clinical trials of the same sponsor and with the same medicinal product which could have consequences for the safety of the participants involved in the clinical trial which were assessed by the IEC/competent authority

The sponsor reported in an expedited manner all SUSARs to the competent regulatory authorities in other Member States according to the requirements of the Member States. The expedited reporting occurred within 15 days after the sponsor first gained knowledge of the adverse reaction. For any fatal or life threatening cases, the timeframe was reduced to maximal 7 days for a preliminary report with another 8 days for completion of the report.

II. RECORDING ADVERSE EVENTS and SERIOUS ADVERSE EVENTS

A diary was given to participants to record AE in the first 4 days after vaccination. The size of the injection site reaction was measured using a supplied measuring device (Figure 7.1). The diary contained a list of solicited AE as listed in Table 6.2. The participant completed the diary 1hr, 24hr, 48hr, 72hr and 7 days post-vaccination, or until AE had resolved, or until follow-up was deemed unnecessary by the investigator. Participants were asked to contact the investigator in the case of any possible SAE until 6 months after vaccination. A follow-up phone call will be made by the investigator and any SAEs reported until 6 months after vaccination.

Figure 7.1. Measuring device used to measure size of a possible vaccination site reaction

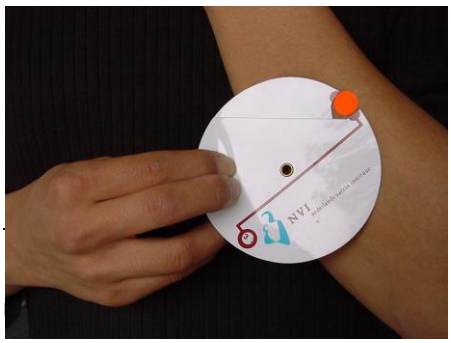


Table 7.1. Categories of severity of AE after vaccination

Local vaccination site reactions		
Pain	Mild	pain only when injection site is touched or pressed (mild pain)
	moderate	pain with movement of arm/leg (moderate pain)
	Severe	pain at rest (severe pain)
Impaired movement of injected arm/leg	Mild	bit less than normal
	moderate	much less than normal
	Severe	hardly, or not at all
Redness/erythema (greatest diameter)	Mild	> 5-25 mm
	moderate	≥ 25-50 mm
	Severe	> 50 mm
Swelling (greatest diameter)	Mild	> 5-25 mm
	moderate	≥ 25-50 mm
	Severe	> 50 mm
Induration (greatest diameter)	Mild	> 5 25 mm
	moderate	≥ 25-50 mm
	Severe	> 50 mm
Systemic reactions:		
Fever (absolute temperature is measured)	Mild	38.0-38.4 °C
	moderate	38.5-39.4 °C
	Severe	≥ 39.5
Headache	Mild	easy to bear, no interference with activity
	moderate	some interference with activity
	Severe	prevents daily activity
Fatigue	Mild	easy to bear, no interference with activity
	moderate	some interference with activity
	Severe	prevents daily activity
Myalgia	Mild	easy to bear, no interference with activity
	moderate	some interference with activity
	Severe	prevents daily activity
Other AE	Mild	easy to bear, no interference with activity
	moderate	some interference with activity
	Severe	prevents daily activity

Table 7.2. Solicited AEs as addressed in the AE diary

Local reactions at injection site	
Pain	If present indicate intensity: Mild-severe*
impaired movement of injected arm/leg	If present indicate intensity: Mild-severe*
Redness/erythema (greatest diameter)	Greatest diameter
Swelling (greatest diameter)	Greatest diameter
Solid disk underneath skin (Induration) (greatest diameter)	Greatest diameter
General/systemic adverse events	
Fever (absolute temperature is measured)	If present indicate intensity: Mild-severe*
Headache	If present indicate intensity: Mild-severe*
Fatigue	If present indicate intensity: Mild-severe*
Muscle pain (myalgia)	If present indicate intensity: Mild-severe*
Other AE	If present indicate nature and intensity: Mild-severe*

*The classification of AE was provided in the diary

III. DATA SAFETY and MONITORING BOARD (DSMB)

The need for a DSMB was assessed according to the European Medicines Authority (EMA) guidelines on data monitoring committees and it was determined that the DSMB of WHO would monitor the trial: EMEA/CHMP/EWP/5872/03 d.d. 27 July 2005, http://www.emea.europa.eu/pdfs/human/ewp/587203en.pdf.

The participants were followed up for four weeks after each vaccination. AE that occurred during the study which had not resolved were followed until they abated, or until a stable situation was reached. In addition Data on SAEs were collected up to 6 months after the last vaccination. Given the trial population consisted of healthy adult volunteers and IPV was a well-studied vaccine, there were no special considerations or concerns which required additional follow-up.

IV. REPORTING ADVERSE EVENTS

All AE were reported by the participant or observed and reported by the investigator or the investigator's staff. The relationship between the adverse event and vaccination was classified into the following categories:

- Very likely/certain - a clinical event with a plausible time relationship to vaccine administration and which cannot be explained by concurrent disease or other drugs or chemicals
- Probable - a clinical event with a reasonable time relationship to vaccine administration; is unlikely to be attributed to concurrent disease or other drugs or chemicals
- Possible – a clinical event with a reasonable time relationship to vaccine administration, but which could also be explained by concurrent disease or other drugs or chemicals

- Unlikely – a clinical event whose time relationship to vaccine administration makes a causal connection improbable, but which could be plausibly explained by underlying disease or other drugs or chemicals
- Unrelated – a clinical event with an incompatible time relationship and which could be explained by underlying disease or other drugs or chemicals
- Unclassifiable – a clinical event with insufficient information to permit assessment and identification of the cause

V. MANAGEMENT of ADVERSE EVENTS

All AE were managed until they were stable or resolved. Depending on the event as needed, management could involve additional tests, medical procedures and referral to the appropriate physician or medical specialist.

