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Can novel oral polio vaccine type 2 (nOPV2) put us back on track towards global polio eradication?

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Can novel oral polio vaccine type 2 (nOPV2) put us back on track towards global polio eradication?

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“Vaccines and antibiotics have made many infectious diseases a thing of the past; we’ve come to expect that public health and modern science can conquer all microbes. But nature is a formidable adversary.”

Tom Frieden, Centers for Disease Control and Prevention (CDC), USA, 2016

“It’s often said that vaccines save lives, but this is not strictly true; it is vaccination that saves lives. A vaccine that remains in the vial is 0% effective even if it is the best vaccine in the world. Thus, it is imperative that we all work together to assure that a high level of coverage is obtained among populations for whom vaccines are recommended.”

Walter Orenstein, Emory University, USA, 2017

Content

Summary

For over 60 years both Salk inactivated poliovirus vaccine (IPV) and Sabin oral poliovirus vaccine (OPV) have been successfully used globally in the prevention of poliomyelitis, resulting in the certified eradication of wild-type serotype 2 poliovirus (WP2) and wild-type serotype 3 poliovirus (WP3), and endemic circulation of wild-type serotype 1 poliovirus (WP1) only continues in specific areas of two countries (Pakistan and Afghanistan). Although OPV has always been the vaccine of choice for the Global Polio Eradication Initiative (GPEI) because of its specific characteristics, it contains live attenuated ribonucleic acid (RNA) viruses which are capable of mutating during replication and so becoming neurovirulent again. In the late 1990s it became clear that on top of sporadically causing vaccine-associated paralytic poliomyelitis (VAPP) in recently vaccinated individuals or their contacts, Sabin strains can also regain transmissibility and start spreading as circulating vaccine-derived polioviruses (cVDPVs), causing outbreaks in under-vaccinated communities. To maintain population immunity in absence of or very low circulation of wild polioviruses a sufficiently high routine immunization coverage needs to be achieved. From 2000 onwards an increasing number of industrial countries switched to IPV while use of trivalent OPV (tOPV) continued in low- and middle-income countries (LMIC). However, while wild-type poliovirus cases decreased environmental conditions of low sanitation and crowding together with insufficient coverage resulted in increasing number of cVDPV cases. To achieve global polio eradication all kinds of polioviruses will need to be eradicated and therefore it became clear that this could only be accomplished by a gradual global transition to IPV. Because WP2 eradication was certified in 2015 and an important number of VAPP cases and most cVDPV outbreaks were due to Sabin type 2 a global withdrawal of Sabin vaccine serotype 2 has taken place in 2016. Supported by the recommendation of the Strategic Advisory Group of Experts on Immunization (SAGE) trivalent OPV has been replaced in routine immunization schedules by bivalent OPV (bOPV) with the addition of at least 1 IPV dose. The switch from tOPV to bOPV was expected to also enhance the immunogenicity of bOPV against types 1 and 3, with which OPV2 is known to interfere, and adding IPV should reduce the risk of paralytic polio in case of exposure to a type 2 poliovirus afterwards. Following this cessation of OPV2 use in May 2016, monovalent OPV2 only remains stockpiled for outbreak response.

This stockpile is necessary because IPV induces only limited primary intestinal mucosal immunity and therefore cannot contribute significantly to stopping outbreaks. Yet, use of mOPV2 in outbreak response carries the inherent risk of seeding new circulating strains that eventually will lead to new outbreaks. In addition, if sufficient upscaling of IPV could not be achieved in a timely manner to meet the global need the overall immunity, including intestinal mucosal immunity, to PV2 on a population level would decrease in regions lacking this supply, so increasing the risk of cVDPVs. In order to meet these risks researchers developed new IPV and OPV vaccine candidates to address current and future needs.

The aim of this thesis was to evaluate the novel oral polio vaccine candidates nOPV2-c1 and nOPV2-c2 in healthy adults for safety, immunogenicity, viral shedding and genetic stability in order that further evaluation could be continued in children and infants in Panama. In addition, a novel adjuvanted IPV vaccine has been evaluated for safety, humoral immunogenicity and the ability to generate mucosal responses in comparison with IPV.

In the **third chapter** of this thesis, I describe the infrastructure that had to be built and all the procedures that were necessary to conduct the first in-human study in containment conditions in 2017. In the previous year, all OPV containing serotype 2 had been globally withdrawn with the remaining stock of mOPV2 restricted to outbreak response. Since then the 3th Global Action Plan (GAPIII) of the WHO with specific containment requirements for all facilities that process samples or retain materials that contain or potentially contain polioviruses was in effect. The aim of GAPIII is to minimize poliovirus facility-associated risk to re-introduce poliovirus type 2 into the environment. As the facility of the Center for the Evaluation of Vaccination at that time was only equipped for conduct of ambulatory trials and because of the urgent need for starting up a phase 1 trial in humans a temporary quarantine facility had to be built completely from scratch to conform the biosafety restrictions of GAPIII. We designed a facility composed of 66 specially designed linked modules, suitable for a 28-day stay for two sequential groups of 15 adult volunteers. Unique standard operating procedures (SOP) and emergency plans were developed to ensure that the vaccine strains could not enter the environment through excretion of the virus in the feces or other body fluids of the vaccinated participants or through transmission by study personnel. All biological samples that might contain polioviruses had to be captured and contained for shipment to central labs or subsequent decontamination and destruction. All wastewater was collected in 2 large external tanks and decontaminated by chlorine dioxide treatment. Between occupation by the two cohorts and at the

end of the study the unit was decontaminated using chlorine dioxide gas. All solid waste was collected in medical waste containers and destroyed according to the local hospital protocol. In addition to all facility requirements, special attention was given to the volunteers and their mental health. Psychological screening beforehand and support during the study was foreseen to make the long duration stay feasible for the subjects.

