

# Immunogenicity of Fractional Dose Inactivated Poliovirus Vaccine in India

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**Introduction.** Following the withdrawal of Sabin type 2 from trivalent oral poliovirus vaccine (tOPV) in 2016, the introduction of  $\geq 1$  dose of inactivated poliovirus vaccine (IPV) in routine immunization was recommended, either as 1 full dose (0.5 mL, intramuscular) or 2 fractional doses of IPV (fIPV—0.1 mL, intradermal). India opted for fIPV. We conducted a comparative assessment of IPV and fIPV.

**Methods.** This was a 4-arm, open-label, multicenter, randomized controlled trial. Infants were enrolled and vaccines administered according to the study design, and the blood was drawn at age 6, 14, and 18 weeks for neutralization testing against all 3 poliovirus types.

**Results.** Study enrolled 799 infants. The seroconversion against type 2 poliovirus with 2 fIPV doses was 85.8% (95% confidence interval [CI]: 80.1%–90.0%) when administered at age 6 and 14 weeks, 77.0% (95% CI: 70.5–82.5) when given at age 10 and 14 weeks, compared to 67.9% (95% CI: 60.4–74.6) following 1 full-dose IPV at age 14 weeks.

**Conclusion.** The study demonstrated the superiority of 2 fIPV doses over 1 full-dose IPV in India. Doses of fIPV given at 6 and 14 weeks were more immunogenic than those given at 10 and 14 weeks.

**Clinical Trial Registry of India (CTRI).** Clinical trial registration number was CTRI/2017/02/007793.

**Key words:** EPI schedule; fractional dose inactivated poliovirus vaccine; immunogenicity; India.

The World Health Assembly resolved in 1988 to eradicate poliomyelitis by the year 2000 [1]. Since the resolution, substantial progress has been made towards eradication. Four of the six World Health Organization (WHO) Regions have been certified free of wild poliovirus (WPV) by Regional Certification Commissions [2], and the number of paralytic cases associated with WPV has decreased by >99% compared to the estimated cases before the launch of global polio eradication initiative (GPEI) [3]. The number of WPV type 1 (WPV1) cases reported in 2 WPV endemic countries of Pakistan and Afghanistan [4] in the year 2019 was 151 and 38, respectively (data as of February 2, 2020). However, there has been a steep increase in the number of

WPV1 cases in Pakistan compared to the year 2018. Nigeria, another WPV-endemic country has not reported WPV-associated cases since the last detection in 2016 [5]. WPV type 2 (WPV2) in 2015 [6] and type 3 (WPV3) in 2019 [7], respectively, were certified eradicated by the Global Certification Commission.

On the other hand, polioviruses that emanate from the use of oral poliovirus vaccine (OPV), designated as circulating vaccine-derived poliovirus (cVDPV) [8], continue to circulate. Eradication requires the absence not only of WPVs but also of all cVDPVs in the communities. The GPEI called for sequential cessation of all Sabin strains starting with serotype 2 and introduction of  $\geq 1$  dose of inactivated poliovirus vaccine (IPV), administered intramuscularly (IM), in routine immunization (RI), in all OPV using countries [9]. However, poliovirus type 2 cVDPV (cVDPV2) has emerged in many Sub-Saharan countries and some Asian Countries in the recent years, facilitated by the low immunity following the withdrawal of type 2-containing oral poliovirus vaccine (OPV2) in 2016. Since the switch from trivalent OPV (tOPV) to bivalent types 1 and 3 (bOPV) 439 cVDPV2 cases have been reported from 17 countries (as of January 21, 2020).

Due to a global shortage, IPV could not be supplied to many low-risk countries until late 2017 and 2018 [10]. Though the

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IPV supply situation in the year 2019 had improved, an estimated 43 million children need to receive IPV catch-up vaccination [11]. In the face of IPV short supply and favorable data on efficacy of fractional dose (0.1 mL intradermal [ID], 1/5th of the regular 0.5 mL dose IM), the Strategic Advisory Group of Experts on Immunization (SAGE) recommended that 2 fIPV doses may be adopted in RI instead of 1 full dose [12]. However, the administration of fIPV ID using BCG needle and syringe (N&S) is challenging [13], but several options are now available using needle-free jet injectors and needle adaptors to facilitate the ID administration of fIPV [14, 15].

India, which many thought would face the greatest challenge to eradication, as it contributed 60% of the global caseload in the first decade of 2000 [16], reported the last case of WPV1 in January 2011. The South-East Asian Region of the WHO which includes India was certified polio-free in 2014 [2, 17]. India, introduced IPV (IM) in 2015, however, due to the continued IPV shortage, it adopted a schedule of 2 doses of fIPV at age 6 and 14 weeks in RI [13].

The current Expanded Programme on Immunization (EPI) schedule in India includes bOPV doses at birth, and 6, 10, and 14 weeks and 2 fIPV doses at 6 and 14 weeks. There are approximately 27 million new births in India each year—the largest national birth cohort in the world [18]. Since fIPV immunogenicity data were not available from Indian infants, the India Expert Advisory Group (IEAG) for Polio Eradication recommended conducting clinical trials to generate fIPV immunogenicity data for the current EPI schedule and assess the operational challenges of ID injections in the country [19]. Hence the main objective of the trial was to assess immunogenicity in terms of seroconversion of 2 fIPV doses as compared to 1 full-dose IPV in India. This trial also addressed the immunogenicity of 2 doses of fIPV when administered at different ages and intervals in the EPI schedule and compared immunogenicity when fIPV is administered intradermally with two different devices.

