Inactivated poliovirus vaccine given alone or in a sequential schedule with bivalent oral poliovirus vaccine in Chilean infants: a randomised, controlled, open-label, phase 4, non-inferiority study

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Summary

Background Bivalent oral poliovirus vaccine (bOPV; types 1 and 3) is expected to replace trivalent OPV (tOPV) globally by April, 2016, preceded by the introduction of at least one dose of inactivated poliovirus vaccine (IPV) in routine immunisation programmes to eliminate vaccine-associated or vaccine-derived poliomyelitis from serotype 2 poliovirus. Because data are needed on sequential IPV–bOPV schedules, we assessed the immunogenicity of two different IPV–bOPV schedules compared with an all-IPV schedule in infants.

Methods We did a randomised, controlled, open-label, non-inferiority trial with healthy, full-term (>2·5 kg birthweight) infants aged 8 weeks (± 7 days) at six well-child clinics in Santiago, Chile. We used supplied lists to randomly assign infants (1:1:1) to receive three polio vaccinations (IPV by injection or bOPV as oral drops) at age 8, 16, and 24 weeks in one of three sequential schedules: IPV–bOPV–bOPV, IPV–IPV–bOPV, or IPV–IPV–IPV. We did the randomisation with blocks of 12 stratified by study site. All analyses were done in a masked manner. Co-primary outcomes were non-inferiority of the bOPV-containing schedules compared with the all-IPV schedule for seroconversion (within a 10% margin) and antibody titres (within two-thirds $\log_2$ titres) to poliovirus serotypes 1 and 3 at age 28 weeks, analysed in the per-protocol population. Secondary outcomes were seroconversion and titres to serotype 2 and faecal shedding for 4 weeks after a monovalent OPV type 2 challenge at age 28 weeks. Safety analyses were done in the intention-to-treat population. This trial is registered with ClinicalTrials.gov, number NCT01841671, and is closed to new participants.

Findings Between April 25 and August 1, 2013, we assigned 570 infants to treatment: 190 to IPV–bOPV–bOPV, 192 to IPV–IPV–bOPV, and 188 to IPV–IPV–IPV. 564 (99%) were vaccinated and included in the intention-to-treat cohort, and 537 (94%) in the per-protocol analyses. In the IPV–bOPV–bOPV, IPV–IPV–bOPV, and IPV–IPV–IPV groups, respectively, the proportions of children with seroconversion to type 1 poliovirus were 166 (98·8%) of 168, 95% CI 95·8–99·7; 178 (100%), 97·9–100·0; and 175 (100%), 97·9–100·0. Proportions with seroconvsion to type 3 poliovirus were 163 (98·2%) of 166, 94·8–99·4; 177 (100%), 97·9–100·0, and 172 (98·9%) of 174, 95·9–99·7. Non-inferiority was thus shown for the bOPV-containing schedules compared with the all-IPV schedule, with no significant differences between groups. In the IPV–bOPV–bOPV, IPV–IPV–bOPV, and IPV–IPV–IPV groups, respectively, the proportions of children with seroprotective antibody titres to type 1 poliovirus were 168 (98·8%) of 170, 95% CI 95·8–99·7; 181 (100%), 97·9–100·0; and 177 (100%), 97·9–100·0. Proportions to type 3 poliovirus were 166 (98·2%) of 169, 94·9–99·4; 180 (100%), 97·9–100·0; and 174 (98·9%) of 176, 96·0–99·7. Non-inferiority comparisons could not be done for this outcome because median titres for the groups receiving OPV were greater than the assay's upper limit of detection ($\log_2$ titres >10·5). The proportions of children seroconverting to type 2 poliovirus in the IPV–bOPV–bOPV, IPV–IPV–bOPV, and IPV–IPV–IPV groups, respectively, were 130 (77·4%) of 168, 95% CI 70·5–83·0; 169 (96·0%) of 176, 92·0–98·0; and 175 (100%), 97·8–100. IPV–bOPV schedules resulted in almost a 0·3 log reduction of type 2 faecal shedding compared with the IPV-only schedule. No participants died during the trial; 81 serious adverse events were reported, of which one was thought to be possibly vaccine-related (intestinal intussusception).

Interpretation Seroconversion rates against polioviruses types 1 and 3 were non-inferior in sequential schedules containing IPV and bOPV, compared with an all-IPV schedule, and proportions of infants with protective antibodies were high after all three schedules. One or two doses of bOPV after IPV boosted intestinal immunity for poliovirus type 2, suggesting possible cross protection. Additionally, there was evidence of humoral priming for type 2 from one dose of IPV. Our findings could give policy makers flexibility when choosing a vaccination schedule, especially when trying to eliminate vaccine-associated and vaccine-derived poliomyelitis.

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Research in context

Evidence before this study

In 2012, the Strategic Advisory Group of Experts on Immunization (SAGE), the world's main policy formulation body for vaccination, recommended the withdrawal of the type 2 component of oral polio vaccine (OPV) with introduction of bivalent OPV (bOPV) in all countries by 2016, preceded by the introduction of at least one dose of inactivated poliovirus vaccine (IPV) in routine immunisation programmes. This recommendation triggered a clinical trial of IPV–bOPV in Chile in 2012, when no published studies of sequential schedules of IPV and bOPV were available. We subsequently searched the scientific literature for English-language reports published up to June, 2015, with the terms "IPV", "OPV", "bOPV", and "schedule", for other studies of IPV and bOPV in mixed schedules. We did not find any studies that used a sequential schedule of IPV and bOPV in infants. Mixed IPV–OPV schedules have been assessed in Chinese infants at age 2, 3, and 4 months, but with trivalent OPV (tOPV) and not bOPV. Additionally, in India, intestinal immunogenicity from bOPV was compared with IPV or no vaccine in children older than 1 year after previous tOPV, as part of a routine immunisation programme or supplementary immunisation activities. We are not aware of any other published study in which sequential infant routine immunisation schedules of IPV and bOPV were investigated, and where mOPV2 was used to assess intestinal immunity in such schedules.