8. Statistical methods and data analyses

I. SAMPLE SIZE DETERMINATION

Taking into consideration the primary objective of the study, the sample size calculation was based on the NRAs' requirements for acceptable data, with the following assumptions:

- alpha 5% (two-sided)
- power of 90%
- expected boosting immune response of at least 85%, after one dose of IM IPV or ID fIPV
- 10% non-inferiority limit

This yielded a sample size of 269 for each group. Assuming a dropout rate < 11% (31 participants), the total target sample size required was 600 participants (300 per study group).

II. PARAMETERS MEASURED

Sera were collected and tested for neutralizing antibodies at days 7, 28 and 56, for both study groups. Parameters for immune response and median antibody titers were measured.

- **Seroconversion** in seronegative participants: change in neutralizing antibodies from seronegative to seropositive (i.e. from reciprocal antibody titers of <8 to ≥8)
- **Boosting** in seropositive participants: 4 fold increase of neutralizing antibodies

III. EVALUATION CRITERIA

Participants who met the study requirements including attending all study visits and providing sufficient quantities of sera, were included in the analysis (per protocol).

IV. STATISTICAL METHODS

All analyses were undertaken using statistical application "R 3.1.2" [15].

a. Descriptive statistics

Demographic and baseline seroprevalence were analysed by study group and poliovirus serotype.

b. Seroconversion and immune response rates, and median antibody titers

The proportion of participants who seroconverted, who had immune response, and the median antibody titers, were analysed by day, study group and poliovirus serotype. Differences in seroconversion rates between the study groups were tested using Fisher's exact test; for immune response, Chi-squared test was used. For median antibody titers p-values were calculated using Wilcoxon rank-sum test with 95% confidence interval (CI) calculated using bootstrapping with 10,000 replications.

c. Non-inferiority of immune response

Non-interiority of immune response rates were assessed by day, study group and poliovirus serotype; the non-inferiority limit was defined as the difference in proportion with immune response between IM-ID study groups <10%.

d. Reverse cumulative distribution (RCD) curves

RCD curves summarising antibody distribution by day, study group and poliovirus serotype were plotted.

9. Results:

Study population, baseline seroprevalence, immune response

I. STUDY POPULATION

The flowchart of the study participants showing numbers excluded, enrolled and lost to follow up by study groups, is presented in **Figure 9.1**.

a) Participants screened for eligibility, enrolled and randomized

Among 676 adults 576 met the eligibility criteria, among which 534/576 (79.0%) were enrolled in the study; 268/534 and 266/534 were randomized to the ID and IM study groups respectively. Overall 30/534 (6%) participants dropped out of the study, with a total of 504/534 (94%) completing the study.

N=504 (ID group=268, IM group=266)

b) Drop outs and per protocol (PP) participants

After randomization in the ID study group, 261/268 participants met study requirements at day 7; 256/268 at day 28 and 253/268 at day 56. In the IM study group, 254/266 met study requirements at day 7; 259/266 at day 28 and 251/266 at day 56. The list of participants who dropped out (including reasons for dropping out), and numbers enrolled and randomized are presented in **Tables 9.1a and 9.1b.** Participants who completed the study including those who provided sufficient quantity of serum for analysis, by study site and study visit are presented in **Tables 9.2a and 9.2b.** The number of participants with complete data and the number of participants who provided sufficient quantity of blood for analysis by study group and study visit is presented in **Tables 9.2a and 9.2b.**

Table 9.1a. Participants who dropped-out of the study and reasons for dropping out, by study site and study group

Study site	Drop out	ID	Study group	Reason
Mella	6	147	IM	Withdrew consent
		468	IM	Withdrew consent
		20	IM	Withdrew consent
		34	IM	Withdrew consent
		502	ID	Withdrew consent
		438	ID	Withdrew consent
Oeste	6	230	ID	Withdrew consent
		399	IM	Withdrew consent
		138	IM	Withdrew consent
		74	IM	Withdrew consent
		582	ID	Withdrew consent
		150	IM	Withdrew consent
Agramonte	4	202	IM	Withdrew consent

		199	ID	Withdrew consent
		174	IM	Tonsillitis
		153	IM	Moved out of province
Este	3	132	ID	Moved out of province (to Havana)
		9	ID	Moved out of province (to Santiago de Cuba)
		590	IM	Moved out of province (to Havana)
Pirre	3	242	ID	Withdrew consent
		274	IM	Moved out of the province (to Santiago de Cuba)
		367	ID	Moved out of the province (to Santiago de Cuba)
Florida Norte	2	581	ID	Herpes Zoster
		248	ID	Treatment with prednisolone (infected tattoo)
Nuevitas	2	286	ID	Withdrew consent
		436	ID	Moved out of municipality
Vertientes	1	287	ID	Out of the country (in Venezuela)
Centro	1	557	ID	Moved out of province (to Santiago de Cuba)
Finlay	1	136	IM	Tooth abscess – antibiotic treatment
Minas	1	362	ID	Withdrew consent
Total	**30**			

Table 9.1b. Participants enrolled and randomized by study site and study group

Flow of participants							
Study site	**Signed informed consent**	**Randomized**	**A**	**B**	**Non randomized**	**Dropouts**	**Complete follow-up**
Este	68	41	20	21	27	3	38
Norte	62	40	19	21	22	0	40
Florida Sur	35	35	19	16	0	0	35
Vertientes	51	51	26	25	0	1	50
Centro	63	30	16	14	33	1	29
Finlay	45	40	19	21	5	1	39
Nuevitas	42	29	16	13	13	2	27
Minas	46	42	21	21	4	1	41
Mella	32	11	7	4	21	6	5
Previsora	28	28	9	19	0	0	28
Oeste	42	42	22	20	0	6	36
Pirre	51	51	28	23	0	3	50
Agramonte	69	52	26	26	17	4	48
Florida Norte	42	42	20	22	0	2	40
Total	**676**	**534**	**268**	**266**	**142**	**30**	**504**