## METHODS

### Study Design and Participants

The study design was an open-label, 4-arm, superiority, multicenter, randomized controlled trial that involved medical institutions across India with good experience, infrastructure, and support system for vaccine trials. The study was conducted between July 2017 and January 2018 at 5 medical institutions: (1) King Edward Memorial Hospital, Pune, Maharashtra; (2) K.L.E Academy of Higher Education and Research, Belagavi, Karnataka; (3) Mysore Medical College, Mysore, Karnataka; (4) Mahatma Gandhi Institute of Medical Sciences, Wardha, Maharashtra; and (5) Malankara Orthodox Syrian Church Medical College, Ernakulum, Kerala.

Healthy infants 6-7 weeks of age, who visited the study site immunization clinic for OPV1/Pentavalent 1 immunization,

weighed at least 3.2 kg and, whose parents presented with a documented evidence that their child received the birth dose of bOPV were eligible for enrollment.

During the first visit (week 6 or 7), the study nurse and the study physician explained the trial purpose to the parents of the eligible infants at the immunization clinics of their respective institutions. Once the parents consented, and written informed consent was obtained, infants were randomized into specific study arm allocation, and a baseline questionnaire was administered, and a blood sample was collected.

The study included 4 arms (3 experimental arms [Arm A, B, and C] and 1 control arm [Arm D]): (1) Arm A received fIPV at age 6 and 14 weeks using BCG N&S; (2) Arm B received fIPV at age 6 and 14 weeks using West/Helm ID adapter; (3) Arm C received fIPV at age 10 and 14 weeks with BCG N&S; and (4) Arm D received 1 full dose of IPV at 14 weeks. In addition, all study subjects received bOPV at birth, 6, 10, and 14 weeks. The Consort Statement (Figure 1) displays enrollment and drop-outs or withdrawal by study arm.

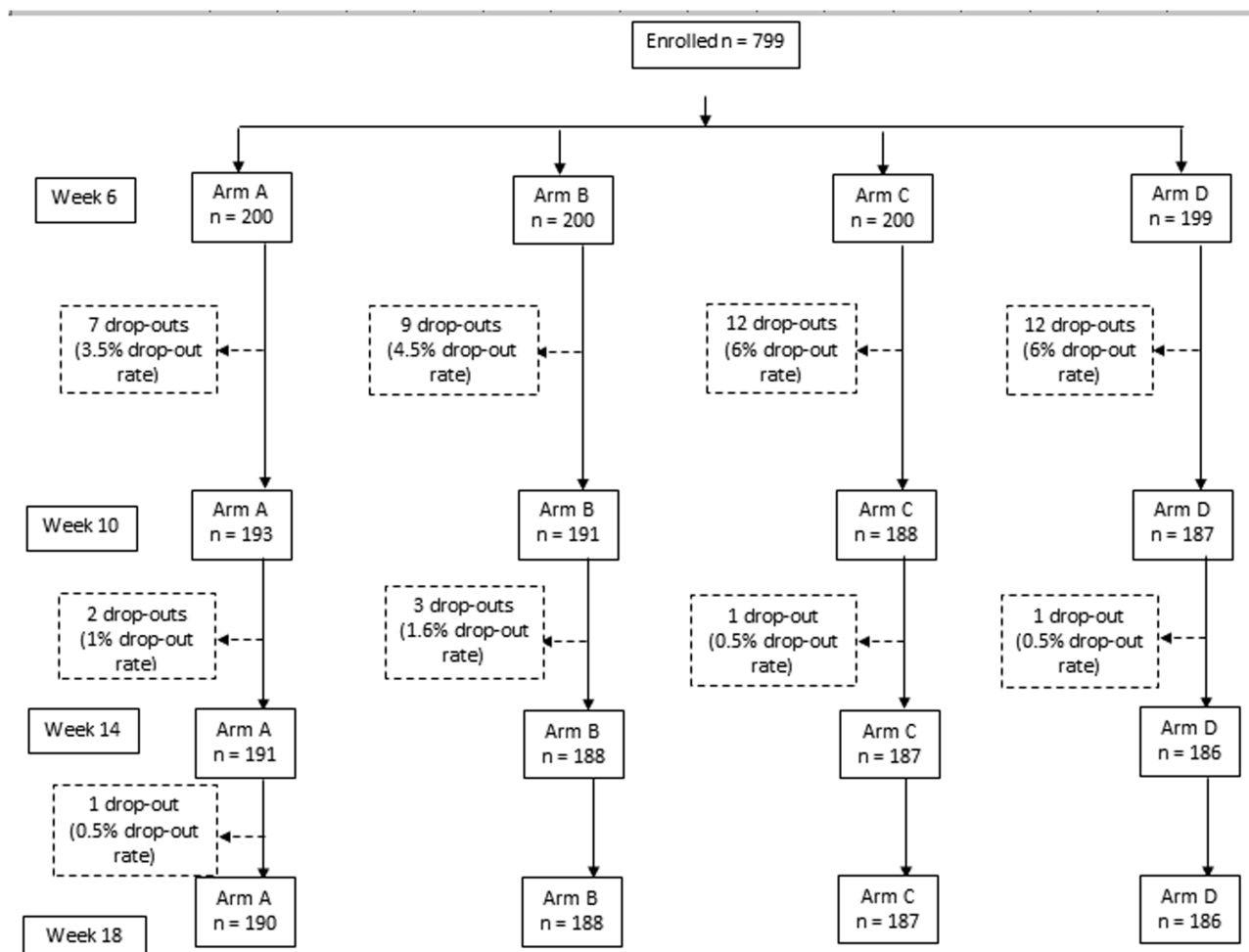
The protocol followed good clinical practice standards and ethical approval was obtained from the WHO Ethics Committee at Geneva and the Ethics Committees of all study institutions involved. The Drugs Controller General (India) provided the regulatory clearance for the clinical trial. The clinical trial registration number was CTRI/2017/02/007793, registered with the Clinical Trial Registry of India (CTRI).