In the **fourth chapter**, I describe the investigation of the safety, immunogenicity and viral shedding of the two candidate vaccines, nOPV2-c1 and nOPV2-c2, in a first in human study (UAM4a) in the containment facility. Two cohorts of 15 healthy IPV-primed adults each, were sequentially enrolled and in each cohort participants were vaccinated with the same vaccine candidate and had to remain in the facility for a maximum of 28 days or until all participants of that cohort reached shedding cessation (PCR-negative viral shedding on 3 consecutive stool samples), whatever occurred first. If shedding persisted after 28 days subjects were allowed to leave the facility but had to remain in Belgium and comply with restrictive measurements until they reached shedding cessation. We showed in this study that the vaccines were safe and well-tolerated with the exception of temporary liver enzyme and creatine kinase elevations that were most probably due to intensive sport activities in the unit. In all subsequent studies this was never reported again. Although high baseline titers were present in these immunized cohorts immunogenicity of the vaccines could be demonstrated with high increase of neutralizing antibody titers and seroconversion in most participants. Because of their IPV-only background most participants started shedding in their stools after vaccination and all volunteers were followed up until shedding cessation. The shedding duration and the magnitude were higher after nOPV2-c1 than after nOPV2-c2 but for both candidates only a few individual samples showed a titer above the threshold for reduced risk of transmission and never lasted longer than 2 days in a sample. In our study we observed resumption of shedding in some individuals after 3 consecutive negative samples, which is the common definition of shedding cessation. This could possibly be due to re-infection in the unit for some samples although it has also been reported in a long-term shedder who returned home. Two subjects exceeded the expected shedding duration time by much longer than expected which was probably due to individual shedding variability as we could not identify any medical cause. Genetic stability and neurovirulence was studied in the latest samples that reached the threshold for reduced risk of transmission of all participants and these results were very promising. Neurovirulence of shed samples was either absent or very low in animal testing and no variants were seen in domain V, the most important

adaptation in the new vaccine candidates. These results supported progression with the candidates into the larger phase II study, with administration to non-IPV vaccinated individuals, and was influential in recommendation of the WHO Containment Advisory Committee that subsequent studies could be done outside of containment.

In a **fifth chapter**, I describe the results of safety, immunogenicity and shedding investigations of both novel vaccine candidates when administrated in a larger phase 2 study (UAM4) in Belgian adults compared with results of a historical control study with mOPV2. The control study UAM1 was done in 2016, in anticipation of the global withdrawal of OPV2, because the novel vaccine candidates were not yet then ready to be tested. As such, UAM1 and UAM4 had similar study designs. In the UAM1 study 100 OPV-primed healthy adults were randomized to receive 1 or 2 doses of mOPV2 . In 2018 the UAM4 trial was conducted in which 200 OPV-primed subjects were randomly assigned to receive 1 or 2 doses of either nOPV2-c1 or nOPV2-c2 and 50 IPV-primed subjects were randomized to receive 2 doses of nOPV2-c1, nOPV2-c2 or placebo. The study was carried out in 2 centers in Belgium (CEV, Antwerp and CEVAC, Ghent), each center enrolling half of the participants. Both UAM1 and UAM4 studies were specifically designed to enable comparison between both nOPV2 candidates and Sabin mOPV2. From these studies we could confirm the safety and acceptable tolerability of both candidates, similar to the safety-profile of mOPV2, and no significant lab abnormalities were reported. Non-inferior immunogenicity was demonstrated for both candidates in comparison with mOPV2 in the OPV-primed groups. Moreover, PV2-specific geometric mean titers (GMT) and seroconversion rates at Day 28 in OPV primed groups indicate that the immunogenicity of nOPV2-c1 and nOPV2-c2 at the 10^6 $CCID_{50}$ level may be superior to a standard dose of mOPV2 (10^5 $CCID_{50}$). The IPV cohorts were relatively small in this study and no comparator control data were available but the results showed a trend similar to the OPV cohorts with high levels of immunity before and after vaccination and high seroconversion rates.

In this ambulatory trial stool samples for assessment of viral shedding were obtained on predefined days. In the OPV-primed participants overall shedding rates and extent after vaccination with either of the nOPV2 candidates were similar and not increased compared with mOPV2. In UAM1, shedding did not last longer than 14 days after first vaccination. This was also the case in the UAM4 study for nOPV2-c1 after which only one subject was still shedding at Day 27. After the second vaccination only a few subjects showed any shedding which was of

short duration and with a similar magnitude of shedding for the three vaccines. In IPV-primed groups shedding rates were higher and of longer duration in comparison with the OPV-primed groups in the UAM4 study, but were lower than the results of the UAM4a study. Decreased shedding after the second dose compared with the first was clearly shown, providing some indirect evidence of mucosal immunity being generated by the first dose of nOPV2. Regarding neurovirulence and genetic sequencing in the modified transgenic mouse model we observed no or limited increases in neurovirulence for shed nOPV2-c1 and nOPV2-c2 compared with the bulk vaccine, which contrasts with a marked loss of attenuation that would be expected from corresponding samples from Sabin OPV2 vaccinees after 7 days. No significant changes to the primary attenuation site domain V were seen for either candidate vaccine, regardless of prior vaccination history of the participants.