Added value of this study

We are the first to report data about humoral and intestinal immunogenicity after sequential schedules of IPV–bOPV, to provide scientific evidence related to the SAGE recommendations to introduce IPV globally by 2015, and replace tOPV with bOPV by 2016. Our data establish that infants who receive sequential IPV–bOPV schedules are adequately protected against all three poliovirus types, which is essential evidence for policymakers deciding on which new schedule to adopt.

Implications of all the available evidence

An absence of immunity to type 2 poliovirus after giving bOPV can be compensated by giving one or more doses of IPV before bOPV, which could also prevent vaccine-associated paralytic poliomyelitis and vaccine-derived poliomyelitis from type 2. This strategy will ensure infants will have adequate protection against accidental exposure to type 2 virus after the withdrawal of all type 2-containing live vaccines, as recommended by SAGE. Additionally, our study provides detailed information on the effect of IPV and bOPV in inducing type 2 intestinal immunity—an issue of essential public health importance for better understanding of polio transmission, as we prepare for the global introduction of IPV and switch to bOPV.

Introduction

Successful immunisation programmes with trivalent oral poliovirus vaccines (tOPV) or inactivated poliovirus vaccines (IPV) containing poliovirus types 1, 2, and 3 have eliminated wild-type poliomyelitis in many regions, including the Americas.[1] However, polio-free is not polio risk-free because live-attenuated Sabin viruses from OPV could revert to virulence causing vaccine-associated paralytic poliomyelitis (VAPP), or acquire neurovirulence and transmissibility as circulating vaccine-derived polioviruses (cVDPV).[2]

Although no cases of poliomyelitis caused by naturally circulating wild-type poliovirus type 2 have been reported for more than 15 years, type 2 vaccine-related viruses continue to induce paralysis, causing 26% of cases of VAPP in vaccinees and 31% in contacts, and more than 90% of all cVDPVs in recent years.[3] Thus, WHO's Strategic Advisory Group of Experts on Immunization (SAGE) recommends eliminating type 2 vaccine virus by replacing tOPV with bivalent OPV (bOPV) vaccine containing only types 1 and 3 by April, 2016. To prime new birth cohorts against potential type 2 exposure after withdrawal of the Sabin type 2 vaccine, bOPV should be given with at least one IPV in a sequential or mixed primary schedule.[4] No data are available on intestinal and humoral immunogenicity of IPV–bOPV sequential schedules when used in a 2–4–6-month primary series, but policy makers need to know how effective such schedules will be in providing both individual and population immunity against potential exposure to wild-type polioviruses 1 and 3, and type 2 cVDPV.

Many countries in South and Central America are considering a switch to sequential IPV–bOPV schedules from their three-dose tOPV primary series for protection against polio. Chile is one such representative middle-income country with high three-dose tOPV coverage (90%), which is considering the switch to a sequential IPV–bOPV schedule.[5] Because the first vaccination dose will have the highest compliance rate and the risk of VAPP is highest with the first dose of OPV, countries choosing such sequential schedules will probably use one or two doses of IPV followed by bOPV. In this phase 4 study we therefore aimed to examine immunogenicity of an all-IPV schedule or sequential IPV and bOPV schedules in Chilean infants, focusing on humoral responses and intestinal immunity.

Methods

Study design and participants

We did this multicentre, randomised, controlled, three-arm, open-label, non-inferiority study at six well-child clinics in community health-care centres in Santiago, Chile. We undertook the study under the auspices of the Chilean Ministry of Health following guidelines from Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by local ethics committees

from the Faculty of Medicine at the University of Chile, the Servicio de Salud Metropolitano Norte, and the Servicio de Salud Metropolitano Sur (all located in Santiago, Chile).

Parents of infants attending the clinics for their first polio vaccinations were approached to enrol their child. Eligible participants were healthy, full-term infants aged 8 weeks (±7 days) with no obvious medical disorders, who weighed more than 2·5 kg at birth. At screening, infants were excluded if they had a sibling who had received, or was scheduled to receive, tOPV during the 6 months before or after the study, to avoid passive exposure to vaccine viruses. Other exclusion criteria were typical for vaccine studies—ie, any disorder or treatment likely to interfere with normal immune responses to vaccination, or known allergy to vaccine components. Participants were excluded from any supplementary polio immunisation activity during the study. Parents or guardians of the participants gave written informed consent before their enrolment. During the study period no mass campaigns were undertaken with trivalent OPV (tOPV). The only use of tOPV was in routine immunisation for children who were not part of the study.

Randomisation and masking

We randomly allocated eligible infants (1:1:1) to one of three polio vaccination schedules: IPV at age 8 weeks and bOPV at age 16 and 24 weeks (IPV–bOPV–bOPV); IPV at age 8 and 16 weeks and bOPV at age 24 weeks (IPV–IPV–bOPV); or IPV at age 8, 16, and 24 weeks (IPV–IPV–IPV). We did the allocation using randomisation lists supplied by the study funder with blocks of 12, stratified for the six study sites (appendix).

Although the families of the study participants and the study physicians could not feasibly be masked to treatment at vaccination because of the evident differences between the bOPV and IPV vaccines, all subsequent analyses were done in a masked manner.

Procedures

IPV was delivered by injection, and bOPV was given as oral drops; both were provided by Sanofi Pasteur. We gave the polio vaccinations concomitantly with the diphtheria, tetanus, pertussis, hepatitis B, and haemophilus influenzae type b (DTP-HBV-Hib) vaccine (Quinvaxem, Novartis Vaccines, Marburg, Germany) and ten-valent pneumococcal conjugate vaccine (Synflorix, GlaxoSmithKline, Rixensart, Belgium), in accordance with the Chilean national childhood vaccination schedule. We also provided oral rotavirus vaccine (Rotarix, GlaxoSmithKline, Rixensart, Belgium) at ages 8 and 16 weeks (full details of all vaccines are provided in the appendix). For all groups, we obtained blood samples at age 8 weeks before the first vaccination and age 28 weeks for assessment of seroconversion and seroprotection. We also obtained a blood sample at age 16 weeks from infants in the IPV–bOPV–bOPV group, and at age 24 weeks from infants in the IPV–IPV–bOPV and the IPV–IPV–IPV groups. We challenged participants with monovalent OPV type 2 poliovirus (mOPV2 [GlaxoSmithKline]) given as oral drops at age 28 weeks, and obtained another blood sample 1 week later at age 29 weeks.