### Randomization and Masking

Eligible infants were randomly assigned to a study arm using permuted block randomization with block sizes of 4, 8, and 12. The random sequence was obtained using SAS 9.4. The allocation concealment was achieved by serially numbered, opaque sealed envelopes. These envelopes were opened by the study nurse and the enrolled child was vaccinated as per the allocation provided in the envelope. Neither the parents of the study participants nor the study investigators had any choice to opt for a specific vaccine arm. The study was open label as the vial size, appearance and route, and age of administration were different. The study participants and the study investigators could not be masked. The laboratory technicians who were the outcome assessors were blinded to the treatment allocation.

### Study Procedures

At enrollment (6-7 weeks of age), a study questionnaire was administered to parents and 1 mL of blood was collected from each participant by venipuncture following necessary aseptic precautions. Every study participant was issued a specific immunization card and all routine vaccinations were administered by study staff during the study period. The study vaccines were administered per the study design and the arm. Whatman blotting papers (circular, 110 mm) were used to measure the wetness of ID injections. Once the vaccine was administered



**Figure 1.** Consort flowchart.

to each child, the remainder of each vial was labeled with the subject's identification number and stored back in cold chain. All vaccines were stored at the study site where power backup facilities were available.

Once the blood sample was collected, serum was separated from the blood clot and was stored at  $-20^{\circ}\text{C}$ . Sera from these samples were labeled with subject identification number. Blood samples were also collected at subsequent visits of 14 and 18 weeks and all sera were stored in cold chain at the respective study sites. The sera were later transported in cold chain to the ICMR-National Institute of Virology (NIV), Mumbai Unit, India (a Global Specialized Laboratory for Polio Eradication). Serum specimens were tested with microneutralization assay (W.C. Weldon, 2016) of antibody titers to poliovirus types 1 and 3 at ICMR-NIV, Mumbai. Because of containment requirements, type 2 testing was carried out at ICMR-NIV, Pune, India, by the staff from ICMR-NIV Mumbai Unit.

Immediate local or systemic hypersensitivity reactions were captured through observation for 30 minutes following vaccination at each visit. The primary caretakers used a diary

card to record any local and systemic reactions between study visits. Adverse events were also recorded by the study physician during the next follow-up visits also capturing any events in the inter-visit intervals or during household contacts made by the study staff during the study period.

Parents were informed about the follow-up visits during the previous visits and reminders through phone calls or in-person household visits were arranged. A gap of 4 weeks was maintained for all subsequent follow-up visits. A window period of +4 days was provided for all visits from week 10 onwards if the participant did not turn up on the scheduled date. The participants were followed until the age of 18 weeks.

#### Study Vaccines

bOPV contained at least  $10^{6.0}$  CCID<sub>50</sub> of Sabin poliovirus type 1 and at least  $10^{5.8}$  CCID<sub>50</sub> of Sabin poliovirus type 3 that was formulated by Panacea Biotec Ltd., New Delhi, India using imported bulk from Sanofi Pasteur, France. IPV with a potency of 40-8-32 D-antigen units produced by Serum Institute of India Ltd. from the bulk provided by Bilthoven Biologicals,

Netherlands, was used. West ID Adapter, manufactured by West Pharmaceutical Services, and Helmject, an auto-disable syringe manufactured by Helm Medical GmbH were used in the study. Both were marketed together by Helm Medical GmbH, Berlin, Germany. The conventional dose of bOPV (2 drops) orally, IPV (0.5 mL IM) and fIPV intradermally (0.1 mL) were used.

### Outcomes

The primary outcome of the study was seroconversion against poliovirus type 2. Seroprevalence was defined as the presence of neutralizing antibodies, that is, reciprocal titer of  $\geq 8$ . A change from non-detectable (reciprocal titer  $< 8$ ) to detectable titer ( $\geq 8$ ) was considered as seroconversion. For subjects with detectable antibodies, seroconversion was defined as the 4-fold increase over the expected decline of maternally derived antibodies (half-life assumed to be 28 days) at that point of time. The secondary outcomes of the study were comparing titer distribution and adverse events across the study arms. Dose-response trend was another outcome assessed.

Vaccine loss from the ID injection site was quantified from the wetness measurement of blotting paper and was classified as  $< 10\%$  and  $\geq 10\%$ .