In a **sixth chapter**, I present the investigation of the safety and humoral and mucosal immunogenicity of IPV adjuvanted with double mutant Enterotoxigenic *Escherichia coli* heat labile toxin (dmLT) in a phase 1 clinical trial. IPV is known to generate a strong humoral response, protective against symptomatic polio disease, but induces only limited mucosal response. Therefore, it has shown little impact where there is predominantly faeco-oral transmission. Since 2016 tOPV has been replaced by bOPV with at least one dose of IPV to ensure a minimal type 2 immunity as a first step in the global transition process to IPV-only vaccination. However, since then population type 2 mucosal immunity has been decreasing with a consequent increase in cVDPV2 outbreaks and leading to the current SAGE recommendation to add a second IPV dose to the bOPV/IPV schedule. Furthermore, the necessary upscaling of IPV production after the switch encountered difficulties resulting in global IPV shortages affecting many low- and middle-income countries. Adding an adjuvant to IPV with the potential of enhancing intestinal immunity and reducing the amount of antigen to allow use of fractional doses could solve both problems. As pre-clinical and clinical studies showed the potential of dmLT to improve mucosal immunity we studied the effects of IPV+dmLT in comparison with IPV after 1 dose, followed by a challenge of bOPV. This study showed that the IPV + dmLT formulation is safe and well tolerated, but did not find any beneficial effect on humoral or mucosal immunity of the adjuvant at the dose level used. Addition of dmLT to IPV did not improve seroprotection or seroconversion over IPV alone and the time to cessation for shedding was similar for both IPV groups. In addition, no meaningful differences were seen for fecal neutralization responses and fecal IgA in type specific responses for both OPV groups.

In **conclusion**, in this thesis I demonstrate that both nOPV2 candidates are safe and show non-inferior immunogenicity in comparison with mOPV2. In addition, in the presented studies both candidates demonstrated enhanced genetic stability of shed viruses with low neurovirulence in animal testing and no reversion of domain V, the most dominant mutation site. These results have led to further testing in children and infants with ultimate selection and roll-out of the current nOPV2 vaccine.

Currently, WPV2 and WPV3 are eradicated and WPV1 circulation is reduced to sub-areas of 2 endemic countries. Yet, due to waning type 2 immunity and insufficient vaccination coverage, many countries have struggled in the last few years with increasing outbreaks of cVDPV2. These communities could only rely on mOPV2 use for outbreak response, although the risk of seeding new cVDPVs exist when insufficient number of children are reached. The development and fast distribution of nOPV2 in many countries affected by cVDPV2s can change this. In addition, the development and EUL (Emergency Use Listing) process of this novel vaccine has paved the way for much faster development of other more genetically stable vaccines for polio type 1 and 3. Only by eliminating cVDPV outbreaks we will eventually be able to stop OPV use and move on to IPV use only. The ideal IPV vaccine would also induce mucosal immunity and we investigated one possible candidate adjuvanted with dmLT, though with negative results and further research will be needed.

The nOPV2 has proven to be a very important vaccine that due to its enhanced genetic stability and safety profile can be one of the final keys to global polio eradication. If cVDPVs can be strongly reduced (eliminated) the transition phase to IPV only vaccination can be further continued in a much safer way. Yet, a vaccine is only effective when administered. The risk of reversion is lower than with mOPV2 but the longer the vaccine viruses can circulate chances to reversion and recombination with other enteroviruses increase. Therefore, enhanced efforts to reach sufficiently high national vaccination coverage with specific attention to communities hard to reach and underserved remain key priority in our goal to global polio eradication.

Samenvatting

Al meer dan 60 jaar worden zowel het Salk geïnactiveerd poliovirusvaccin (IPV) als het Sabin oraal poliovirusvaccin (OPV) wereldwijd met succes gebruikt voor de preventie van poliomyelitis, wat geleid heeft tot de gecertificeerde uitroeiing van het poliovirus van het wilde serotype 2 (WP2) en het wilde serotype 3 (WP3); het poliovirus van het wilde serotype 1 (WP1) circuleert alleen nog in bepaalde gebieden in twee landen (Pakistan en Afghanistan). Hoewel OPV vanwege zijn specifieke kenmerken altijd het voorkeursvaccin is geweest voor het Global Polio Eradication Initiative (GPEI), bevat het levende verzwakte ribonucleïnezuur (RNA)-virussen die tijdens de replicatie kunnen muteren en zo opnieuw neurovirulent kunnen worden. Eind jaren negentig werd duidelijk dat Sabin-stammen niet alleen sporadisch vaccin-geassocieerde paralytische poliomyelitis (VAPP) kunnen veroorzaken bij recent gevaccineerde personen of hun contacten, maar ook terug hun besmettelijkheid kunnen herwinnen en zich kunnen gaan verspreiden als circulerende vaccin-afgeleide poliovirussen (cVDPVs), die uitbraken veroorzaken in onder-gevaccineerde gemeenschappen. Om de immuniteit van de bevolking in stand te houden wanneer er geen of zeer weinig wilde poliovirussen circuleren, moet een voldoende hoge routine-immunisatiegraad worden bereikt. Vanaf 2000 schakelde een toenemend aantal industriële landen over op IPV, terwijl het trivalent OPV (tOPV) verder gebruikt werd in landen met een laag en gemiddeld inkomen. Terwijl het aantal gevallen van poliovirus van het wilde type afnam, leidden omgevingsfactoren zoals slechte hygiëne en overbevolking in combinatie met onvoldoende vaccinatiegraad tot een toenemend aantal gevallen van cVDPV. Om polio wereldwijd uit te roeien moeten alle soorten poliovirussen worden uitgeroeid en daarom werd duidelijk dat dit alleen kon worden bereikt door een geleidelijke wereldwijde overgang naar IPV. Omdat WP2 in 2015 uitgeroeid was verklaard en een belangrijk aantal VAPP-gevallen en de meeste cVDPV-uitbraken te wijten waren aan Sabin type 2, heeft men in 2016 wereldwijd serotype 2 Sabin-vaccin teruggetrokken. Ondersteund door de aanbeveling van de Strategic Advisory Group of Experts on Immunization (SAGE) is trivalent OPV in routine-immunisatieschema's vervangen door bivalent OPV (bOPV) met toevoeging van ten minste 1 dosis IPV. De overschakeling van tOPV naar bOPV zou naar verwachting ook de immunogeniciteit van bOPV tegen de types 1 en 3, waarvan men weet dat OPV2 mee interfereert, verbeteren, en de toevoeging van IPV zou