We obtained stool samples (5–10 mg) before mOPV2 challenge at week 28, then once per week up to week 32. Blood and stool samples were transported within 24 h in appropriate cold-chain conditions to a central laboratory (Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile) for processing and storage before sending to the Polio and Picornavirus Laboratory (US Centers for Disease Control and Prevention, Atlanta, GA, USA) for analysis. Under appropriate cold-chain conditions, blood samples were allowed to coagulate at the participating sites and then sent to the central laboratory where serum was separated by centrifuge (2500 rpm for 10 min) into three aliquots and sent to the Polio and Picornavirus Laboratory. Stool samples were processed and poliovirus isolated in L20b cells using a modification of the WHO polio diagnostic algorithm,[6] with poliovirus confirmed by real-time PCR (rtPCR) in samples positive for virus in cell-culture isolation.[7] We measured viral titres in mOPV2-positive stool samples using a modified WHO cell sensitivity assay.[6] We used dilutions resulting in at least 80% destruction of the cell monolayer to calculate the viral titre as the 50% endpoint cell-culture infectious dose ($CCID_{50}$), expressed as the log ($CCID_{50}$).

Outcomes

The coprimary objectives were non-inferiority comparisons of the sequential schedules, IPV–bOPV–bOPV, or IPV–IPV–bOPV, with an all-IPV regimen for seroconversion and antibody titres to poliovirus serotypes 1 and 3 at week 28 (age 28 weeks) in the per-protocol population.

See Online for appendix

Secondary objectives were poliovirus serotype 2 responses (ie, titres, seroconversion and seroprotection rates, and viral shedding) in the three study groups after the three-dose vaccination series at age 28 weeks and 29 weeks (1 week after the mOPV2 challenge). mOPV2 shedding in stool samples was examined from age 28 to 32 weeks. Results are expressed as proportions with seroprotective titres (≥8) and seroconversion rates. Seroconversion was judged to have been achieved by a subsequent timepoint if the type-specific titre measured at that time was ≥8 and more than four times higher than expected titres of maternally derived antibodies, which were computed from the recorded titre at baseline, assuming an exponential decay with a half-life of 24 days.[8]

We monitored participants for 30 min after each vaccination to ensure no allergic reactions occurred, but because all the study vaccines are licensed we did not formally record local or systemic reactions unless these resulted in a medical consultation. Safety was assessed in

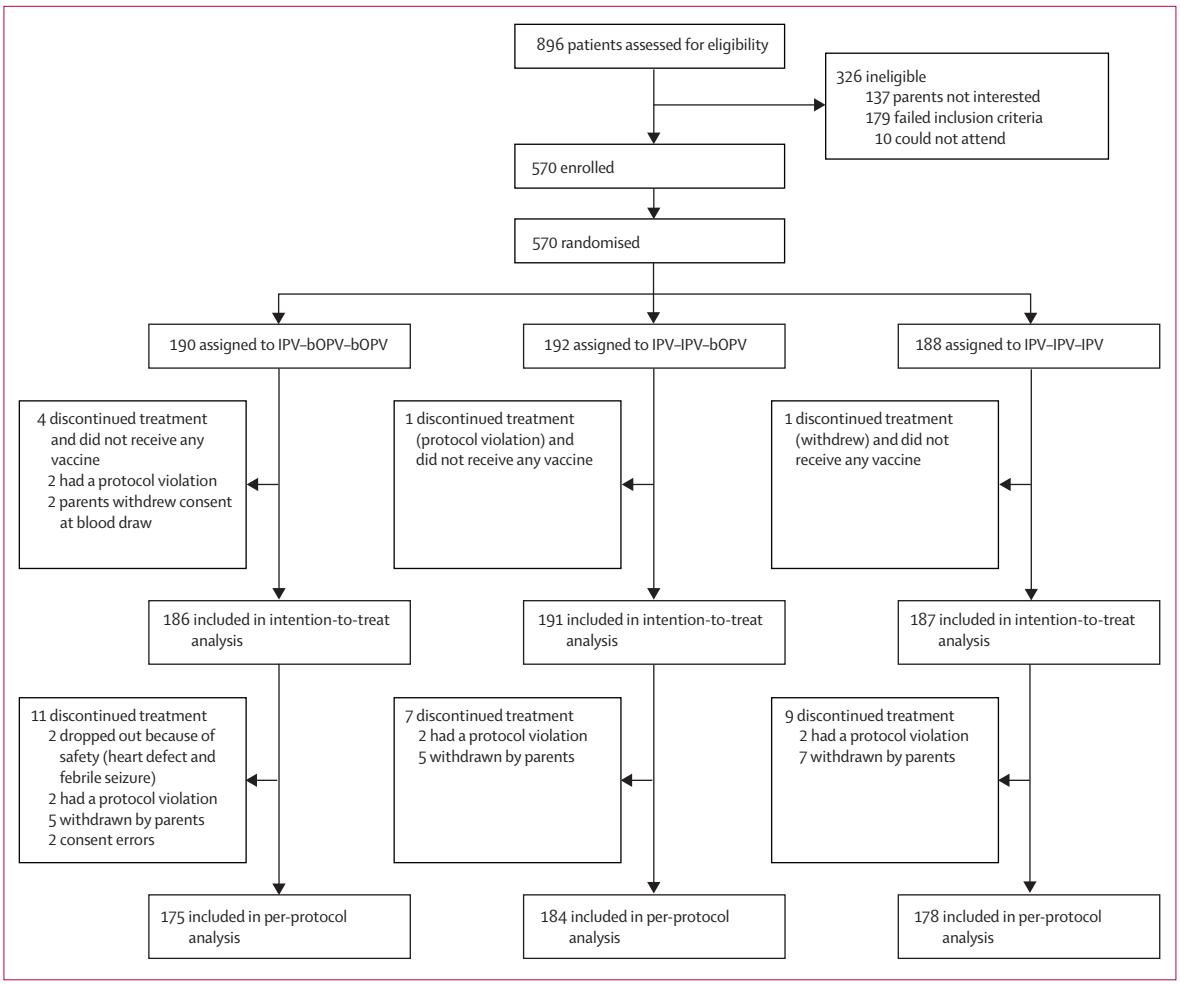

Figure 1: **Trial profile**
IPV=inactivated poliovirus vaccine. bOPV=bivalent oral poliovirus vaccine.

participants in the intention-to-treat cohort who received at least one vaccination, for serious adverse events (defined as death or life-threatening events that needed admission to hospital or caused persistent or significant disability) and important medical events (defined as medically significant events that were not serious adverse events but needed medical intervention or consultation). We monitored safety up to and including a final follow-up about 5 months after the last vaccination. Serious adverse events and important medical events were reported to an independent data safety monitoring board to monitor for unexpected safety signals. Members of the board were experts from different countries who met at periodic intervals in different cities throughout the study.