### Sample Size

The sample size was calculated taking into consideration the overriding primary objective of the comparison of immunogenicity against type 2 poliovirus with 2 doses fIPV given at 6 and 14 weeks to 1 full-dose IPV given at 14 weeks. Considering seroconversion of 80% based on the trial conducted in Bangladesh with 2 doses of fIPV when given at 6 and 14 weeks [20] and assuming about 70% seroconversion from full-dose IPV at 14 weeks based on a previous study in India [21], 5% level of significance, a power of 80%, and adjusting for drop-out rate, the estimated sample size was 200 in each arm, and a total of 800 subjects were to be enrolled in 4 arms.

### Statistical Analysis

All analyses were done on both intention-to-treat (ITT) and per-protocol (PP) basis. If the lower limit of 95% confidence interval (CI) of the difference in poliovirus type 2 seroconversion proportion between 2 arms excluded 0, superiority was concluded. Analysis of the efficacy of endpoints was based on proportions using Fisher's exact test for proportions with corresponding 95% CIs. The median titer with the 10 000 bootstrapped samples CI was computed. Secondary outcome was compared using the Wilcoxon rank sum test. Adverse or severe adverse events were noted and reported as percentages in each arm. The association of doses of fIPV on seroconversion rate was assessed using the Cochran Armitage trend test. The analysis was performed separately for serotypes 1, 2, and 3. Baseline

titers (at 6 weeks) that were equal to  $\geq 1448$  for any poliovirus type were excluded. All *P* values were 2-sided. All analyses were done using SAS 9.4.

## RESULTS

A total of 827 children were screened, among whom, 799 eligible subjects were enrolled. A number of subjects randomized into the respective arms were 199 in Arm D that received bOPV and full-dose IPV at 14 weeks and 200 each, respectively, in the remaining 3 arms (Arms A, B, and C). There were only 47 (5.9%) participants who dropped out of the study as shown in the consort flowchart (Figure 1).

The baseline characteristics of the study participants are shown in Table 1. The distribution of demographic characteristics like gender of the subject, mothers' education level, religion was comparable across the study arms. It was observed that stunted subjects were more prevalent in Arm C while wasted subjects were seen more in Arm A. Seroprevalence of serotypes 1, 2, and 3 were distributed equally across the study arms. The median titers also were similar in all the study arms at baseline. A total of 14 subjects with poliovirus type 1 titers  $\geq 1448$  at baseline (week 6) were excluded from all further analyses.

The cumulative seroconversion of the study arm with current EPI schedule (receiving 2 fIPV at 6 and 14 weeks using BCG N&S—Arm A), against type 2 poliovirus, was 85.8% (95% CI: 80.1-90.0). There were 22 subjects with seroconversion in Arm D despite not having received any type 2-containing vaccine before age 14 weeks. These 22 subjects were removed from any further analysis. Then the study Arm D demonstrated seroconversion of 67.9% (95% CI: 60.4-74.6) (Table 2) against type 2 poliovirus. The difference in the cumulative seroconversion between Arms A and D was 17.9% (95% CI: 9.2-26.6; *P* value  $< .001$ ) (Figure 2). PP analysis and the ITT were similar; hence, the PP results are presented here. The cumulative seroconversion rate when 2 doses of fIPV were administered at 10 and 14 weeks (Arm C) was 77.0% (95% CI: 70.5-82.5). The difference in the seroconversion rate between the arms C and D that received 2 doses of fIPV at 10 and 14 weeks and full-dose IPV at 14 weeks, respectively, was 9.1% (95% CI: -0.2 to -18.5; *P* value = .057).

The seroconversion against type 2 poliovirus at 14 weeks when first dose of fIPV was given at 10 weeks (Arm C) and 6 weeks (Arm A), respectively, were 28.9% (95% CI: 22.9-35.7) and 14.2% (95% CI: 10.0-19.9) with the difference of 14.7% (95% CI: 6.5-22.9; *P* value  $< .0011$ ) between the two. The difference in cumulative seroconversion rate at 18 weeks with 2 fIPV doses received at 10 and 14 weeks (Arm C) and 2 fIPV at 6 and 14 weeks administered in the current EPI schedule (Arm A) was -8.8% (95% CI: -16.6 to -0.98; *P* value = .028).

Table 3 provides the median titers in all study arms. The median titer at 18 weeks in the EPI schedule (Arm A) for serotype