het risico van paralytische polio in geval van een latere blootstelling aan een type 2-poliovirus moeten verminderen. Na deze stopzetting van OPV2 in mei 2016 blijft monovalent OPV2 alleen voorradig voor uitbraakbestrijding. Deze voorraad is nodig omdat IPV slechts beperkte primaire intestinale mucosale immuniteit induceert en daarom niet significant kan bijdragen aan het indammen van uitbraken. Toch houdt het gebruik van mOPV2 bij uitbraakbestrijding het inherente risico in dat er nieuwe circulerende stammen ontstaan die uiteindelijk tot nieuwe uitbraken zullen leiden. Als bovendien niet tijdig voldoende IPV productie kan worden opgeschaald om aan de wereldwijde behoefte te voldoen, zou de algemene immuniteit, inclusief de intestinale mucosale immuniteit, voor PV2 op bevolkingsniveau afnemen in regio's waar deze voorziening ontbreekt, waardoor het risico van cVDPVs toeneemt. Om aan deze risico's tegemoet te komen, ontwikkelden onderzoekers nieuwe kandidaten voor IPV- en OPV-vaccins om in de huidige en toekomstige behoeften te voorzien.

Het doel van deze thesis was het evalueren van de nieuwe kandidaten voor het orale poliovaccin nOPV2-c1 en nOPV2-c2 op veiligheid, immunogeniciteit, virusuitscheiding en genetische stabiliteit, zodat verdere evaluatie bij kinderen en zuigelingen in Panama kan worden voortgezet. Daarnaast werd een nieuw IPV-vaccin met adjuvans onderzocht op veiligheid, humorale immunogeniciteit en het vermogen om mucosale immuunreacties te genereren in vergelijking met IPV.

In het **derde hoofdstuk** van dit proefschrift beschrijf ik de infrastructuur die moest worden opgebouwd en alle procedures die nodig waren om in 2017 de fase 1 studie in quarantaine omstandigheden uit te voeren. In het voorgaande jaar waren alle OPV met serotype 2 wereldwijd teruggetrokken en was de resterende voorraad mOPV2 uitsluitend voorbehouden voor uitbraakbestrijding. Sindsdien was het 3e Global Action Plan (GAPIII) van de WHO van kracht met specifieke inperkingseisen voor alle faciliteiten die stalen verwerken of materialen bewaren die poliovirussen bevatten of kunnen bevatten. Het doel van GAPIII is het risico van herintroductie van poliovirus type 2 in het milieu, dat zo'n faciliteit met zich meebrengt, tot een minimum te beperken. Aangezien de faciliteit van het Centrum voor de Evaluatie van Vaccinatie op dat moment alleen was uitgerust voor het uitvoeren van ambulante studies en vanwege de dringende noodzaak om een fase 1-studie bij mensen op te starten, moest een tijdelijke quarantainevoorziening volledig vanaf nul worden gebouwd om te voldoen aan de bioveiligheidsbeperkingen van GAPIII. Wij ontwierpen een faciliteit bestaande uit 66 speciaal ontworpen, gekoppelde modules, geschikt voor een verblijf van 28 dagen voor twee opeenvolgende groepen van 15 volwassen vrijwilligers. Er

werden unieke standaard operationele procedures (SOP) en noodplannen opgesteld om ervoor te zorgen dat de vaccinstammen niet in het milieu terecht konden komen door uitscheiding van het virus in de feces of andere lichaamsvloeistoffen van de gevaccineerde deelnemers of door overdracht door studiepersoneel. Alle biologische stalen die mogelijk poliovirussen bevatten, moesten worden opgevangen en ingeperkt voor verzending naar centrale laboratoria of voor latere ontsmetting en vernietiging. Al het afvalwater werd verzameld in 2 grote externe tanks en ontsmet door behandeling met chloordioxide. Tussen de bezetting door de twee cohorten en aan het eind van de studie werd de eenheid ontsmet met chloordioxidegas. Al het vaste afval werd verzameld in containers voor medisch afval en vernietigd volgens het plaatselijke ziekenhuisprotocol. Naast alle facilitaire vereisten werd speciale aandacht besteed aan de vrijwilligers en hun geestelijke gezondheid. Er was een psychologische screening vooraf voorzien en ondersteuning tijdens de studie om het lange verblijf voor de deelnemers haalbaar te maken.