Statistical analysis

We assumed a seroconversion rate of at least 90% for the all-IPV schedule for poliovirus types 1 and 3 and a 20% dropout rate, and therefore estimated a sample size of 190 (152 assessable) to provide 80–86% power (depending on the degree of correlation among the type-specific tests) to declare overall non-inferiority of each sequential IPV–bOPV regimen compared with the all-IPV regimen. We used nominal, one-sided α levels of 0·05 for these tests. Defined non-inferiority criteria were seroconversion rates being no more than 10% less than those noted for the all-IPV regimen, and median log antibody titres no more than two-thirds $\log_2$ less than those for the all-IPV regimen. Overall, a regimen was deemed non-inferior if statistical non-inferiority was shown for both serotypes 1 and 3 for seroconversion and antibody endpoints. We tested differences between groups at week 28 using a log-rank test.[9]

Immunogenicity and shedding analyses were done for the per-protocol population who received all vaccinations. 95% CIs for immunogenicity assessments were based on the Wilson score method.

We assessed the effect of IPV–bOPV and all-IPV schedules on type 2 poliovirus shedding after the mOPV2 challenge using a shedding index. We computed this endpoint for each participant as the area under the virus-concentration curve (AUC), based on stool $\log_{10}$ viral

titres at days 7, 14, 21, and 28 after mOPV2 challenge, using a trapezoid rule for computing area and assigning to zero values of $\log_{10}$ viral titre for measurements below the assay limit of detection (10^2 $CCID_{50}$ per mL). We tested differences between shedding indices by the Wilcoxon test; p values were assessed by ANOVA.

Neutralising antibodies against poliovirus types 1, 2, and 3 were assessed according to established protocols[10,11] in all groups at baseline (age 7–8 weeks), after the full course (28 weeks), and 1 week after challenge with mOPV2 (29 weeks). Responses were also measured 2 months after one dose (at age 16 weeks in the IPV–bOPV–bOPV group) or two doses (at age 24 weeks in IPV–IPV–bOPV and IPV–IPV–IPV groups) of IPV. Neutralisation titres, estimated by the Spearman-Kärber method,[12] were reported as the reciprocal of the calculated 50% endpoint, with a maximum value of 10·5 $\log_2$ titre. Since many individuals had immune responses at or above the assay's upper limit of detection, we calculated median rather than geometric mean titres.

	IPV–bOPV–bOPV (n=184)	IPV–IPV–bOPV (n=189)	IPV–IPV–IPV (n=185)
Age (days)	57 (54–61)	57 (55–61)	58 (54–61)
Sex			
Male	92 (50%)	100 (53%)	91 (49%)
Female	92 (50%)	89 (47%)	94 (51%)
Weight (kg)	5·2 (4·8–5·6)	5·3 (4·9–5·8)	5·2 (4·8–5·7)
Proportion being breastfed			
At week 8	171/184 (93%)	181/189 (97%)	176/185 (95%)
At week 16	149/180 (83%)	163/186 (88%)	154/176 (88%)
At week 24	131/176 (74%)	145/185 (79%)	146/176 (83%)
At week 29	120/175 (69%)	135/185 (73%)	129/176 (73%)
Proportion in day care			
At week 8	1/184 (1%)	0	0
At week 16	0	0	1/176 (1%)
At week 24	7/175 (4%)	4/185 (2%)	3/176 (2%)
At week 29	14/175 (8%)	12/185 (6%)	8/176 (5%)
Number of family members	5 (4–6)	5 (4–6)	5 (4–6)

Data are median (IQR) range or n (%).

Table 1: **Baseline characteristics of vaccinated infants in the intention-to-treat population**

Role of the funding source

One of the authors of this report (ASB) is an employee of the funder of the study, and was involved in study design, data analysis, data interpretation, and writing of the report. The funder had no role in data collection. All authors had full access to all the data in the study and shared final responsibility for the decision to submit for publication.

Results

Between April 25, and Aug 1, 2013, we screened 896 infants, and enrolled and randomly assigned 570 to treatment (figure 1). Six of these infants did not receive any vaccination because of protocol violations or they were withdrawn by parents before vaccination. 564 (99%) infants were therefore included in the intention-to-treat cohort and 537 (94%) were vaccinated according to protocol (and thus included in per-protocol analyses; figure 1). Across groups, the 33 dropouts were mainly due to withdrawal by parents (n=20) or protocol deviations (n=9), with two being incorrectly enrolled, and two safety dropouts. Demographic characteristics of the three study groups were similar for all variables (table 1).

At baseline (age 8 weeks), 288 (54%) of 530 infants had seroprotective titres against type 1 and 111 [21%] of 529 infants (one sample was excluded because not enough volume was available to confirm results) had seroprotective titres against type 3, presumably due to maternally derived antibodies. Seroconversion and seroprotection rates after each dose are shown in table 2. The non-inferiority objective for seroconversion was met for both IPV–bOPV regimens, with seroconversion to serotypes 1 and 3 being more than 98% in all three groups after completion of the three-dose series. The non-inferiority comparison for antibody titres could not be done because median antibody titres for groups receiving any OPV were greater than the assay's upper limit of detection ($\log_2$ titres >10·5). In the IPV–bOPV–bOPV group, two participants did not seroconvert to poliovirus type 1, and three did not seroconvert to type 3. In the IPV–IPV–IPV group, two children did not seroconvert to type 3.