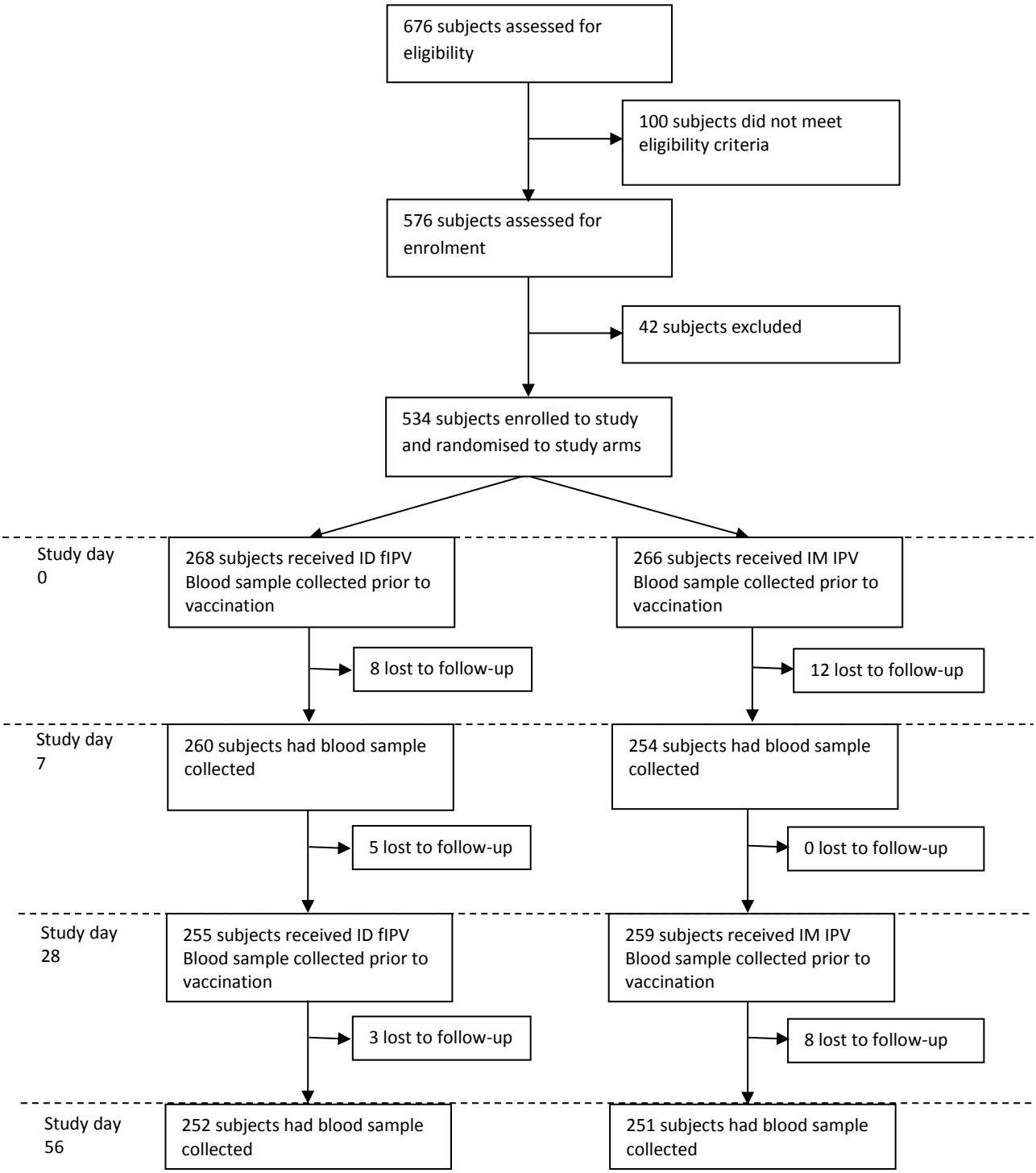
Table 9.2a. Participants with complete data by day and study group

		Complete Cases			
No. of subjects		**Day 0**	**Day 7**	**Day 28**	**Day 56**
ID IPV	268	268	260	255	252
IM IPV	266	266	254	259	251

Table 9.2b. Participants who provided sufficient quantity of blood for analysis by study site and study visit

	Blood extraction			
Study site	**1st**	**2nd**	**3rd**	**4th**
Este	41	37	37	38
Norte	40	39	39	40
Florida Sur	35	34	35	35
Vertientes	51	51	50	50
Centro	30	27	29	29
Finlay	40	38	40	39
Nuevitas	29	28	27	27
Minas	42	41	41	41
Mella	11	10	11	5
Previsora	28	27	28	28
Oeste	42	41	40	36
Pirre	51	50	49	48
Agramonte	52	50	49	48
Florida Norte	42	42	40	40
Total	**534**	**515**	**515**	**504**

Figure 9.1. Flowchart of study participants



RESULTS of PER PROTOCOL ANALYSIS

II. BASELINE DEMOGRAPHICS and SEROPREVALENCE

The demographics of the participants and their baseline seroprevalence to poliovirus serotypes 1, 2 and 3, is presented in **Table 9.3.** The median age was 24.0 years in both study groups. There were no statistically significant differences in baseline seroprevalence between the study groups (p≤0.05). Seroprevalence was highest for poliovirus types 1 (87.6%-91.0%) and 2 (87.2%-88.4%) and lowest for type 3 (66.9%-70.2%) in IM and ID study groups, respectively.

Table 9.3. Baseline demographics and seroprevalence by study group and poliovirus serotype

	ID fIPV		IM IPV		
Variable	**N**	**Mean (95% CI)**	**N**	**Mean (95% CI)**	**P-value**
Age		23.9 (23.5-24.4)		24.1 (23.7-24.6)	0.538
	n/N	**% (95% CI)**	**n/N**	**% (95% CI)**	
P1 Seroprevalence	244/268	91.0 (87.0-93.9)	233/266	87.6 (83.1-91.0)	0.575
P2 Seroprevalence	237/268	88.4 (84.1-91.7)	232/266	87.2 (82.7-90.7)	0.964
P3 Seroprevalence	188/268	70.2 (64.4-75.3)	178/266	66.9 (61.1-72.3)	0.734

All participants were male. ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine; n=number of subjects seropositive; N=total number of subjects; CI=confidence interval; P1=poliovirus type 1; P2=poliovirus type 2; P3=poliovirus type 3; P-value calculated using Student's t-test for continuous variables and Chi-squared test for categorical variables.

