# Peer Review File

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**Reviewer Comments**

**Comment 1: This study is a post-hoc analysis of previous data from China. As such, it is not clear what additional information this paper provides.**

Reply 1: The data in these two clinical trials have not been published previously. There are some reports about clinical trials of sequential immunization with inactivated poliovirus vaccine (IPV) and oral polio vaccine (OPV) in other studies; however, most of the previous studies used conventional IPV (cIPV, Salk strain-based inactivated poliovirus vaccine), whereas data about the use of Sabin strain-based inactivated poliovirus vaccine (sIPV) in sequential immunization have been less reported. To investigate the efficacy and safety of sequential immunization with sIPV and bivalent OPV (bOPV) in China, we carried out this clinical trial. The switch of polio vaccination from using trivalent OPV (tOPV) to using a sequential schedule combining IPV and bOPV will result in changes to immunity against polio in infants, so we performed a post-hoc analysis of two clinical trials to observe the changes that may occurred after switching and to provide guidance for the application of sequential immunization in China.

Changes in the text: We have modified our text. Because Sabin strain-based inactivated poliovirus vaccine (sIPV) was licensed in China in 2015, data about the use of Sabin strain-based inactivated poliovirus vaccine (sIPV) in sequential immunization have been less reported. To investigate the efficacy and safety of sequential immunization with sIPV and bivalent OPV (bOPV) in China, we carried out the clinical trial. In the other hand, the switch of polio vaccination from using trivalent OPV (tOPV) to using a sequential schedule combining IPV and bOPV will result in changes to immunity against polio in infants, so we performed a post-hoc analysis of two clinical trials to observe the changes and to provide guidance for the application of sequential immunization in China.(see page 5, line71-84 in revised text)

**Comment 2: If considered for publication, major revisions are, in my opinion, needed.**

Reply 2: Thank you for your questions and suggestions. We have made substantial changes to the original text.
Changes in the text: For the modified parts, please see the red text in the revised manuscript.

**Comment 3: In the abstract, it provides a false conclusion that at least 2 IPV doses should be provided before bOPV to avoid emergence of polio. Not clear how the authors arrived at that**

**conclusion.**

Reply 3: Because the bivalent oral polio vaccine lacks live attenuated type II (i.e., it contains only types I + III) and only one dose of IPV was administered in the IPV+2bOPV program, this is equivalent to only one dose of polio vaccine containing type II components being administered for the IPV+2bOPV program. The clinical data from this study indicate that the seroconversion against poliovirus type II was 60.2% in cIPV-bOPV-bOPV and 51.8% in sIPV-bOPV-bOPV, rates which are significantly lower than the 81.0% in cIPV-cIPV-bOPV and 79.4% in sIPV-sIPV-bOPV. Only the group inoculated with three doses of polio vaccine containing type II components achieved a seroconversion against poliovirus type II of nearly 100%. Therefore, the schedules of one IPV dose followed by two (type I/III) bOPV doses failed to induce high-level immunity against type II poliovirus, thus increasing the risk of transmission of poliovirus type II in infants.
Changes in the text: Two or more doses of IPV should be administered before vaccination with bOPV in a sequential schedule to improve immunity against type II poliovirus, if the capacity of IPV can be increased.(see page 3, line 48-50)

**Comment 4: In introduction, it refers to "pervasive" existence of VAPP while this is an extremely rare occurrence; and it talks about VDPVs "in some developing areas" while VDPV was detected in China itself in 2019.**

Reply 4: Thank you for your question. You are correct that the probabilities of VDPV and VAPP occurring are rare. We have removed the word "pervasive" from the manuscript accordingly. Additionally, we have also replaced "the pervasive existence of VDPVs in some developing areas" with "the existence of VDPVs in some areas that use OPV".
Changes in the text: "pervasive" has been deleted.( see page 5, line55)
"the existence of VDPVs in some developing areas" has been changed to "the existence of VDPVs in some areas that use OPV". (see page 5, line 60)

**Comment 5: In introduction, there is a mention of "switch from OPV to IPV" in China. As per my information, this is not yet being implemented. The authors should better inform themselves about the plans of the Chinese EPI program.**

Reply 5: At present, China is in a transitional stage, and the sequential immunization program of IPV and bOPV is in the process of being adopted. The main idea here is to say that China is in a transitional stage.
Changes in the text: "switch from OPV to IPV-only" has been changed to "switch from tOPV to IPV-bOPV". (see page 5-6, lines 72, 91)