Figure 2 shows that against a background of waning maternally derived antibodies, median titres rose slightly (<1 log) after one IPV dose (in the IPV–bOPV–bOPV group at 16 weeks), but showed larger responses (>5 logs) at 24 weeks after a second IPV dose (in the IPV–IPV–bOPV and all-IPV groups) and a further small (about 1 log) increase after a third dose. At week 28, median log titres for serotype 1 were significantly higher in the IPV–bOPV–bOPV and IPV–IPV–bOPV groups (>10·5 [95% CI 10·5–10·5] for both) than in the all-IPV group (9·5 [95% CI 9·2–9·8]), and median log titres for serotype 3 were greater than 10·5 in all groups.

Overall, 337 (63·7%) of 529 participants had seroprotective titres against poliovirus serotype 2 at baseline (table 2). 4 weeks after the full vaccination series (ie, age 28 weeks), more than 75% of the IPV–bOPV–bOPV group, and more than 96% of the IPV–IPV–bOPV and all-IPV groups had seroconverted and had seroprotective titres. From week 16, 2 months after their only IPV dose, to week 28, seroconversion and seroprotection rates in the IPV–bOPV–bOPV group against serotype 2 rose substantially (table 2); eg, the seroprotection rate rose from 62·1% (95% CI 54·7–68·9) to 80·6% (74·0–85·8; p<0·0001), with a commensurate increase in median log titres from 3·5 (95% CI 3·2–3·8) to 4·8 (4·2–5·5, p<0·0001; figure 2).

At week 28, after completing their vaccinations, 33 of 170 (19%) children assessable for serology in the

	Polio serotype 1			Polio serotype 2			Polio serotype 3		
	IPV–bOPV–bOPV	IPV–IPV–bOPV	IPV–IPV–IPV	IPV–bOPV–bOPV	IPV–IPV–bOPV	IPV–IPV–IPV	IPV–bOPV–bOPV	IPV–IPV–bOPV	IPV–IPV–IPV
Week 8									
Seroprotective antibody titres	93/173 (53·8%, 46·3–61·0)	99/181 (54·7%, 47·4–61·8)	96/176 (54·5%, 47·2–61·7)	112/173 (64·7%, 57·4–71·5)	112/180 (62·2%, 54·9–69·0)	113/176 (64·2%, 56·9–70·9)	39/172 (22·7%, 17·1–29·5)	35/181 (19·3%, 14·2–25·7)	37/176 (21·0%, 15·7–27·6)
Week 16									
Seroprotective antibody titres	121/174 (69·5%, 62·3–75·9)	..	..	108/174 (62·1%, 54·7–68·9)	..	..	73/174 (42·0%, 34·9–49·4)	..	..
Seroconversion	86/172 (50·0%, 42·6–57·4)	..	..	77/172 (44·8%, 37·5–52·0)	..	..	62/171 (36·3%, 29·4–43·7)	..	..
Week 24									
Seroprotective antibody titres	..	179/182 (98·4%, 95·3–99·4)	174/176 (98·9%, 96·0–99·7)	..	179/182 (98·4%, 95·3–99·4)	174/176 (98·9%, 96·0–99·7)	..	172/182 (94·5%, 90·2–97·0)	169/175 (96·6%, 92·9–98·4)
Seroconversion	..	169/179 (94·4%, 90·0–96·9)	167/174 (96·0%, 91·9–98·0)	..	166/178 (93·3%, 88·6–96·1)	158/174 (90·8%, 85·6–94·3)	..	168/179 (93·9%, 89·3–96·5)	167/173 (96·5%, 92·6–98·4)
Week 28									
Seroprotective antibody titres	168/170 (98·8%, 95·8–99·7)	181/181 (100·0%, 97·9–100)	177/177 (100%, 97·9–100·0)	137/170 (80·6%, 74·0–85·8)	177/180 (98·3%, 95·2–99·4)	177/177 (100·0%, 97·9–100)	166/169 (98·2%, 94·9–99·4)	180/181 (100·0%, 97·9–100·0)	174/176 (98·9%, 96·0–99·7)
Seroconversion	166/168 (98·8%, 95·8–99·7); p=0·1477 *vs* IPV–IPV–IPV	178/178 (100%, 97·9–100·0); p=0·1443 *vs* IPV–bOPV–bOPV, p=1·0000 *vs* IPV–IPV–IPV	175/175 (100%, 97·9–100·0)	130/168 (77·4%, 70·5–83·0); p<0·0001 *vs* IPV–IPV–IPV	169/176 (96·0%, 92·0–98·0); p<0·0001 *vs* IPV–bOPV–bOPV p=0·0077 *vs* IPV–IPV–IPV	175/175 (100·0%, 97·8–100·0)	163/166 (98·2%, 94·8–99·4); p=0·6145 *vs* IPV–IPV–IPV	177/177 (100%, 97·9–100); p=0·0724 *vs* IPV–bOPV–bOPV p=0·1526 *vs* IPV–IPV–IPV	172/174 (98·9%, 95·9–99·7)
Week 29									
Seroprotective antibody titres	..	..	..	146/159 (91·8%, 86·5–95·2)	172/173 (99·4%, 96·8–99·9)	172/172 (100·0%, 97·8–100·0)	..	..	..
Seroconversion	..	..	..	142/157 (90·4%, 84·8–94·1); p=0·0001 *vs* IPV–IPV–IPV	165/169 (97·6%, 94·1–99·1); p<0·0056 *vs* IPV–bOPV–bOPV p=0·0436 *vs* IPV–IPV–IPV	170/170 (100·0%, 97·8–100·0)	..	..	..

Data are n/N (%, 95% CI). ..=not applicable.

Table 2: **Seroprotection and seroconversion rates**

IPV–bOPV–bOPV group had not seroconverted and remained seronegative for type 2. Only 170 of 175 children were assessable because the parents of the other five participants did not return them at week 28 to provide blood samples. At week 29, 1 week after the mOPV2 challenge, a further 19 of 29 (65·5%) participants in this group who had serum samples available for assessment had seroconverted, suggesting they had been primed. Thus, at week 29, roughly 92% of children in the IPV–bOPV–bOPV group were either seroprotected or primed against poliovirus type 2 (table 2). In the IPV–IPV–bOPV group, three of 180 infants remained seronegative for type 2 after vaccination. Two of these children seroconverted 1 week after mOPV2 challenge, making a total of 21 of 32 (65·6%) assessable infants who seroconverted 1 week after challenge. Median titres for type 2 rose in all three groups after challenge, with the biggest proportional increase in the IPV–bOPV–bOPV group (figure 2).