**Table 1. Baseline Distribution of the Study Characteristics by Study Arms**

|                                  | Arm A (n = 200) |      | Arm B (n = 200) |      | Arm C (n = 200) |      | Arm D (n = 199) |      |
|----------------------------------|-----------------|------|-----------------|------|-----------------|------|-----------------|------|
|                                  | n               | %    | n               | %    | n               | %    | N               | %    |
| <b>Gender</b>                    |                 |      |                 |      |                 |      |                 |      |
| Male                             | 96              | 48.0 | 99              | 49.5 | 102             | 51.0 | 100             | 50.3 |
| <b>Religion</b>                  |                 |      |                 |      |                 |      |                 |      |
| Hindu                            | 154             | 77.0 | 155             | 77.5 | 152             | 76.0 | 161             | 80.9 |
| <b>Mothers' education level</b>  |                 |      |                 |      |                 |      |                 |      |
| Illiterate/primary/middle school | 32              | 16.0 | 32              | 16.0 | 33              | 16.5 | 29              | 14.6 |
| Tenth grade                      | 48              | 24.0 | 60              | 30.0 | 57              | 28.5 | 57              | 28.6 |
| Twelfth grade                    | 49              | 24.5 | 47              | 23.5 | 49              | 24.5 | 53              | 26.6 |
| Graduate/higher                  | 71              | 35.5 | 61              | 30.5 | 61              | 30.5 | 60              | 30.2 |
| <b>Stunting</b>                  |                 |      |                 |      |                 |      |                 |      |
| Normal                           | 125             | 62.5 | 135             | 67.5 | 105             | 52.5 | 107             | 53.8 |
| Mild/moderate/severe             | 75              | 37.5 | 65              | 32.5 | 95              | 47.5 | 92              | 46.2 |
| <b>Wasting</b>                   |                 |      |                 |      |                 |      |                 |      |
| Normal                           | 100             | 50.0 | 124             | 62.0 | 115             | 57.5 | 122             | 61.3 |
| Mild/moderate/severe             | 100             | 50.0 | 76              | 38.0 | 85              | 42.5 | 77              | 38.7 |
| <b>Baseline seroprevalence</b>   |                 |      |                 |      |                 |      |                 |      |
| Type 1                           | 163/200         | 81.5 | 159/200         | 79.5 | 165/200         | 82.5 | 169/199         | 84.9 |
| Type 2                           | 131/200         | 65.5 | 128/200         | 64.0 | 125/200         | 62.5 | 126/199         | 63.3 |
| Type 3                           | 86/200          | 43.0 | 75/200          | 37.5 | 90/200          | 45.0 | 83/199          | 41.7 |
| <b>Baseline median titers</b>    |                 |      |                 |      |                 |      |                 |      |
| Type 1 (median, 95% CI)          | 57 (36-113)     |      | 64 (36-144)     |      | 144 (72-181)    |      | 91 (36-113)     |      |
| Type 2 (median, 95% CI)          | 11 (11-14)      |      | 11 (9-14)       |      | 11 (11-14)      |      | 11 (11-14)      |      |
| Type 3 (median, 95% CI)          | <8 (<8 to 8)    |      | <8 (<8 to <8)   |      | <8 (<8 to 9)    |      | <8 (<8 to <8)   |      |

Abbreviations: BCG, Bacille Calmette-Guérin; bOPV, bivalent oral poliovirus vaccine; CI, confidence interval; fIPV, fractional doses of inactivated poliovirus vaccine; N&S, needle and syringe.

Arm A (4 bOPV + 2 fIPV<sup>6, 14</sup> BCG N&S); Arm B (4 bOPV + 2 fIPV ID adapter); Arm C (4 bOPV + fIPV<sup>10, 14</sup> BCG N&S); Arm D (4 bOPV + IPV).

Stunting: height-for-age z scores >-1—normal; ≤-1—mild/moderate/severe.

Wasting: weight-for-height z scores >-1—normal; ≤-1—mild/moderate/severe.

Seroprevalence: ≥3.

2 was 57 (95% CI: 45-72) while the median titer at 18 weeks for the Arm D was 18 (95% CI: 14-22), and this difference was statistically significant (*P* value < .001). The comparison of median titers of type 2 at week 14 between those subjects who received first dose of fIPV at 6 weeks (Arm A) and subjects who received

first dose fIPV at 10 weeks (Arm C) was also statistically significant (median titers in Arm A was <8 [<8 to <8] vs Arm C was 9 [<8 to 11]; *P* value < .001). At week 18, the comparison of median titers where subjects received 2 doses of fIPV at 6 and 14 weeks with those who received 2 doses fIPV at 10 and

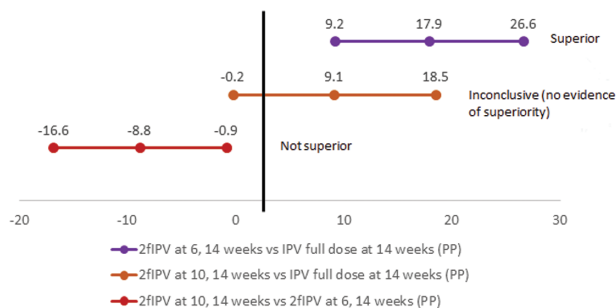
**Table 2. Immune Response (Seroconversion or 4-Fold Rise Over Expected Decline in Maternal Antibodies) for Per-Protocol Population**