**Comment 6: In methods, it is unclear how the study participants were selected in the two**

**studies and what were the differences in the selection between the two studies. This needs to be better described.**

Reply 6: The inclusion and exclusion criteria in both clinical trials were similar. Inclusion criteria: 1) Infants younger than 90 days but older than 60 days; 2) Guardians are informed, agree and sign informed consent; 3) Guardians and the family follow the requirements of the clinical trial protocol; 4) No immune globulin immunization history after birth (except hepatitis B immune globulin), no other live vaccination history 28 days before vaccination; and 5) axillary temperature of <37.1 °C. Exclusion criteria: 1) Allergy, convulsions, epilepsy, encephalopathy, or psychosis history or family history; 2) Allergy to neomycin, streptomycin, or polymyxin B; 3) Immunodeficiency or receiving immunosuppressors; 4) Poliomyelitis history; 5) Acute febrile disease or infectious disease; 6) Abnormal stage of labor, asphyxiation history, congenital malformation, developmental disorder or severe chronic disease; 7) Severe anaphylactic reactions following previous vaccination; 8) Administration of oral steroids for 14 consecutive days within 1 month before the trial; 9) Fever in the previous 3 days (axillary temperature of ≥38.0 °C); 10) Diarrhea within the previous week (defecation frequency of ≥3 times/day); 11) Participation in other drug clinical trials; and 12) other conditions that may influence evaluation.
The clinical trial conducted to examine the immunogenicity and safety of sequential immunization schedules of IPV + bOPV had one more inclusion criterion: no inactivated vaccination history in the 14 days before vaccination.
Changes in the text: The details above have been added to the manuscript. (see page 7-8, lines 113–130)

**Comment 7: In methods, were the children in the first study randomized?**

Reply 7: Yes, the children in the first study were randomized.
Changes in the text: The eligible infants (N = 1200) randomly received three doses of either the tOPV from human diploid cells or the tOPV from monkey kidney cells at the ages of 2, 3, and 4 months. (see page 9, line 153)

**Comment 8: Line 116, reference to the protocol please.**

Reply 8: In accordance with the approved protocol, participants were observed onsite for 30 minutes after vaccination. For the second clinical trial, the guardians were trained to measure the child's body temperature, observe adverse reactions, and record them daily for the 14 days following vaccination. On day 15, the subjects returned to the vaccination site, where their guardians were interviewed and returned the diary card. Their guardians then filled out the contact card during the 15–28-day period to record any adverse events. Any serious adverse events (SAEs) that occurred between 28 days and 6 months after full vaccination were followed up by releasing reminder cards

(especially for vaccine-related cases).

For first clinical trial, at 8 days post-vaccination, the diary cards from days 0–7 were returned. A contact card was released so that any adverse events occurring 30 days after vaccination could be recorded.

Changes in the text: In accordance with the approved protocol, participants were observed onsite for 30 minutes after vaccination. In study NCT02231632, any adverse events occurring 30 days after vaccination were collected. In another clinical trial, the adverse events were collected through 28 days after inoculation, and any serious adverse events (SAEs) occurring between 28 days and 6 months after full vaccination were followed up. (see page 10, line 164-169)

**Comment 9: In methods, was natural decline of maternal antibodies taken into consideration in either study?**

Reply 9: A neutralizing antibody titer of <1:8 before vaccination and of higher than 1:8 against Type I, Type II, and Type III poliovirus after three doses of vaccine is considered as seroconversion. In contrast, a neutralizing antibody titer of ≥1:8 before vaccination is considered to indicate maternally-transferred antibody; for these cases, a four-fold increase of polio-specific antibody response after three doses of the vaccine is defined as seroconversion. The changes in antibody titer after vaccination were analyzed.

Changes in the text: The details above have been added to the revised manuscript.( see page 10-11, line176-181)

**Comment 10: In methods, it is not clear what was the maximum dilution and maximum reported titer.**

Reply 10: Both the maximum dilution and the maximum reported titer were 16384. When the titer was greater than 16384, it was calculated as 16384.

Changes in the text: The details above have been added to the revised manuscript. (see page 11, line 182-183)

**Comment 11: In results, lines 135-137 do not belong to this section; perhaps in the discussion.**

Reply 11: Thank you for your suggestion. We have moved this content into the Discussion in the revised manuscript.

Changes in the text: We have moved the corresponding content into the discussion section.( see page 12, line200-203 and page 15, line273-275)

**Comment 12: Line 139, what does it mean "significantly higher than the rates before polio immunization"?**

Reply 12: There are some inaccuracies here. Before inoculation with three doses of tOPV vaccine was implemented, the positive rates of antibody against polio types I, II, and III were 49.2%, 30.3%, and 24.0%, respectively; these positive cases may be due to maternal antibody.
Changes in the text: We have modified the relevant content in the revised manuscript. (see page 12, line 206-208)

**Comment 13: Again, lines 140-141 do not belong to Results**

Reply 13: There are some inaccuracies here. Here is a description of the results. For the combined sequential immunization programs, the IPV-bOPV-bOPV immunization program had a lower seroconversion against polio type II than did the IPV-IPV-bOPV immunization program, regardless of whether the IPV used in the immunization program was sIPV or cIPV. (Table 2, Supplementary Table 3)
Changes in the text: We have modified the relevant content in the manuscript. (see page 12, lines 212–215)

**Comment 14: In general, in results, please, consider including only results and not discussion points.**

Reply 14: Thank you for your advice. We have reviewed the manuscript and removed the discussion points from the results section as suggested.
Changes in the text: We have modified the relevant content in the revised manuscript.