Interference with vaccine responses due to maternally derived type 2 antibodies at baseline is shown in figure 3A. At week 28, significantly lower seroconversion rates were noted in infants in the IPV–bOPV–bOPV group who were seropositive at baseline compared with those who were seronegative (p=0·0005; appendix). All 11 participants in the IPV–bOPV–bOPV group who were not primed at week 28 were seropositive at baseline, with high antibody titres (range $\log_2$ 4·8–9·2). Median baseline titres (at 8 weeks) were highest in non-responders, then those who only seroconverted after oral

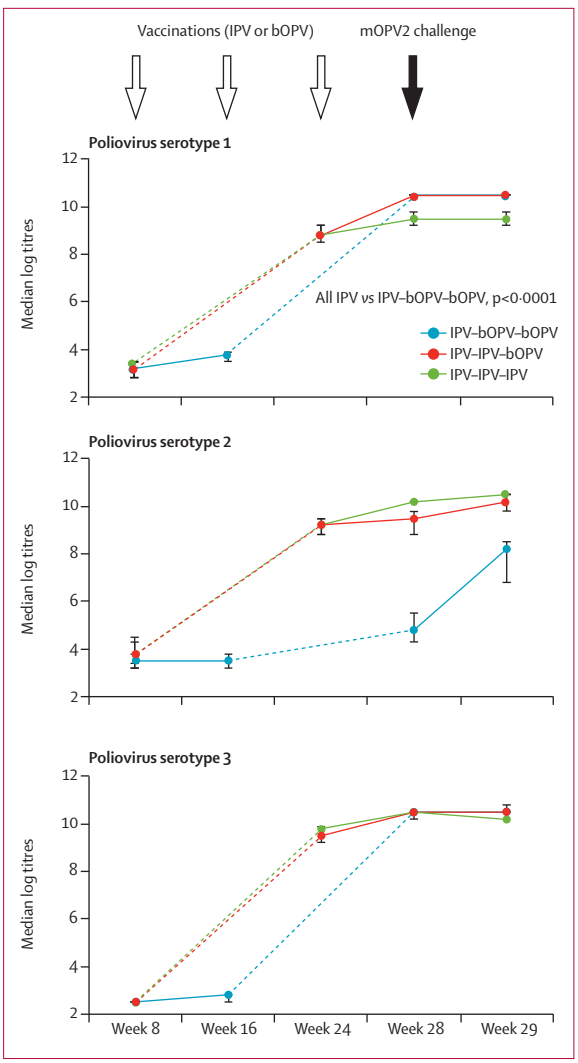

Figure 2: **Median titres of serum neutralising antibodies against poliovirus serotypes 1, 2, and 3 in the three study groups**
Titres are shown at the different timepoints with 95% CI bars. IPV=inactivated poliovirus vaccine. bOPV=bivalent oral poliovirus vaccine. mOPV2=monovalent type 2 oral poliovirus vaccine.

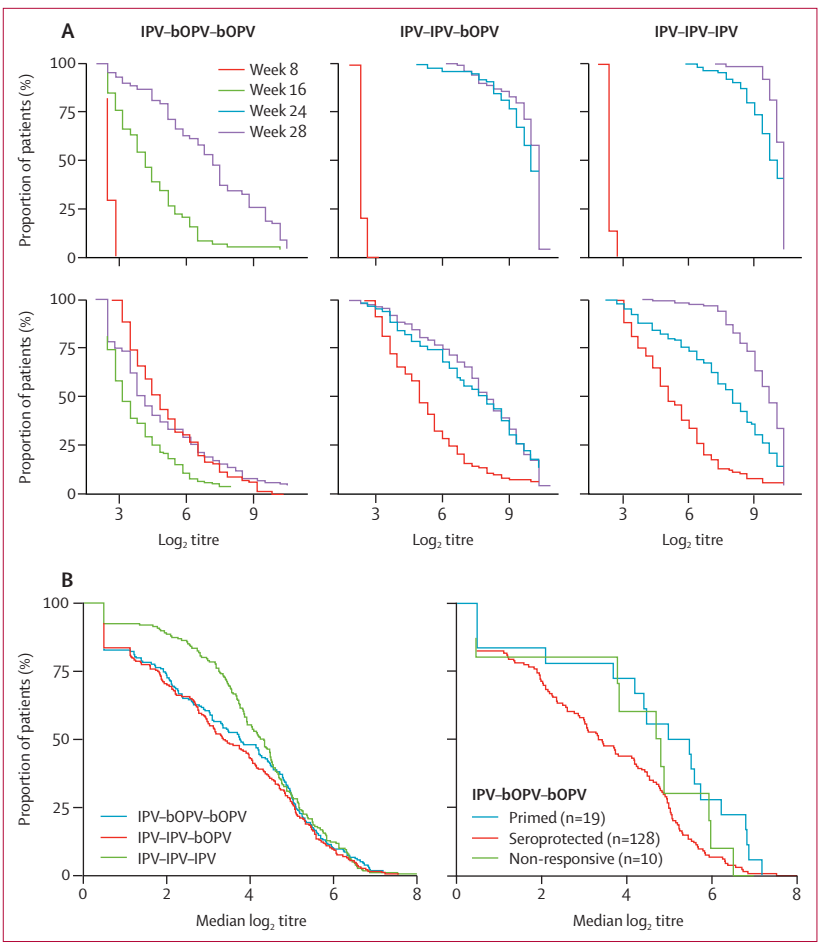

Figure 3: **Serum poliovirus type 2 neutralising antibody titres (A) and faecal shedding indices for serotype poliovirus (B)**
(A) Reverse cumulative distribution curves shown for antibody titres in participants who were initially seronegative (upper row) or seropositive (lower row) at baseline. (B) Proportions (%) with type 2 shedding indices shown for all groups (left panel) and for IPV–bOPV–bOPV according to immune status (right panel). Seroprotected=titre ≥8 at age 28 weeks. Primed=seronegative at age 28 weeks, but seropositive at age 29 weeks. Non-responders=seronegative at age 28 and 29 weeks. IPV=inactivated poliovirus vaccine. bOPV=bivalent oral poliovirus vaccine.

challenge, and lowest in children who had seroconverted by 28 weeks (appendix).