In het **vierde hoofdstuk** beschrijf ik het onderzoek naar de veiligheid, immunogeniciteit en virusuitscheiding (shedding) van de twee kandidaat-vaccins, nOPV2-c1 en nOPV2-c2, in een fase 1 studie (UAM4a) in de quarantaine-unit. Twee cohorten van elk 15 IPV gevaccineerde gezonde volwassenen werden achtereenvolgens geïncludeerd en in elke cohorte werden de deelnemers gevaccineerd met hetzelfde kandidaat-vaccin en moesten zij maximaal 28 dagen in de faciliteit blijven of totdat alle deelnemers van die cohorte virus uitscheiding gestopt hadden (PCR-negatieve virusuitscheiding op 3 opeenvolgende ontlastingstalen), afhankelijk van wat eerst gebeurde. Als de uitscheiding na 28 dagen aanhield, mochten de deelnemers de instelling verlaten, maar moesten zij in België blijven en zich houden aan beperkende maatregelen totdat de uitscheiding was gestopt. In deze studie toonden wij aan dat de vaccins veilig waren en goed werden verdragen, met uitzondering van tijdelijk verhoogde leverenzymen en creatinekinase, die waarschijnlijk het gevolg waren van intensieve sportactiviteiten in de instelling. In alle volgende studies werd dit nooit meer gemeld. Hoewel in deze geïmmuniseerde cohorten hoge basistiters aanwezig waren, kon de immunogeniciteit van de vaccins worden aangetoond met een sterke toename van de neutraliserende antistof-titer en seroconversie in de meeste deelnemers. Vanwege hun exclusieve IPV achtergrond begonnen de meeste deelnemers na de vaccinatie virussen uit te scheiden in hun ontlasting en alle vrijwilligers werden gevolgd totdat de virusuitscheiding ophield. De duur en de omvang van de uitscheiding waren hoger na nOPV2-c1 dan na nOPV2-c2, maar voor beide kandidaten vertoonden slechts enkele individuele stalen een titer

boven de drempel voor een verminderd risico van overdracht en dit duurde nooit langer dan 2 dagen in een staal. In onze studie hebben wij bij sommige personen een hervatting van de uitscheiding vastgesteld na drie opeenvolgende negatieve stalen, wat de gangbare definitie is van stopzetting van de uitscheiding. Voor sommige stalen kan dit mogelijk het gevolg zijn van her-infectie op de afdeling, hoewel het ook is gemeld bij een persoon met langdurige uitscheiding die al naar huis terug gekeerd was. Twee deelnemers overschreden de verwachte uitscheidingsduur veel langer dan verwacht, wat waarschijnlijk te wijten was aan individuele uitscheidingsvariabiliteit, aangezien wij geen medische oorzaak konden vaststellen. Genetische stabiliteit en neurovirulentie werden bestudeerd in de laatste stalen die de drempel voor verminderd risico op overdracht van alle deelnemers bereikten en deze resultaten waren veelbelovend. Er werd geen of zeer weinig neurovirulentie van de uitgescheiden stalen vastgesteld in dierproeven en er werden geen varianten gezien in domein V, de belangrijkste aanpassing in de nieuwe vaccin-kandidaten. Deze resultaten ondersteunden de voortgang van de kandidaten naar de grotere fase II-studie, met toediening aan niet-IPV-gevaccineerde personen, en waren van invloed op de aanbeveling van het Containment Advisory Committee van de WHO dat verdere studies buiten de inperking konden worden uitgevoerd.

In een **vijfde hoofdstuk** beschrijf ik de resultaten van onderzoek naar veiligheid, immunogeniciteit en shedding van beide nieuwe vaccin kandidaten bij toediening in een grotere fase 2 studie (UAM4) bij Belgische volwassenen, in vergelijking met de resultaten van een historische controle studie met mOPV2. De controlestudie UAM1 werd vooraf uitgevoerd in 2016, vóór de wereldwijde terugtrekking van OPV2, omdat de nieuwe kandidaat-vaccins toen nog niet klaar waren om getest te worden. Als zodanig hadden UAM1 en UAM4 een vergelijkbare studieopzet. In de UAM1-studie werden 100 OPV-gevaccineerde gezonde volwassenen gerandomiseerd om 1 of 2 dosissen van mOPV2 te ontvangen. In 2018 werd de UAM4-studie uitgevoerd waarbij 200 OPV-gevaccineerde proefpersonen willekeurig werden toegewezen om gevaccineerd te worden met 1 of 2 dosissen van nOPV2-c1 of nOPV2-c2 en 50 IPV-gevaccineerde proefpersonen werden gerandomiseerd om 2 dosissen van nOPV2-c1, nOPV2-c2 of placebo te ontvangen. De studie werd uitgevoerd in 2 centra in België (CEV, Antwerpen en CEVAC, Gent), waarbij elk centrum de helft van de deelnemers includeerde. De studies UAM1 en UAM4 waren specifiek ontworpen om een vergelijking tussen de nOPV2-kandidaten en Sabin mOPV2 mogelijk te maken. Uit deze studies konden wij de veiligheid en aanvaardbare verdraagbaarheid van beide kandidaten bevestigen, vergelijkbaar met het veiligheidsprofiel van mOPV2, en er werden geen

significante laboratoriumafwijkingen gerapporteerd. Voor beide kandidaten werd niet-inferieure immunogeniciteit aangetoond in vergelijking met mOPV2 in de OPV-gevaccineerde groepen. Bovendien wijzen PV2-specifieke geometrische gemiddelde titer (GMT) en seroconversiepercentages op dag 28 in OPV-gevaccineerde groepen erop dat de immunogeniciteit van nOPV2-c1 en nOPV2-c2 op het niveau van 10^6 $CCID_{50}$ superieur kan zijn aan een standaarddosis mOPV2 (10^5 $CCID_{50}$). De IPV-cohorten waren relatief klein in deze studie en er waren geen vergelijkende controlegegevens beschikbaar, maar de resultaten vertoonden een soortgelijke tendens als de OPV-cohorten met hoge niveaus van immuniteit voor en na vaccinatie en hoge seroconversiepercentages.