|                           | n/N     | Arm A, % (95% CI) | n/N     | Arm B % (95% CI) | n/N     | Arm C, % (95% CI) | n/N     | Arm D, % (95% CI) |
|---------------------------|---------|-------------------|---------|------------------|---------|-------------------|---------|-------------------|
| <b>Type 1</b>             |         |                   |         |                  |         |                   |         |                   |
| 14 weeks                  | 175/184 | 95.1 (91.0-97.4)  | 171/188 | 91.0 (86.0-94.3) | 171/183 | 93.4 (89.0-96.2)  | 166/184 | 90.2 (85.1-93.7)  |
| 18 weeks                  | 2/8     | 25.0 (7.1-59.1)   | 9/17    | 52.9 (31.0-73.8) | 5/11    | 45.5 (21.3-72.0)  | 10/17   | 58.8 (36.0-78.4)  |
| Cumulative seroconversion | 177/184 | 96.2 (92.4-98.1)  | 180/188 | 95.7 (91.8-97.8) | 176/183 | 96.2 (92.3-98.1)  | 176/184 | 95.7 (91.7-97.8)  |
| <b>Type 2</b>             |         |                   |         |                  |         |                   |         |                   |
| 14 weeks                  | 27/190  | 14.2 (10.0-19.9)  | 34/189  | 18.0 (13.2-24.1) | 54/187  | 28.9 (22.9-35.7)  | —/164   | —                 |
| 18 weeks                  | 136/162 | 84.0 (77.5-88.8)  | 134/153 | 87.6 (81.4-91.9) | 90/132  | 68.2 (59.8-75.5)  | 110/162 | 67.9 (60.4-74.6)  |
| Cumulative seroconversion | 163/190 | 85.8 (80.1-90.0)  | 168/189 | 88.9 (83.6-92.6) | 144/187 | 77.0 (70.5-82.5)  | —       | —                 |
| <b>Type 3</b>             |         |                   |         |                  |         |                   |         |                   |
| 14 weeks                  | 180/190 | 94.7 (90.6-97.1)  | 176/189 | 93.1 (88.6-95.9) | 179/187 | 95.7 (91.8-97.8)  | 161/186 | 86.6 (80.9-90.7)  |
| 18 weeks                  | 5/10    | 50.0 (23.7-76.3)  | 8/13    | 61.5 (35.5-82.3) | 6/7     | 85.7 (48.7-97.4)  | 24/25   | 96.0 (80.5-99.3)  |
| Cumulative seroconversion | 185/190 | 97.4 (93.9-98.9)  | 184/189 | 97.4 (94.0-98.9) | 185/187 | 98.9 (96.2-99.7)  | 185/186 | 99.5 (97.0-99.9)  |

Abbreviations: bOPV, bivalent oral poliovirus vaccine; CI, confidence interval; fIPV, fractional doses of inactivated poliovirus vaccine; NS, needle and syringe; WA, West/Helm Adapter; Wks, weeks.

14 weeks: Arm A—3 bOPV-fIPV<sup>NS 6 wks</sup>; Arm B—3 bOPV-fIPV<sup>WA 6 wks</sup>; Arm C—3 bOPV-fIPV<sup>10 wks</sup>; Arm D—3 bOPV.

18 weeks: Arm A—4 bOPV-2 fIPV<sup>NS 6, 14 wks</sup>; Arm B—4 bOPV-2 fIPV<sup>WA 6, 14 wks</sup>; Arm C—4 bOPV-2 fIPV<sup>10, 14 wks</sup>; Arm D—4 bOPV-IPV.



**Figure 2.** Type 2 cumulative seroconversion difference between the different comparison arms with 95% CI for the difference.

14 weeks were 57 (45-72) and 23 (18-28), respectively, and this difference was highly significant ( $P < .001$ ). The reverse cumulative antibody distribution curves (Figure 3) also demonstrate the higher seroconversion of fIPV when administered at 6 and 14 weeks of age.

Comparing the ID administration with 2 devices, the seroconversion with 2 doses of fIPV at 6 and 14 weeks, using Helm ID adapter was 88.9% (95% CI: 83.6-92.6) against type 2 poliovirus while the cumulative seroconversion with 2 doses fIPV at the same vaccination contacts (6 and 14 weeks) administered using N&S was 85.8% (95% CI: 80.1-90.0). The difference in the seroconversion was found to be 3.1% (95% CI: -3.6 to -9.8;  $P$  value = .364).

The cumulative seroconversion at week 18 for serotypes 1 and 3 in all 4 arms were >95%. The median titers against polioviruses types 1 and 3 also significantly increased over time.

Successful ID injection was defined as injection resulting in a bleb with diameter  $\geq 5$  mm. 91% had bleb size diameter  $\geq 5$  mm when administered using BCG N&S or ID adapter at 6 and 14 weeks, but 97% resulted in bleb size diameter  $\geq 5$  mm in the arm with BCG N&S at 10 and 14 weeks ( $P = .004$ ).

Higher vaccine loss (ie, wetness) was recorded for BCG N&S (47%) compared to ID adapter (29%) ( $P < .001$ ). In addition, the average time taken for preparation process before giving the injection was slightly more when ID adapters were used as compared to BCG N&S (103.7 vs 93.3 seconds,  $P$  value = .031).

A total of 830 adverse events were recorded. The distribution of adverse events was similar in all study arms (Arm A = 22.5%; Arm B = 26.0%; Arm C = 24.2%; and Arm D = 27.0%). The adverse events were categorized as mild in all the study arms. There were 6 serious adverse events where 5 were reported from bOPV + full-dose IPV at 14 weeks (Arm D) while only one was reported from fIPV arm at 6 and 14 weeks (Arm A). None of these were attributed to the trial interventions by the investigators or the Data and Safety Monitoring Board.

## DISCUSSION

The main finding of the trial was that 2 doses of fIPV administered at 6 and 14 weeks were superior in inducing seroconversion to poliovirus type 2 than 1 full-dose IPV provided at 14 weeks. The trial demonstrated that the routine schedule in use in India of 2 doses of fIPV at 6 and 14 weeks, administered using BCG N&S, together with bOPV at birth, and age 6, 10, and 14 weeks is effective, providing >95% seroconversion against poliovirus types 1 and 3 and >85% seroconversion against type 2 poliovirus. These findings confirm the superiority of 2 fractional doses of IPV over 1 full-dose IPV and support the continued use of fIPV in the 6- and 14-week schedule.