Of 531 infants tested on the day of mOPV2 challenge, ten (1·9%) were already shedding type 2 virus (table 3). Viral shedding peaked 1 week after challenge, with virus present in stools from 80·5% of group IPV–bOPV–bOPV, 77·7% of group IPV–IPV–bOPV, and 92·4% of group IPV–IPV–IPV. Despite a gradual decline in these proportions, 42–58% were still shedding type 2 virus 4 weeks after challenge. Median faecal titres in those shedding the virus (table 3) also peaked at 1 week, and despite declining by day 14, concentrations were still higher than baseline through day 28.

The median type 2 shedding index was significantly higher for children who received the all-IPV schedule compared with the children in the groups receiving IPV and bOPV (p=0·0131 compared with the IPV–bOPV–bOPV group and p=0·0005 compared with the IPV–IPV–bOPV group; table 3). The largest differences took place at the lower concentrations of shedding (figure 3B). When the immune response of infants in the IPV–bOPV–bOPV group at age 28 and 29 weeks was assessed, only those seroprotected at week 28, but not those who were primed (seroconversion between weeks 28 and 29 after challenge), had a lower shedding index than seronegative children (figure 3).

Importantly, the pre-challenge serum antibody titres showed a significant linear relation with the shedding index (appendix). Although the slopes of the linear relations were not significantly different, they shifted down incrementally with each bOPV dose received such that the predicted shedding index for the same antibody titre was

	(IPV–bOPV–bOPV)		(IPV–IPV–bOPV)		(IPV–IPV–IPV)	
	Shedders (% [n/N])	Median (95% CI) log_2 titres in shedders	Shedders (% [n/N])	Median (95% CI) log_2 titres in shedders	Shedders (% [n/N])	Median (95% CI) log_2 titres in shedders
Days from mOPV2 challenge						
0	2·9% (5/173)	3·2 (··)	1·1% (2/181)	6·3 (··)	1·7% (3/177)	2·9 (··)
7	80·5% (132/164)	6·5 (6·2-6·7)	77·7% (139/179)	6·4 (6·2-6·6)	92·4% (158/171)	6·7 (6·5-6·9)
14	66·7% (110/165)	4·8 (4·3-5·0)	68·2% (122/179)	4·9 (4·4-5·2)	84·4% 146/173	5·2 (4·9-5·5)
21	60·9% (103/169)	4·6 (4·3-5·0)	52·8% (94/178)	4·6 (4·0-4·9)	66·5% 115/173	4·0 (3·7-4·5)
28	45·9% (79/172)	4·8 (3·9-5·3)	42·0% (76/181)	4·5 (3·8-5·1)	58·0% 102/176	3·9 (3·5-4·4)
Shedding index endpoint	··	(n=154) 3·5 (2·8-4·2); p=0·4713 *vs* IPV–IPV–bOPV, p=0·0131 *vs* IPV–IPV–IPV	··	(n=170) 3·1 (2·6-3·8); p=0·0005 *vs* IPV–IPV–IPV	··	(n=166) 4·1 (3·7-4·3)

Data from the per-protocol population. ··=not applicable.

Table 3: **Proportions shedding poliovirus serotype 2 after mOPV2 challenge**

lower in IPV–bOPV–bOPV than in IPV–IPV–bOPV, and lower in IPV–IPV–bOPV than in IPV–IPV–IPV (p<0·0001). Thus, after adjusting for the effect of pre-challenge titres, mean type 2 shedding among children who received at least one dose of bOPV was significantly lower than those who had received 3 doses of IPV.

In general, all vaccines given during the study were well tolerated, with no fatalities. Overall, 81 serious adverse events were reported up to visit 8 (ie, 28 days from the mOPV2 challenge at age 28 weeks), 66 of which occurred between weeks 8 and 23. No significant differences in occurrences of serious adverse events were noted between the study groups. Only one serious adverse event was considered as vaccine related (appendix) and was judged as indeterminate in a child admitted for surgery for intestinal intussusception 4 days after receiving the mOPV2 challenge at age 7 months. Similarly, 249 important medical events occurred, distributed equally across the study groups, and most (199) were reported during weeks 8–23 (appendix). Only one important medical event, a case of pain at the vaccination site, was considered as related to vaccination (appendix).

Discussion

To our knowledge, we are the first to compare the immunogenicity of an all-IPV schedule with sequential IPV and bOPV schedules in a phase 4 study. We showed that seroconversion rates against polioviruses types 1 and 3 were non-inferior in sequential schedules containing IPV and bOPV, compared with an all-IPV schedule, and proportions of protective antibodies were high with all three. For poliovirus type 2, the IPV–bOPV–bOPV schedule with one type 2 immunisation achieved seroconversion in 77·4% of infants at 28 weeks.

With the increasing likelihood of success in global eradication of wild-type poliovirus, evidence-based polio endgame strategies should be agreed upon and implemented. Increased availability and affordability of IPV for developing countries will be important prerequisites to ensure global withdrawal of tOPV in 2016,[13] and eventually of all OPV by 2019 to stop all vaccine-related polio disease, including VAPP, and sustain eradication of all polioviruses. In view of the persisting circulation of both wild-type 1 poliovirus and Sabin type 2 causing cVDPV,[2,3] the SAGE recommends using bOPV (containing types 1 and 3) for routine immunisation to replace tOPV.[4,13] At least one dose of IPV is recommended in such bOPV schedules to prime the population against the risk of type 2 wild-virus disease (eg, from a break in laboratory containment, as happened in India in 2002–03[14]) or emergence of VDPV. For countries opting for a mixed schedule beginning with bOPV with only one subsequent dose of IPV, IPV is recommended to be given at 14 weeks of age with DTP3-HBV-Hib vaccine, or later, to ensure high immunogenicity because interference with maternally derived antibodies in early infancy is regarded as the biggest factor for vaccine failure with IPV.[15] Therefore, although estimates suggest that the coverage with one dose of IPV at age 14 weeks or later will be lower compared with giving it at younger ages, because of children leaving the immunisation schedule, the gain in immunogenicity is believed to outweigh the risk of lower coverage.[16] A previous study has shown that IPV boosts intestinal immunity in Indian children aged 1–4 years previously vaccinated with tOPV.[17] Our study showed for the first time that in infants, sequential schedules of IPV followed by bOPV were non-inferior to an all-IPV schedule in eliciting systemic immune responses to polio serotypes 1 and 3, giving policy makers flexibility in choosing different schedules, particularly when trying to eliminate VDPV and VAPP.