In dit ambulante onderzoek werden op vooraf bepaalde dagen stoelgangsstalen genomen voor de beoordeling van de virusuitscheiding. Bij de OPV gevaccineerde deelnemers waren de totale uitscheidingspercentages en -omvang na vaccinatie met een van beide nOPV2-kandidaten vergelijkbaar en niet verhoogd in vergelijking met mOPV2. In UAM1 duurde de uitscheiding niet langer dan 14 dagen na de eerste vaccinatie. Dit was ook het geval in de UAM4-studie voor nOPV2-c1, waarna slechts één deelnemer op dag 27 nog virussen uitscheidde. Na de tweede vaccinatie vertoonden slechts enkele deelnemers uitscheiding, die van korte duur was en voor de drie vaccins van vergelijkbare omvang. In de IPV-gevaccineerde groepen waren de uitscheidingspercentages hoger en van langere duur in vergelijking met de OPV-gevaccineerde groepen in de UAM4-studie, maar lager dan de resultaten van de UAM4a-studie. Er werd een duidelijk verminderde uitscheiding na de tweede dosis aangetoond in vergelijking met de eerste dosis, hetgeen indirect aantoont dat de eerste dosis nOPV2 een mucosale immuniteit opwekt. Met betrekking tot neurovirulentie en genetische sequentiebepaling in het gemodificeerde transgene muismodel hebben wij geen of een beperkte toename van de neurovirulentie voor uitgescheiden nOPV2-c1 en nOPV2-c2 waargenomen in vergelijking met het bulkvaccin, hetgeen in contrast staat met een duidelijk verlies van attenuatie dat men kan verwachten van overeenkomstige stalen van Sabin OPV2-vaccins na 7 dagen. Er werden geen significante veranderingen in domein V, de hoofdlocatie van attenuatie, gezien voor beide kandidaat-vaccins, ongeacht de voorafgaande vaccinatiegeschiedenis van de deelnemers.

In een **zesde hoofdstuk** presenteer ik het onderzoek naar de veiligheid en de humorale en mucosale immunogeniciteit van IPV met dubbele mutant Enterotoxigene Escherichia coli hittelabiel toxine (dmLT) in een fase 1 klinische studie. Van IPV is bekend dat het een sterke humorale respons opwekt, die

beschermt tegen symptomatische polioziekte, maar slechts een beperkte mucosale respons genereert. Daarom heeft het weinig effect waar overwegend faeco-orale overdracht plaats vindt. Sinds 2016 is tOPV vervangen door bOPV met ten minste één dosis IPV om te zorgen voor een minimale type 2-immuniteit als eerste stap in het wereldwijde overgangsproces naar uitsluitend IPV-vaccinatie. Sindsdien is de mucosale immuniteit van type 2 bij de bevolking echter afgenomen, met een toename van het aantal uitbraken van cVDPV2 als gevolg, wat geleid heeft tot de huidige SAGE-aanbeveling om een tweede dosis IPV toe te voegen aan het bOPV/IPV-schema. Bovendien was de noodzakelijke opschaling van de IPV-productie na de omschakeling niet evident, hetgeen heeft geleid tot wereldwijde IPV-tekorten in veel landen met lage en middeninkomens. Toevoeging van een adjuvans aan IPV met het vermogen om de intestinale immuniteit te versterken en de hoeveelheid antigeen te verminderen om het gebruik van fractionele doses mogelijk te maken, zouden beide problemen kunnen oplossen. Aangezien preklinische en klinische studies het potentieel van dmLT aantoonden om de mucosale immuniteit te verbeteren, onderzochten wij de effecten van IPV+dmLT in vergelijking met IPV na 1 dosis, gevolgd door een toediening van bOPV. Deze studie toonde aan dat de IPV+dmLT- formulering veilig is en goed wordt verdragen, maar vond geen gunstig effect op de humorale of mucosale immuniteit van het adjuvans bij de gebruikte dosis. Toevoeging van dmLT aan IPV verbeterde de seroprotectie of seroconversie niet ten opzichte van IPV alleen en de duur tot stopzetting van de uitscheiding was vergelijkbaar voor beide IPV-groepen. Bovendien werden geen significante verschillen gezien voor fecale neutralisatiereacties en fecale IgA in type-specifieke reacties voor beide OPV-groepen.

Concluderend, toon ik in dit proefschrift aan dat beide nOPV2-kandidaten veilig zijn en een niet-inferieure immunogeniciteit vertonen in vergelijking met mOPV2. Bovendien toonden beide kandidaten in de beschreven studies een verbeterde genetische stabiliteit van de uitgescheiden virussen met een lage neurovirulentie in dierproeven en geen reversie van domein V, de meest dominante mutatieplaats. Deze resultaten hebben geleid tot verder onderzoek bij kinderen en zuigelingen met uiteindelijke selectie en uitrol van het huidige nOPV2-vaccin.