III. IMMUNE RESPONSE

The results for the proportion of participants who seroconverted after 1st and 2nd boosting doses of ID fIPV and full dose IM IPV are presented in **Table 9.4**. The proportion of participants with immune response after 1st and 2nd boosting doses is presented in **Table 9.5** and **Figure 9.2**, and the number of participants with complete data who had the potential to mount an immune response is presented in **Table 9.6.** Differences between the study groups were tested using Fisher's exact test for seroconversion rates, and Chi squared test for immune response rates.

a) Seroconversion

Seroconversion rates were very high, with the majority of participants in both study groups seroconverting by day 7, after 1st boosting dose. Among participants who were seronegative at day 0, 100% seroconverted by day 7 for poliovirus types 1 and 2 by for both study groups, and also for poliovirus type 3 in the IM study group; seroconversion rates for poliovirus type 3 in the ID study group were 98.7% (95% CI 93.1-99.8) at day 7 (p=0.476), 98.7% (95% CI 93.0-99.8) at day 28 (p=0.470), 98.7% (95% CI92.9-99.8) at day 56 (p=0.469), due to 1 participant failing to seroconvert by day 56. There were no statistically significant differences in seroconversion rates between day 0-7, day 0-28 or day 0-56, between the study groups (**Table 9.4**).

Table 9.4. Seroconversion rates in participants between days 0 and 7, days 0 and 28, and days 0 and 56, by study group and poliovirus serotype

		Day 0 to 7			Day 0 to 28			Day 0 to 56		
		n/N	%(95%CI)	P-value	n/N	%(95%CI)	P-value	n/N	%(95%CI)	P-value
P1	**ID IPV**	22/22	100.0 (85.1-100.0)	NA	22/22	100.0 (85.1-100.0)	NA	21/21	100.0 (84.5-100.0)	NA
	IM IPV	33/33	100.0 (89.6-100.0)		33/33	100.0 (89.6-100.0)		31/31	100.0 (89.0-100.0)	
P2	**ID IPV**	31/31	100.0 (89.0-100.0)	NA	31/31	100.0 (89.0-100.0)	NA	31/31	100.0 (89.0-100.0)	NA
	IM IPV	34/34	100.0 (89.9-100.0)		34/34	100.0 (89.9-100.0)		33/33	100.0 (89.6-100.0)	
P3	**ID IPV**	77/78	98.7 (93.1-99.8)	0.476	76/77	98.7 (93.0-99.8)	0.470	75/76	98.7 (92.9-99.8)	0.469
	IM IPV	86/86	100.0 (95.6-100.0)		87/87	100.0 (95.8-100.0)		86/86	100.0 (95.7-100.0)	

ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine; CI=confidence interval; P1=poliovirus type 1; P2=poliovirus type 2; P3=poliovirus type 3; n=number of participants who seroconverted (i.e. antibody titer value <8 to >=8); N=total number of participants with potential to seroconvert; P-value calculated using Fisher's exact test

b) Immune response

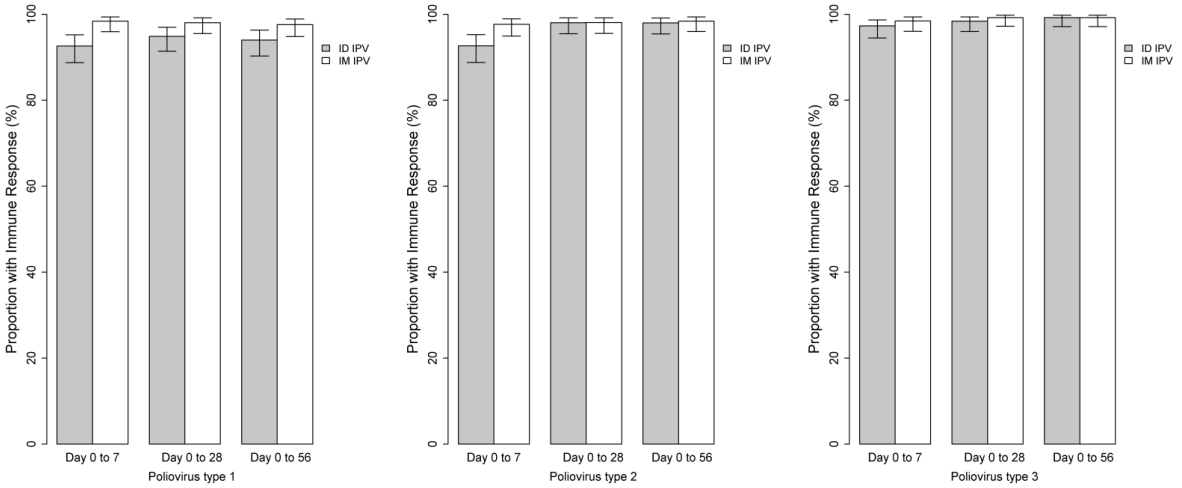
Immune response rates were very high (>92%) by day 7, after 1st boosting dose in both ID and IM study groups: 92.6% (94% CI 88.7-95.2), 98.4% (95% CI 96.0-99.4) for poliovirus type 1; 92.6% (95% CI 88.8-95.2), 97.6% (94.9% CI 94.9-98.9) for poliovirus type 2; 97.3% (95% CI 94.5-98.7), 98.4% (95% CI 96.0-99.4) for poliovirus type 3, in ID and IM study groups respectively. Immune response rates were >94% for all poliovirus serotypes at days 28 and 56 in both study groups. Although differences in immune response rates were found to be statistically significant at day 7 (p=0.002, p=0.012 for poliovirus types 1, 2 respectively), the differences were not statistically significant by day 28 (p=0.058, p=1.000 for poliovirus types 1, 2 respectively) and this was maintained at day 56 (p=0.072, 0.751 for poliovirus types 1 and 2 respectively) (**Table 9.6 and Figure 9.2**).