Moreover, the study by the Program for Appropriate Technology in Health (PATH) organization modeled the potential incremental costs of RI with IPV in India, reported that delivery of fractional (1/5 of full dose) ID dose with either N&S or disposable-syringe jet injectors could result in cost savings

**Table 3. Median Titers With 95% Bootstrap Confidence Intervals**

|                           | Arm A          | Arm B           | Arm C           | Arm D             |
|---------------------------|----------------|-----------------|-----------------|-------------------|
| <b>6 weeks</b>            |                |                 |                 |                   |
| Type 1 <sup>c</sup>       | 45 (28-72)     | 56 (36-144)     | 144 (72-181)    | 72 (36-128)       |
| Type 2                    | 11 (11-14)     | 11 (9-12)       | 11 (11-14)      | 11 (9-14)         |
| Type 3                    | <8 (<8 to 9)   | <8 (<8 to <8)   | <8 (<8 to 9)    | <8 (<8 to <8)     |
| <b>14 weeks</b>           |                |                 |                 |                   |
| Type 1                    | 724 (576-910)  | 724 (576-910)   | 910 (724-910)   | 910 (724-910)     |
| Type 2 <sup>c,d</sup>     | <8 (<8 to <8)  | <8 (<8 to <8)   | 9 (<8 to 10)    | <8 (<8 to <8)     |
| Type 3 <sup>a,d</sup>     | 362 (288-455)  | 362 (288-362)   | 362 (362-455)   | 288 (228-362)     |
| <b>18 weeks</b>           |                |                 |                 |                   |
| Type 1 <sup>a,b</sup>     | 910 (910-1152) | 1152 (910-1152) | 1152 (910-1152) | 1152 (1152-≥1448) |
| Type 2 <sup>a,b,c,d</sup> | 57 (45-72)     | 81 (57-114)     | 23 (18-28)      | 18 (14-23)        |
| Type 3 <sup>a,b</sup>     | 576 (455-576)  | 576 (576-724)   | 576 (455-576)   | 910 (724-910)     |

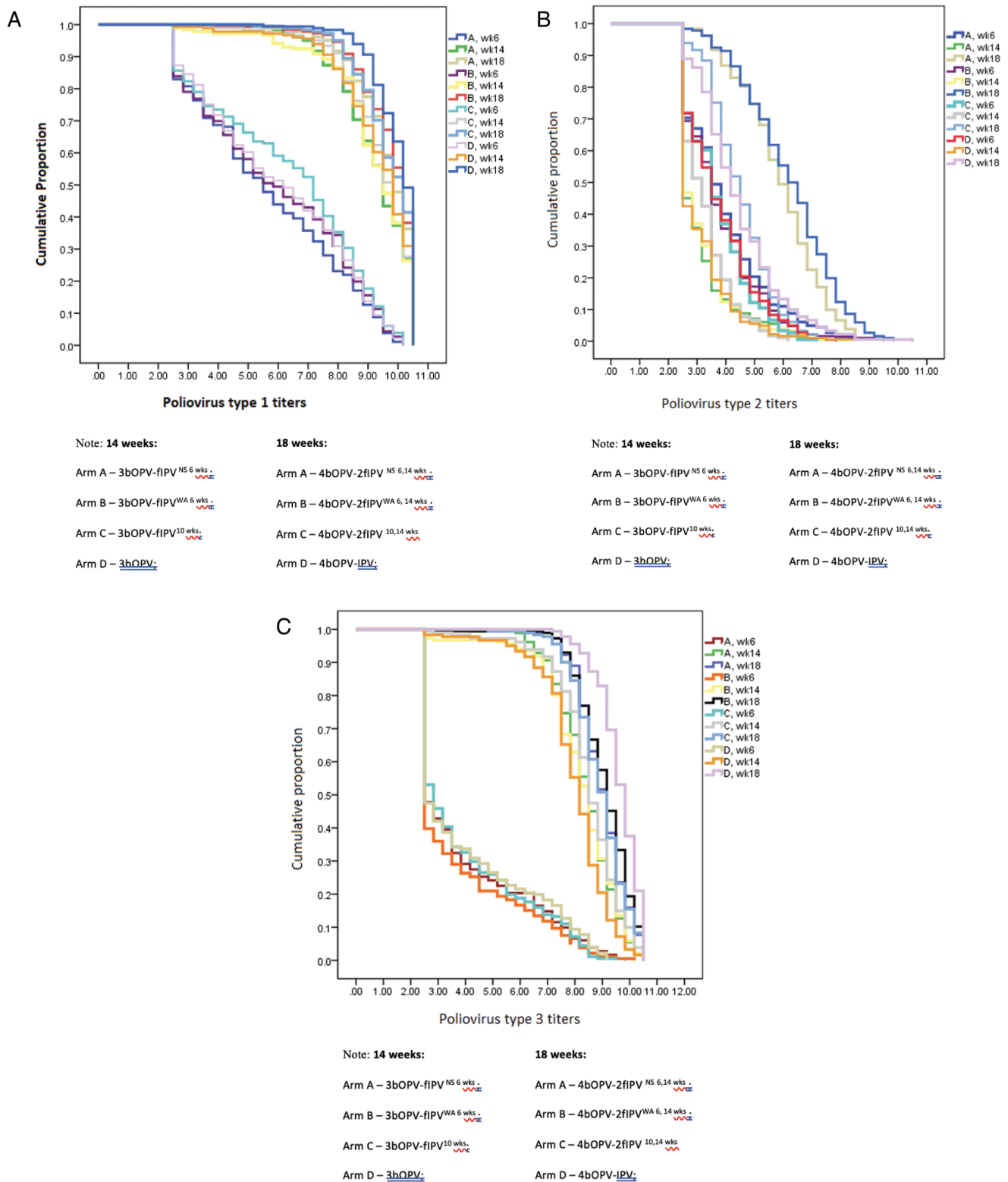
Analyses were done for complete set of observations.