Momenteel zijn WPV2 en WPV3 uitgeroeid en is de circulatie van WPV1 beperkt tot deelgebieden van 2 endemische landen. Echter, als gevolg van de afnemende immuniteit voor type 2 en de ontoereikende vaccinatiegraad hebben veel landen de afgelopen jaren te kampen gehad met toenemende uitbraken van cVDPV2. Deze gemeenschappen konden alleen vertrouwen op het gebruik van mOPV2

voor uitbraakbestrijding, hoewel het risico bestaat dat nieuwe cVDPVs ontstaan wanneer onvoldoende kinderen worden bereikt. De ontwikkeling en snelle verdeling van nOPV2 in vele landen die getroffen zijn door cVDPVs kan hier verandering in brengen. Bovendien hebben de ontwikkeling en het proces voor toelating voor gebruik in noodtoestanden (EUL) de weg vrij gemaakt voor veel snellere ontwikkeling van andere genetisch meer stabiele poliovaccins type 1 en 3. Enkel door cVDPV uitbraken te elimineren zullen we uiteindelijk geen OPV meer hoeven te gebruiken en kunnen overgaan op het gebruik van IPV alleen. Het ideale IPV-vaccin zou ook mucosale immuniteit opwekken en daarom hebben wij een mogelijke kandidaat met dmLT als hulpstof onderzocht, maar met negatieve resultaten en verder onderzoek is nodig.

Het nOPV2 heeft bewezen een zeer belangrijk vaccin te zijn dat, dank zij de verbeterde genetische stabiliteit en veiligheidsprofiel, 1 van de finale sleutels kan zijn tot wereldwijde polio eradicatie. Als cVDPVs sterk gereduceerd (geëlimineerd) kunnen worden, kan de overgangsfase naar vaccinatie met enkel IPV op een veel veiliger manier verder gezet worden. Een vaccin is echter alleen effectief als het wordt toegediend. Het risico op reversie is kleiner dan met mOPV2 maar hoe langer de vaccinvirussen kunnen circuleren, hoe groter de kans op reversie en recombinatie met andere enterovirussen. Daarom blijven verhoogde inspanningen om een voldoende hoge nationale vaccinatiegraad te bereiken, met specifieke aandacht voor moeilijk bereikbare en achtergestelde gemeenschappen, belangrijke prioriteiten in ons streven naar wereldwijde polio-uitroeiing.

List of Abbreviations

AE	Adverse event
AFP	Acute flaccid paralysis
ALT	Alanine transaminase
AST	Aspartate transaminase
aVDPV	Ambigous vaccine derived poliovirus
BBB	Blood brain barrier
BMGF	Bill & Melinda Gates Foundation
bOPV	bivalent oral poliovirus vaccine
$CCID_{50}$	50% cell culture infective dose
CDC	Centers for Disease Control and prevention
CK	Creatine kinase
CNS	Central nervous system
Cre	Cis-acting replication element
CSF	Cerebrospinal fluid
cVDPV	Circulating vaccine derived polioviruses
DCR	Democratic Republic of Congo
dmLT	Double Mutant Heat-Labile Toxin
DTaP	Diphtheria+ tetanus+ acellular pertussis vaccine
EES	Exploratory endpoint specimen
EPI	Expanded program on Immunization
EUL	Emergency use listing
FIH	First in human
fIPV	Fractional dose of IPV
GAP III	WHO Global Action Plan III
GAVI	Global Alliance for Vaccines and Immunizations
GGT	Gamma-glutamyl transferase
GPEI	Global Polio Eradiation Initiative
Hib	Haemophilus influenzae type b vaccine
hPVR	Human poliovirus receptor
IFN-1	Interferon type 1
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IPV	Inactivated poliovirus vaccine
IPV-Al	IPV adjuvanted with aluminum

IRES	Internal ribosome entry site
IV	intravenous
iVDPV	immunodeficiency-associated vaccine derived poliovirus
LLOQ	Lower limit of quantitation
LMIC	Low-and middle income countries
ml	Milliliter
mOPV	monovalent oral poliovirus vaccine
mTmNVT	Modified transgenic mouse neurovirulence test
nAB	Neutralizing serum antibodies
NIDs	National immunization days
nOPV	Novel oral poliovirus vaccine
nOPV2-c1	Novel oral poliovirus vaccine candidate 1
nOPV2-c2	Novel oral poliovirus vaccine candidate 2
OPV	Oral poliovirus vaccine
PAHO	Pan American Health Organization
PHEIC	Public health emergency of international concern
PPS	Post-polio syndrome
PV	Poliovirus
RNA	Ribonucleic acid
SAE	Serious adverse event
SAGE	Strategic Advisory Group of Experts on Immunization
SCR	Seroconversion rate
SIA	Supplementary immunization activity
sIPV	Sabin-IPV
SPR	Seroprotection rate
TCID	Tissue culture infective dose
TNF-a	Tumor necrosis factor alpha
tOPV	Trivalent oral poliovirus vaccine
ULOQ	Upper limit of quantitation
UNICEF	United Nations Children's fund
UTR	Untranslated region
VAPP	Vaccine associated paralytic poliomyelitis
VDPV	Vaccine derived polioviruses
VP	Viral protein
WHO	World Health Organization
WPV	Wild-type poliovirus

Chapter 1 Introduction

Chapter 1: Introduction

1.1 The poliovirus

Poliomyelitis is an infectious disease caused by the poliovirus (PV), a small non-enveloped enterovirus belonging to the Picornaviridae family. The poliovirus consists of a single-stranded, positive sense RNA genome surrounded by a capsid composed of 60 copies of 4 capsid proteins (VP1, VP2, VP3,VP4) forming an icosahedral structure. The 7500 nucleotides long RNA genome contains a long 5' untranslated region (UTR) followed by the coding sequence and a short 3' UTR *with poly(A) tail*. (1)

Three serotypes of poliovirus exist (PV1, PV2 and PV3), sharing for 70% an identical genome. (2) While the sequences encoding for the capsid proteins are unique for the polioviruses, the flanking sequences frequently differ because of recombination with C enteroviruses during circulation in nature. (3)