Table 9.5. Immune response rates in participants between days 0 and 7, days 0 and 28, and days 0 and 56, by study group and poliovirus serotype

		Day 0 to 7			Day 0 to 28			Day 0 to 56		
		n/N	% (95%CI)	P-value	n/N	% (95%CI)	P-value	n/N	% (95%CI)	P-value
P1	**ID IPV**	238/257	92.6 (88.7-95.2)	0.002	239/252	94.8 (91.4-97.0)	0.058	234/249	94.0 (90.3-96.3)	0.072
	IM IPV	246/250	98.4 (96.0-99.4)		250/255	98.0 (95.5-99.2)		241/247	97.6 (94.8-98.9)	
P2	**ID IPV**	239/258	92.6 (88.8-95.2)	0.012	248/253	98.0 (95.5-99.2)	1.000	245/250	98.0 (95.4-99.1)	0.751
	IM IPV	248/254	97.6 (94.9-98.9)		254/259	98.1 (95.6-99.2)		247/251	98.4 (96.0-99.4)	
P3	**ID IPV**	250/257	97.3 (94.5-98.7)	0.545	248/252	98.4 (96.0-99.4)	0.445	247/249	99.2 (97.1-99.8)	1.000
	IM IPV	249/253	98.4 (96.0-99.4)		256/258	99.2 (97.2-99.8)		248/250	99.2 (97.1-99.8)	

ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine; CI=confidence interval; P1=poliovirus type 1; P2=poliovirus type 2; P3=poliovirus type 3; n=number of participants who seroconverted (i.e. antibody titer value <8 to >=8); N=total number of participants with potential to seroconvert; P-value calculated using Chi-squared test

Figure 9.2. Proportion of participants with immune response between days 0 and 7, days 0 and 28, and days 0 and 56, by study group and poliovirus serotype



ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine.

Table 9.6. Participants with complete data with potential for immune response by day and study group

		No. of subjects	Day 0	Day 7	Day 28	Day 56
P1	**ID IPV**	268	265	258	253	250
	IM IPV	266	261	249	254	246
P2	**ID IPV**	268	266	259	254	251
	IM IPV	266	266	254	259	251
P3	**ID IPV**	268	262	255	250	247
	IM IPV	266	263	251	256	248

c) Median antibody titers

The results of median antibody titers by day (0, 7, 28 and 56) by study group are presented in **Table 9.7.** The median antibody titers at day 28, of participants who were seronegative or seropositive at day 0, by study group are presented in **Table 9.8**.

Median antibody titers were low at day 0 for all poliovirus types (45 for poliovirus type 1 and 36 for poliovirus type 2, for both study groups), and lowest at day 0 for poliovirus type 3 (14 for both study groups). There were no statistically significant differences in titers at day 0 between the study groups for all poliovirus serotypes. Median antibody titers greatly increased at day 7 after 1st boosting dose of ID fIPV with high titers >2800 in both study groups for all poliovirus types. There were significantly significant differences at day 7 ($p<0.001$ for all poliovirus serotypes for both study groups).

By day 28 median antibody titers remained high, >2800 for all poliovirus serotypes and although the differences between the study groups remained statistically significant, this had decreased ($p=0.040$, $p=0.006$, $p=0.035$ for poliovirus type 1, 2 and 3, respectively). Median antibody titers declined between day 28 and 56 however titers remained high >2800 for all poliovirus serotypes for both study groups ($p=0.023$, $p=0.024$, $p=0.384$ for poliovirus types 1 and 2, respectively) **(Table 9.7)**.

Median titer values at day 28 for subjects who were seropositive at day 0 were higher than those for subjects who were seronegative at day 0 for all poliovirus serotypes for both study groups **(Table 9.8)**.

Table 9.7. Median antibody titers at day 0 in seronegative and seropositive participants by study group

		Day 0		Day 7		Day 28		Day 56	
		Median (95% CI)	P-value	Median (95% CI)	P-value	Median (95% CI)	P-value	Median (95% CI)	P-value
P1	ID IPV	45 (36-57)	0.895	3573 (2839-4499)	<0.001	4499 (3573-4499)	0.040	2839 (2839-3573)	0.023
	IM IPV	45 (36-57)		4499 (4499-≥5664)		4499 (3573-4499)		3573 (2839-3573)	
P2	ID IPV	36 (28-45)	0.839	2839 (1791-3573)	<0.001	2839 (2839-3573)	0.006	2839 (2255-2839)	0.024
	IM IPV	36 (28-45)		4499 (4499-≥5664)		4499 (3573-4499)		2839 (2839-3573)	
P3	ID IPV	14 (11-18)	0.745	≥5664 (4499-≥5664)	<0.001	≥5664 (4499-≥5664)	0.035	3573 (3573-4499)	0.384
	IM IPV	14 (11-18)		≥5664 (≥5664-≥5664)		≥5664 (≥5664-≥5664)		3573 (3573-4499)	

ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine; CI=confidence interval; P1=poliovirus type 1; P2=poliovirus type 2; P3=poliovirus type 3; P-value calculated using Wilcoxon rank-sum test. 95% CIs calculated using bootstrapping with 10,000 replications. Highest serial dilution=5664.

Table 9.8. Median antibody titers in participants at days 0, 7, 28 and 56, by study group and poliovirus serotype

			Day 7		Day 28		Day 56	
		Sero status day 0	Median (95% CI)	P-value	Median (95% CI)	P-value	Median (95% CI)	P-value
P1	ID fIPV	SN	1607 (450-4499)	0.035	2023 (713-3573)	0.004	898 (566-2255)	<0.001
	ID fIPV	SP	3573 (2839-4499)		4499 (3573-4499)		2839 (2839-3573)	
	IM IPV	SN	2255 (1130-2839)	<0.001	2255 (1130-4499)	<0.001	1423 (713-1791)	<0.001
	IM IPV	SP	≥5664 (4499-≥5664)		4499 (4499-≥5664)		3573 (3573-4499)	
P2	ID fIPV	SN	1423 (713-1791)	0.003	2839 (1130-3573)	0.063	2255 (898-2839)	0.068
	ID fIPV	SP	2839 (2255-3573)		2839 (2839-3573)		2839 (2255-2839)	
	IM IPV	SN	2839 (1423-≥5664)	0.011	2839 (2839-3573)	0.014	1423 (898-2839)	0.002
	IM IPV	SP	4499 (4499-≥5664)		4499 (3573-4499)		3573 (2839-4499)	
P3	ID fIPV	SN	2547 (1791-3573)	<0.001	4499 (3573-4499)	0.001	2255 (1791-2839)	<0.001
	ID fIPV	SP	≥5664 (≥5664-≥5664)		≥5664 (3573-≥5664)		4499 (4499-4499)	
	IM IPV	SN	4499 (4499-≥5664)	<0.001	≥5664 (≥5664-≥5664)	0.007	2839 (2255-3573)	<0.001
	IM IPV	SP	≥5664 (≥5664-≥5664)		≥5664 (≥5664-≥5664)		4499 (4499-4499)	

ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine; CI=confidence interval; P1=poliovirus type 1; P2=poliovirus type 2; P3=poliovirus type 3; P-value calculated using Wilcoxon rank-sum test. 95% CIs calculated using bootstrapping with 10,000 replications. Highest serial dilution=5664.