**Comment 15: All of the results were already reported in the other two trials so again, it is unclear to me why this is being done.**

Reply 15: The data from these clinical trials has not yet been published in a journal. There are some published reports about other clinical trials on sequential immunization with inactivated poliovirus vaccine (IPV) and oral polio vaccine (OPV); however, most of the previous studies used conventional IPV (cIPV, Salk strain-based inactivated poliovirus vaccine), while data about the use of Sabin strain-based inactivated poliovirus vaccine (sIPV) in sequential immunization have been less reported. Since sIPV was licensed in China in 2015, it has become the main IPV used in China. This study supplements the existing data regarding sIPV use in sequential immunization. During 2011–2012, we developed a clinical trial to evaluate the safety and immunogenicity of the live attenuated OPV (human diploid cell; ClinicalTrials.gov number: NCT02231632). Since then, global immunization strategies for polio have changed. The other trial was carried out during 2015–2016 in Guangxi Province, China to examine the immunogenicity and safety of sequential immunization

schedules with sIPV+bOPV (ClinicalTrials.gov number: NCT03614702). This article is a comprehensive analysis of these two clinical trials in which the changes induced by switching the immunization strategy from tOPV to IPV+bOPV can be observed.
Changes in the text: We have modified the relevant content in the revised manuscript. (see page 15, line271-284)

**Comment 16: In AEFIs, I think it is tricky to compare AEFIs between the trials. The conclusion that in trial one there were fewer fevers than in trial two may or may not be real. Were they done in the same period of the year? And, obviously, in full-oral schedule, there will be less local reactions (redness, swelling) than in a schedule that includes an injection.**

Reply 16: Thank you for your question. Although the two clinical trials were completed in the same region, they were not conducted during the same period, and there were also some small differences in AEs collection, so the AE differences may be caused by factors other than the immunization procedure. Additionally, as the reviewer points out, immunization with IPV can cause more local reactions (e.g., pain, repattern, specify product), which may be related to injection.
Changes in the text: The incidences of fever were 28.0% (335/1195) in the tOPV-tOPV-tOPV schedule and 49.9% (598/1198) for all sequential schedules combined. Additionally, the incidences of abnormal irritability were 5.9% and 20.9%, the incidences of local reactions (e.g., pain, redness, swelling, induration) were 0% and 1.3%; and the incidences of gastrointestinal symptoms were 10.0% and 6.7% in the tOPV-tOPV-tOPV schedule and for all sequential schedules combined, respectively. (see page 14, lines 253–258 and page 17, line 315-321)

**Comment 17: In discussion, discussing merits of tOPV is a bit off the mark since this vaccine has been withdrawn globally in 2016.**

Reply 17: Thank you for your advice. We did not mean to extol the tOPV. In fact, although OPV did contribute significantly to the rapid decline in polio cases, it also has important disadvantages. Immunization procedures need to be selected based on polio prevalence, polio vaccine capacity, and other factors. As stated at the beginning of our manuscript, OPV induces high levels of polio immunity in the population, but it can cause VAPP and VDPV. In the context of a significant reduction in cases caused by wild virus infection, the WHO has decided to gradually replace OPV with IPV to reduce the incidence of VAPP and VDPV. The first step is to remove the type II component from OPV and to administer at least one dose of IPV before administering bOPV. Because bivalent oral polio vaccine lacks the live attenuated type II (i.e., it contains only types I + III) and only one dose of IPV was included in the IPV+2bOPV program, the schedule of one IPV dose followed by two bOPV (type I/III) doses failed to induce high-level immunity against type II poliovirus. Although the WHO emphasizes the synchronous cessation of tOPV use worldwide, the

occurrence of VDPV II tended to increase after using the schedule of IPV+2bOPV. Based on the VDPV II prevalence reported by the WHO and the analysis of our clinical trials, it is necessary to increase the doses of IPV to improve infant immunity against type II poliovirus.
Changes in the text: We have modified the relevant content in the revised manuscript. (see page 5, line69-71; page 12, 200-203 and page15, line 274)

**Comment 18: The conclusion that "The use of IPV-bOPV-bOPV schedule in China must be reconsidered cautiously." is misleading.**

Reply 18: Thank you for pointing out this issue. We have revised our statement accordingly. The schedule of one IPV dose followed by two bOPV (types I/III) doses failed to induce high-level immunity against type II poliovirus, consequently increasing the risk of poliovirus type II transmission in infants. Two or more doses of IPV should be administered before bOPV in a sequential schedule to improve immunity against type II poliovirus, if the capacity of IPV can be increased.
Changes in the text: There is still wild poliovirus strain infection and VDPV II cases in at least two countries bordering China. To improve immunity against type II poliovirus, two or more doses of IPV should be administered before bOPV in a sequential schedule.( see page 16, line302-304)