We noted that 54·3%, 63·7%, and 21·0% of infants aged 8 weeks had seroprotective antibody titres against poliovirus serotypes 1, 2, and 3 respectively, most probably suggesting high titres of maternal antibodies in the Chilean population. Despite the interference of

maternal antibodies with vaccination, as suggested by the lower responses in initially seropositive infants, all three schedules achieved high levels of humoral immunity against serotypes 1 and 3. Quantitatively, sequential IPV–bOPV schedules resulted in higher antibody titres against serotypes 1 and 3 than the all-IPV schedule. Non-inferiority for antibody titres could not strictly be established because of the very high rate of censored responses at the upper limit of assay detection. Any differences are unlikely to be clinically relevant, in view of the amount of protection associated with $10 \cdot 5$ $\log_2$ antibody titres and seroprotection rates of 98–100%, although some benefits could arise from enhanced mucosal immunity due to bOPV use.

For type 2 poliovirus, the IPV–bOPV–bOPV schedule with one type 2 immunisation achieved seroconversion in 77·4% of infants at 28 weeks, which is a reassuring finding. This included a rise of roughly 30% in seroconversion rate between weeks 16 and 28, without giving a type-2-containing vaccine, although the corresponding increases in median titres between weeks 16 and 28 in this group were only slight. One possible explanation could be the response of IPV-vaccinated infants to passive exposure from Sabin type 2 virus circulating in the environment due to the concurrent use of tOPV in the national vaccine schedule. However, the exclusion of infants living with a sibling scheduled to receive tOPV during the study should have minimised this factor. Alternatively, the rise in type 2 seroconversion could be due to heterotypic immune responses in children previously primed by one IPV dose, an occurrence previously noted both naturally and experimentally.[18,19] A delayed response to vaccination in children with maternal antibodies might also play a part, as suggested by the fact that almost 80% of children who only seroconverted between weeks 28 and 29 were seropositive at baseline.

The rapid seroresponse noted 1 week after the mOPV2 challenge in seronegative children suggests immune priming by IPV for type 2. Our data suggest that by following the global recommendation to use mOPV2 to control outbreaks of wild-type 2 or cVDPV2 after the switch to bOPV, systemic immunity against type 2 could be achieved in more than 90% of infants previously given one dose of IPV containing type 2.

Faecal shedding of type 2 poliovirus after mOPV2 challenge was significantly lower in the sequential IPV–bOPV schedules compared with the all-IPV schedule, despite fewer doses with type-2-containing vaccines. This induction of cross-protective intestinal immunity from bOPV to type 2 in IPV–bOPV regimens would not only be expected to provide enhanced individual immunity,[20] but could decrease transmission of type 2 wild virus or vaccine-related virus after tOPV is withdrawn from vaccination programmes. However, although the difference is significant, the magnitude of the difference in shedding index endpoints between IPV–bOPV and all-IPV groups was small (about $0 \cdot 3$ logs during days 7 to 14). The clinical and epidemiological effect of this cross-protection for type 2 from bOPV is unknown.

A limitation of our study was the inability to assess the extent of passive exposure from type 2 poliovirus derived from tOPV being used in routine immunisation, on the immune responses noted in participants. Some exposure to Sabin viruses from tOPV in the environment almost certainly occurred, despite the effort to minimise this effect in the exclusion criteria. Other limitations are the uncertainties about the maternal origin of the 8-week serum antibodies, and the significance of the recorded seroconversion 1 week after mOPV2 challenge. Although most of this seroconversion was probably caused by priming, it could also be partly due to prompt immune responses in non-primed children. The results of this study are relevant for Latin America, and regions and countries where the infant schedule is 8, 16, and 24 weeks, but are less so for regions that use the 6, 10, and 14 weeks schedule from the Expanded Program on Immunization, for which maternal antibodies will have a more important role. However, as the shift from tOPV to bOPV in routine immunisation leads to elimination of type 2 circulation and lower maternal exposure, maternal antibodies to type 2 will wane over time, thereby decreasing any effect.

Our prospective assessment showed that serological responses against poliovirus types 1 and 3 were non-inferior in sequential schedules containing IPV and bOPV, compared with an all-IPV regimen. If priming, elicited by one dose of IPV to type 2 poliovirus and shown by the rapid seroconversion after mOPV2 challenge is protective against paralytic disease, the overall protection rate against type 2 after an IPV–bOPV–bOPV schedule would be up to 92%, compared with 98–100% noted after schedules with two or three IPV doses. Also, because mOPV2 will be the vaccine of choice for type 2 outbreak responses in the future, the rapid antibody response triggered by mOPV2, as shown in this study, would allow for prompt development of immunity in the population at risk in an outbreak situation. All infants who were seronegative at baseline were protected or primed for type 2 by one dose of IPV, but inclusion of bOPV induced better intestinal immunity to type 2 than the all-IPV schedule, suggesting possible heterotypic type 2 immunity derived from types 1 or 3 in bOPV. This novel set of data for IPV–bOPV sequential schedules will be essential for policy formulation by national and global authorities to enable and sustain polio eradication.

Contributors

MO'R, ASB, RV, JN, EJA, RC, WO, JJ, RR, and SACC all contributed to the study design. SS and BRB prepared the initial statistical design and analysed the final data. MO'R, RV, ME, and JN enrolled co-investigators and participants, and coordinated vaccinations, blood draws, and data collection. WCW and MSO did all the viral assays.

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Declaration of interests
We declare no competing interests.

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