IV. NON-INFERIORITY TESTING

Table 9.9. Non-inferiority testing of boosting immune response following intradermal IPV compared to intramuscular IPV administration, between days 0 to 7, days 0 to 28, and days 0 to 56, by poliovirus serotype

	Day 0 to 7	Day 0 to 28	Day 0 to 56
	% Difference (95% CI)	% Difference (95% CI)	% Difference (95% CI)
P1	5.8 (2.2-9.4)	3.2 (-0.02-6.4)	3.6 (0.1-7.1)
P2	5.0 (1.3-8.7)	0.1 (-2.4-2.4)	0.4 (-1.9-2.7)
P3	1.1 (-1.4-3.7)	0.8 (-1.1-2.7)	0.0 (-1.6-1.6)

Non-inferiority defined by a 10% margin difference in immune response IM-ID; ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine; CI=confidence interval; P1=poliovirus type 1; P2=poliovirus type 2; P3=poliovirus type 3

The % difference in proportion of participants with immune response between the study groups was calculated with 95% CI, between day 0-7, day 0-28, and day 0-56. The non-inferiority margin <10% was met for all poliovirus serotypes for all time periods. The greatest % difference was seen between day 0-7 for poliovirus

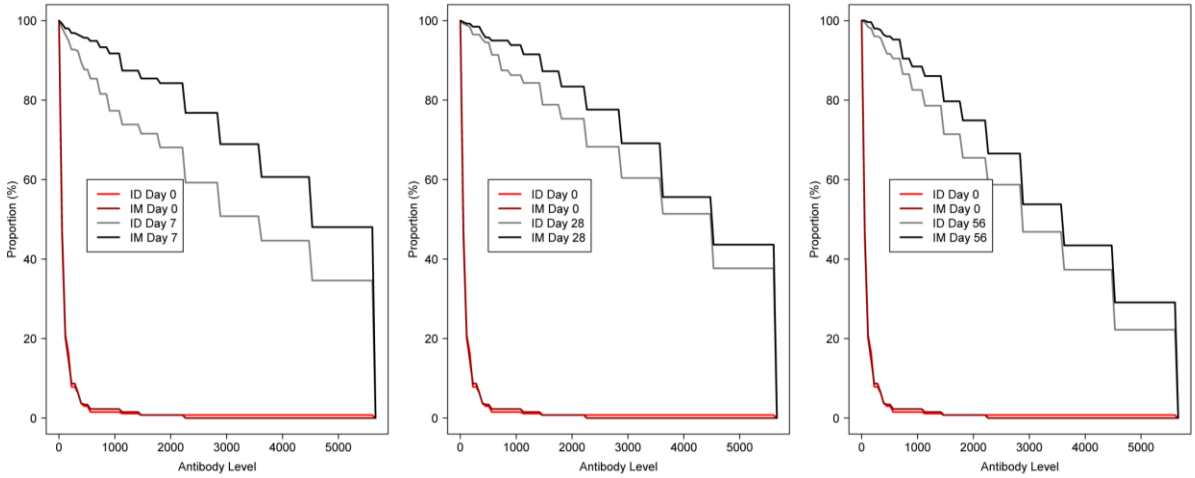
type 1: 5.8% (95% CI 2.2-9.4); this % difference decreased by day 28 3.2 (95% CI -0.02-6.4) and was maintained at day 56 3.6(95% CI 0.1-7.1).

V. REVERSE CUMULATIVE DISTRIBUTION (RCD) CURVES

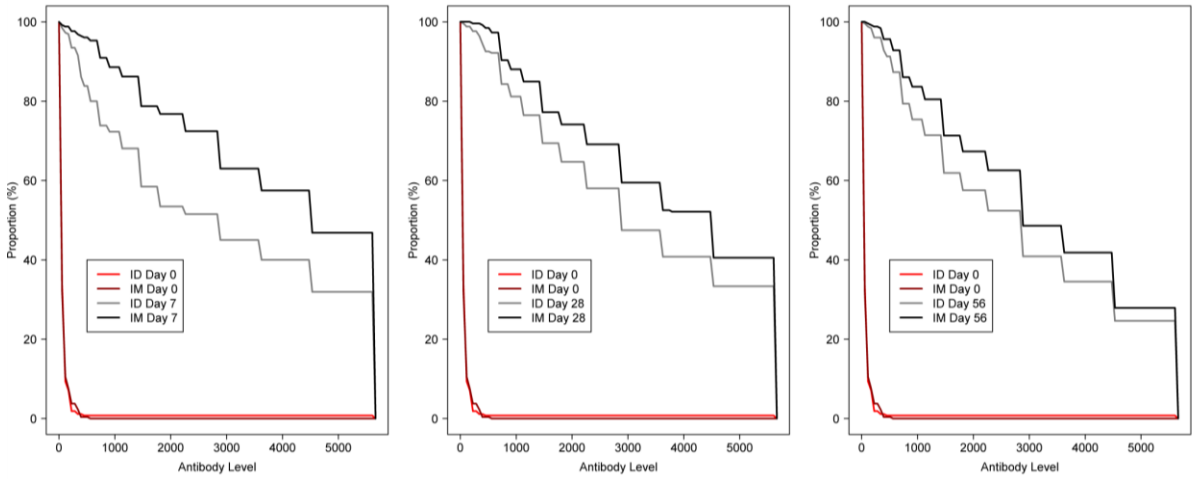
The RCD curves summarising antibody distribution by day and study group, are presented in **Figure 9.3.** The RCD curves for all poliovirus serotypes demonstrated similar trends in both study groups: RCD curves showed significant increase in titers between day 0 to 7, 0-28 and 0-56, for both study groups; although titers were lower for ID compared to IM study group at day 7, this difference decreased by day 28 which was maintained at day 56; indeed there was little difference in titers between day 28 and 56 in both study groups.