<sup>a</sup>Significant difference between the arms D and C.

<sup>b</sup>Significant difference in titers between arms D and A.

<sup>c</sup>Significant difference in titers between the arms A and C.

<sup>d</sup>Significant difference in titers between the arms D and A.



**Figure 3.** (a) Reverse cumulative distributions for poliovirus type 1 titers in log 2 scale. (b) Reverse cumulative distributions for poliovirus type 2 titers in log 2 scale. (c) Reverse cumulative distributions for poliovirus type 3 titers in log 2 scale.

of 71%-73% per immunized child [22] compared to full single dose of IPV.

The seroconversion of IPV is sensitive to levels of maternally derived antibodies [23–27]. In our study, we confirmed that the

seroconversion rates were higher when fIPV dose was provided at a later age (10 weeks as compared to 6 weeks). However, the findings from Cuba trial had demonstrated even higher seroconversion rates than the current trial wherein fIPV first dose

was administered at much later age of 4 months (~17 weeks) as compared to 6 weeks) or 10 weeks in the current trial.

This trial demonstrated the importance of administering fIPV doses at longer intervals of time. The immunogenicity against poliovirus type 2 when 2 doses of fIPV were administered at 6 and 14 weeks were significantly higher than 2 doses at 10 and 14 weeks (Table 2). The immunogenicity of fIPV administered at 8-week interval outweighed the immunogenicity of fIPV administered at 4-week interval, though the first dose was administered at a later age. This is in line with the finding from a systematic review [27] and a study conducted in Puerto Rico [24].

The cumulative seroconversion for serotypes 1 and 3 at 18 weeks suggested that 2 doses fIPV even closed the remaining immunity gaps to poliovirus types 1 and 3 very effectively. This finding was similar to the study findings from an earlier trial conducted in India [28].

There are several options to facilitate ID administration of fIPV. A study conducted in Pakistan in the year 2015 [14], in low-income areas demonstrated 74% and 68% immune response for serotype 2 with 1 dose fIPV administered using with west/Helm ID adapter and BCG N&S, respectively. The current trial on the other hand showed very low levels of seroconversion rate following 1 dose of fIPV. The possibility of the low seroconversion rates could be explained by the fact that this trial was implemented a year after the switch whereby subjects born in 2017 were naïve (or less likely) to any type 2 exposure as opposed to the pre-switch timings of the Pakistan study.

In terms of ID injection quality, though, there was a variation in the loss of vaccine, wet injections, or quality of ID injections with BCG N&S and West/Helm adapters, there was no significant difference with the use of either device in terms of seroconversion. No correlation of bleb size and loss of antigen (ie, wetness) was found with seroconversion, similar to the finding reported from the Cuban trial [15, 29]. ID administration is technically demanding, and many countries hesitate to adopt their use in RI due to additional health staff training needs. The vaccinators in the trial were highly experienced and trained, they could deliver quality ID injections either with BCG N&S or with the adapters, but the injection quality may vary under field conditions when vaccination is done through out-reach sessions.

Our study had limitations. Because of containment requirements, the type 2 neutralization had to be conducted in a BSL-3 facility, though testing protocols were similar for all 3 serotypes [30]. Unexpectedly, type 2 seroconversion was about 12% (22 subjects) in the Arm D at 14 weeks which had no exposure to type 2 antigen till that time. These subjects had received only bOPV at birth, 6 and 10 weeks. These 22 subjects were distributed across all the 5 study sites as follows—2, 6, 6, 4, and 4 participants each per site, respectively. We explored these findings in greater detail and noted: (1) all of these subjects had very low

antibody titers, just above detectable levels; (2) the definition of seroconversion included a change from non-detectable to detectable antibody (ie, from a reciprocal titer of <8 to >8). The inherent sensitivity and reproducibility of the tests themselves could account for some of the positive subjects; and (3) some exposure of type 2-containing poliovirus vaccines or exposure to type 2 poliovirus cannot be excluded, but this seems very unlikely (based on limited availability of IPV in India during the study implementation phase or the absence of detection of poliovirus type 2 from case and environmental surveillance). Thus, all hypotheses were investigated, and no specific reasons could be uncovered.

In summary, the trial confirmed the excellent immunogenicity of a 2-dose fIPV schedule when administered at 6 and 14 weeks in India. This schedule is both antigen- and cost-saving and could potentially be used in other countries with good immunization programs that may be interested in both, the increased immunogenicity and the cost savings.

## Notes

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