Figure 9.3. Reverse cumulative distribution plot by study group (ID or IM), day and poliovirus serotype

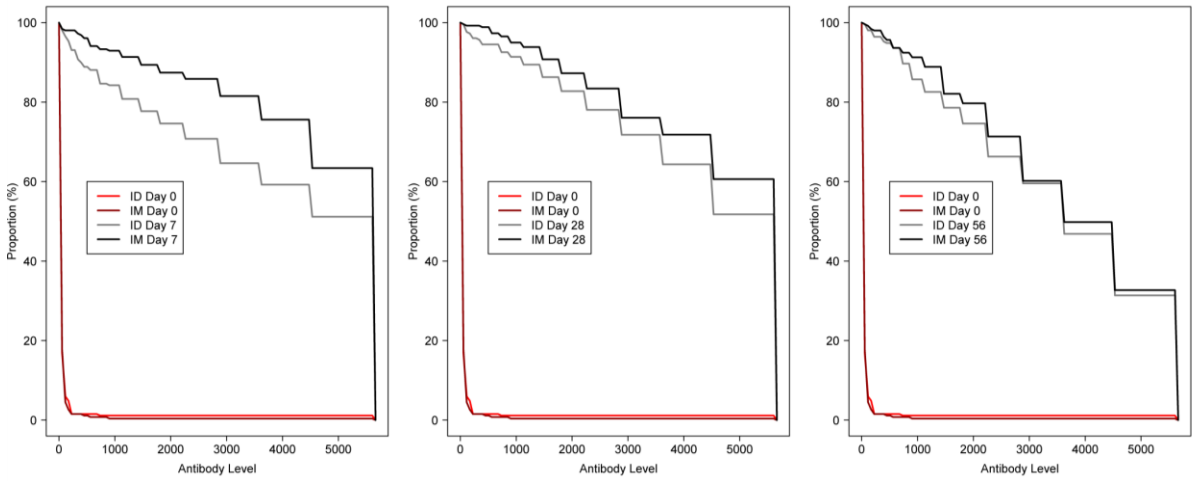
Type 1 **Day 0-7** **Day 0-28** **Day 0-56**



Type 2



Type 3



10. Results:

Adverse events

I. MINOR ADVERSE EVENTS

There were 73 adverse events reported in total between 1 hr and 7 days after 2nd vaccination; 56/73 (77%) AEs were reported after the first vaccination (31 from ID and 25 from IM study groups) and 17/73 after the 2nd vaccination (all from the ID study group). Half the symptoms reported [38/73 (52%)] were due to induration among which 33/38 (87%) resolved by 48 hours. The summary of AEs reported is presented in **Table 10.1**.

The number of any AEs reported during the study period by each participant was calculated with 95% CI in **Table 10.2**. Between 7.1%-10% of participants (IM and ID study groups respectively), reported an adverse event at any time point (1hr, 24hr, 48hr, 72hr, 7d) after the first or second vaccine dose. No significant differences were seen between the study groups (p=0.564).

Table 10.2. Any adverse event reported by each participant during the study period, by study group

	Any adverse event		
	n/N	**% (95% CI)**	**P-value**
ID	28/268	10.5 (7.3-14.7)	0.564
IM	19/266	7.1 (4.6-10.9)	

ID=Intradermal; IM=Intramuscular; IPV=Inactivated Polio Vaccine; CI=confidence interval. Adverse events include: temperature >=38 degrees Celsius, weakness, anaphylaxis, erythema, induration, inflammation, pain, immobility, abscess, necrosis or other. Any adverse event is defined as a recorded adverse event at any time point (i.e. 1h, 24hr, 48h, 72h, and 7d) after the first or second vaccine dose.

II. SERIOUS ADVERSE EVENTS

There were no SAEs reported.

III. SUSPECTED UNEXPECTED SERIOUS ADVERSE EVENTS

There were no SUSARs reported.

Table 10.1. Adverse events reported at 1hr, 24hr, 48hr, 72hr and 7 days after receiving 1st and 2nd vaccination

	1st vaccination												2nd vaccination											
	1hr		24hr		48hr		72hr		7 days		**Total**		1hr		24hr		48hr		72hr		7 days		**Total**	
	ID	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**	**ID**	**IM**
Temperature	0	0	1	1	0	1	1	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Weakness	1	0	1	0	0	0	1	0	2	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Anaphylaxis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	3	0	3	1	1	1	0	1	0	0	7	3	1¶	0	1¶	0	1¶	0	1¶	0	0	0	4	0
Induration	8*	13	2* †	1§	1†	1§	0	1§	0	0	11	16	7**	0	2**, ††	0	1††	0	1	0	0	0	11	0
Inflammation	1	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	2	0
Pain	1	1	1	1	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Immobility	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abscess	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Others	1	0	2§§	1¶¶	0	1¶¶	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0
Total	**15**	**14**	**10**	**5**	**2**	**4**	**2**	**2**	**2**	**0**	**31**	**25**	**10**	**0**	**3**	**0**	**2**	**0**	**2**	**0**	**0**	**0**	**17**	**0**

*Participant 77 (ID) reported induration at 1hr and 24hr
†Participant 71 (ID) reported induration at 24hr and 48hr
§Participant 404 ((IM) reported induration at 24hr, 48hr and 72hr
¶ Participant 375 (ID) reported erythema at 1hr, 24hr, 48hr and 72hr
**Participant 416 (ID) reported induration at 1hr and 24hr
††Participant 375 (ID) reported induration at 24hr and 48hr
§§Participant 323 (ID) reported headache and participant 23 (IM) reported itching
¶¶Participant 533 (IM) reported itching at 24hr and 48hr

11. Summary, limitations and conclusions

I. SUMMARY

Baseline seroprevalence

There were no significant differences in baseline seroprevalence between the study groups with high baseline seroprevalence for all poliovirus serotypes. Seroprevalence was highest for poliovirus type 1 91.0% (95% CI 87.0-93.9), 87.6% (95% CI 83.1-91.0) and poliovirus type 2 88.4% (95% CI 84.1-91.7), 87.2% (95% CI 82.7-90.7), and lowest for poliovirus type 3 70.2.0% (95% CI 64.4-75.3) 66.9% (95% CI 61.1-72.3) in ID and IM study groups, respectively.

Seroconversion and immune response rates

There were no significant differences in seroconversion rates between the study groups, however it must be noted that baseline seroprevalence was high for all poliovirus serotypes.

The proportion of participants with immune response was very high (>92%) in both study groups by day 7, with >94% participants with immune response by day 28 in both study groups, which was maintained by day 56. Although there was a statistically significant difference in immune response between the study groups at day 7 for poliovirus types 1 and 2 (p=0.002, p=0.012 for poliovirus types 1 and 2, respectively) this difference was not significant by day 28 (p=0.058, p=1,000 for poliovirus types 1 and 2, respectively), which was maintained at day 56.

Non-inferiority of immune response

The difference in proportion of participants with immune response between the study groups was <10% at day 7, 28 and 56. There % difference in immune response was greatest at day 7: 5.8% (95% CI 2.2-9.4), 5.0% (95% CI 1.3-8.7) 1.1% (95% CI -1.4-3.7) for poliovirus types 1, 2 and 3, respectively, however this decreased by day 28 3.2% (95% CI -0.02-6.4) 0.1% (95% CI -2.4-2.4) 0.8% (95% CI -1.1-2.7) for poliovirus types 1, 2 and 3, respectively, and this was maintained at day 56 for all 3 serotypes.

Median antibody titers

Median antibody titers increased significantly (>2800) between day 0-7 for all poliovirus types in both study groups and although there were significant differences between the study groups at day 7 (p<0.001 for all poliovirus serotypes), the differences decreased by day 28 (p=0.040, p=0.006, p=0.035 for poliovirus types 1, 2 and 3, respectively) and this was maintained by day 56 (p=0.023, p=0.024, p=0.384 for poliovirus types 1, 2 and 3, respectively). It must be noted that median antibody titers were extremely high in both study groups at day 28 (>2800), which was maintained at day 56. Between day 28 and 56, after the 2nd dose of vaccine there was little change in the median antibody titers. This was also demonstrated in the RCD curves, with minimum change in titers observed between day 28 and 56 in both study groups. The median antibody titers were higher at day 28 in participants who were seropositive at day 0 for all poliovirus serotypes.

Adverse events

There were 73 adverse events reported during the study period, all were minor adverse events, with 57/73 (78%) reported within 24hr of any vaccination, of which 33/57 (58%) reported induration. During the study period, induration was reported on 38 occasions of which 22/38 (58%) was reported in the ID study group.

Between 7.1%-10.5% of subjects (in IM and ID study groups, respectively) recorded any adverse event after the first or second vaccine dose, and there were no significant differences in the number of any adverse events reported by a participant, between study groups (p=0.564).

II. LIMITATIONS

The study had some limitations. The study results related to the specific use of BBio vaccine. The participants were recruited from one province in Cuba, a tropical environment and likely homogeneous area. The study was conducted in healthy adults whereas the practical implementation for use of ID fIPV will be for the outbreak response protocol, targeting children aged 0-10 years. for boosting response.

III. CONCLUSIONS

1. Our study found high baseline seroprevalence particularly for poliovirus serotypes type 1 and 2, which is in keeping with previous studies conducted in Camaguey province, Cuba [13, 14, 16].
2. Our study demonstrated the rapidity of immune response, within 7 days, as has been previously demonstrated in other studies [13, 14].
3. Our study demonstrated the non-inferiority of immune response rates following 1 and 2 boosting doses of ID fIPV compared to full dose IM IPV. Our study demonstrated significant immune response following 1 boosting ID fIPV dose with relatively less gain in immune response from 2nd boosting ID fIPV dose. This is in keeping with a study previously conducted in Cuba which demonstrated that a single ID fIPV dose could induce seroconversion and priming in more than 90% of immunized infants [13].
4. Our study demonstrated higher immune response rates for ID group versus IM group, as compared with other boosting studies; however this could be due to the study population which were previously vaccinated healthy adults, compared to boosting study conducted in children aged 6-9 months who were vaccinated at 6, 10 and 14 weeks in India [17] and children aged 12-20 months who were vaccinated through biannual mass vaccination campaigns in Cuba [14].
5. Our study demonstrated lower median antibody titers in the ID versus IM study group, as seen in previously conducted boosting studies [13, 18] as well as studies when IPV was administered ID versus IM in the primary immunization series [12, 16]. Our study also demonstrated a greater Increase in median antibody titers in participants who were seropositive at baseline.
6. Our study demonstrated that AEs were more common in the ID compared to IM study group, primarily related to induration; however the majority of local reactions from ID administration fully resolved within 48 hours, in keeping with other ID fIPV studies [13, 14].

IV. PROGRAMMTIC RELEVANCE

Given the non-inferiority of immune response following boosting ID fIPV dose compared to IM full IPV dose, as well as the rapidity of the immune response demonstrated, 1 boosting dose of ID fIPV can have a critical role in rapidly boosting population immunity particularly in an outbreak response scenario in a population already primed with OPV, as would be the scenario following the global withdrawal of OPV type 2 (the current

outbreak response protocol for cVDPV2 recommends targeting children aged 1-10). This has particular relevance for the polio eradication endgame plan, given the significant tight global IPV supply, with potential to stretch 1 IPV dose 5-fold while ensuring robust immune response. Furthermore, although it was beyond the scope of our study and requires future assessment, in addition to the benefit to humoral immunity as demonstrated in this study, in previously primed OPV recipients, there would be the added benefit of IPV boosting mucosal immunity [10].

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