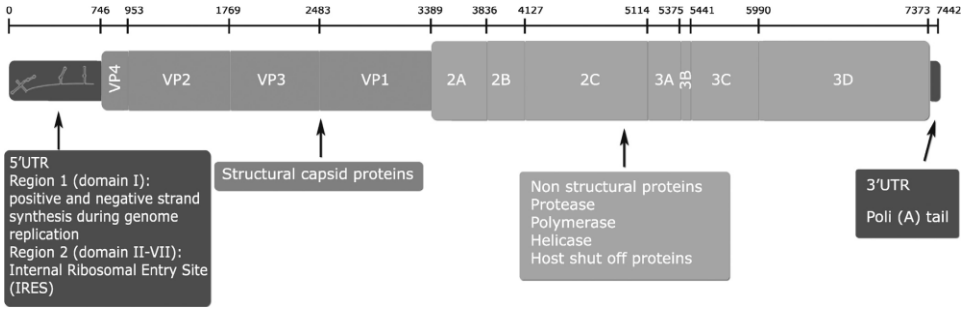

Figure 1. Schematic diagram of the poliovirus genome. Structural and non-structural virus-coded proteins are indicated. The 5' non-translated genome terminus (5'-UTR) regulates virus replication and plays a fundamental role in the synthesis of viral proteins. (4) Reproduced with permission for non-commercial use without adaptation, Creative Commons license.

The surface of the polio virion carries several peptide loops extending from VP1, VP2 and VP3 which form the neutralizing antigenic sites and in addition the attachment site for binding to glycoprotein CD155. (3) This glycoprotein belongs to the immunoglobulin superfamily and can act as human poliovirus receptor (hPVR) located on the cell surfaces. (1) Humans are the only natural host although some primates can be infected by inoculation or oral ingestion of high doses in experimental circumstances. (5)The reason for this species specificity is the

expression of a CD155 variant, capable for poliovirus binding in the intestinal epithelium, more specifically on the M cells of Peyer's patches. (6) After attaching to CD155 on the cell membrane the virus deforms and releases the RNA into the cytoplasm of the cell. Ribosomes bind to the internal ribosome entry site (IRES) of the 5'UTR and start the translation process leading to the creation of new virions which are released 4 to 6 hours later during the apoptosis of the infected cell. (1)

1.2 Pathogenesis

Transmission occurs mostly by faeco-oral and to a lesser extent by oral-oral route, the latter dominates in areas with good sanitation and hygiene. After ingestion, the virus first replicates at the oropharyngeal and intestinal mucosa (tonsils, intestinal M cells and Peyer's patches of the ileum) causing virus shedding that can persist for 1-2 weeks in the oropharynx while continuing for several weeks (4-8 weeks) in the intestine, being the main route of transmission. ((1), (5)) Via the cervical and mesenteric lymph nodes, the virus enters the blood stream and causes a transient viremia spreading the infection to other tissues (muscle, fat, liver spleen, bone marrow). Containment of the virus in this phase limits the disease to subclinical infection. If virus replication persists, the viremia becomes amplified and this enables the virus in rare cases to enter the central nervous system (CNS). (2)

Poliovirus can reach the CNS via two routes: via the blood through the blood-brain barrier (BBB) and via retrograde axonal transport. Several studies in CD155 transgenic and non-transgenic mice have shown that polioviruses are able to pass the BBB in a hPVR independent way although the specific mechanism is still not entirely clear. (6)The BBB is a multilayer barrier of vascular endothelial cells with tight junctions between the cells. Its main function is to protect the CNS from hazardous substances in the blood e.g. pathogens, while on the other hand it has to be permeable for transport of necessary nutrients. These nutrients can pass the barrier by specific carriers such as glucose, amino acids and transferrin. In vitro research has demonstrated that the poliovirus by his capsid protein VP1 can bind to the mouse transferrin receptor 1 and passes the BBB in an intact way. (7) In the CNS, the virus spreads to other cells by viral-induced cell death and this motor neuron destruction results in atrophy of the corresponding muscles and paralysis. (8)

The axonal route has been investigated after several clinical observations. Children who were accidently vaccinated with incomplete inactivated polio vaccine, derived from virulent poliovirus strains in 1954 ('Cutter vaccine incident') showed a high incidence of initial paralysis in the inoculated limb. Studies in transgenic mice revealed that after a hPVR mediated binding to the neuronal synapse, endocytosis of the poliovirus occurs after which the virus, packed in endosomes, is rapidly (>12 cm per day) and retrogradely carried through the axon of the CNS. The reason that the virus stays intact although the binding to the synapse is hPVR related is not known yet. Once the motor neuron cell body is reached uncoating and replication occurs.((1), (6), (8))

The determinant of neurovirulence however, seems not to be defined by the efficiency of those routes but rather by the capability of the virus to replicate in the CNS. (1) Serotype 1 poliovirus is known to be the most paralytic, accounting for almost 80% of paralytic infections in the pre-vaccine era in the US. (9) Molecular genetic analysis showed the importance of certain nucleotide positions in the IRES region of the viral RNA for neurovirulence e.g. Nucleotide position 480 in poliovirus serotype 1 and nucleotide position 481 in poliovirus serotype 2, whereas a mutation from C to U at the nucleotide 472 in the IRES of the Sabin vaccine strain impairs neurovirulence of poliovirus type 3. Many other neurovirulence determinants on the RNA genome are identified but IRES clearly plays an important role in the translation initiation. Mutations at these positions might reduce viral replication in the intestine sufficiently to allow the interferon alfa/beta response to limit viral replication more effectively. (1)

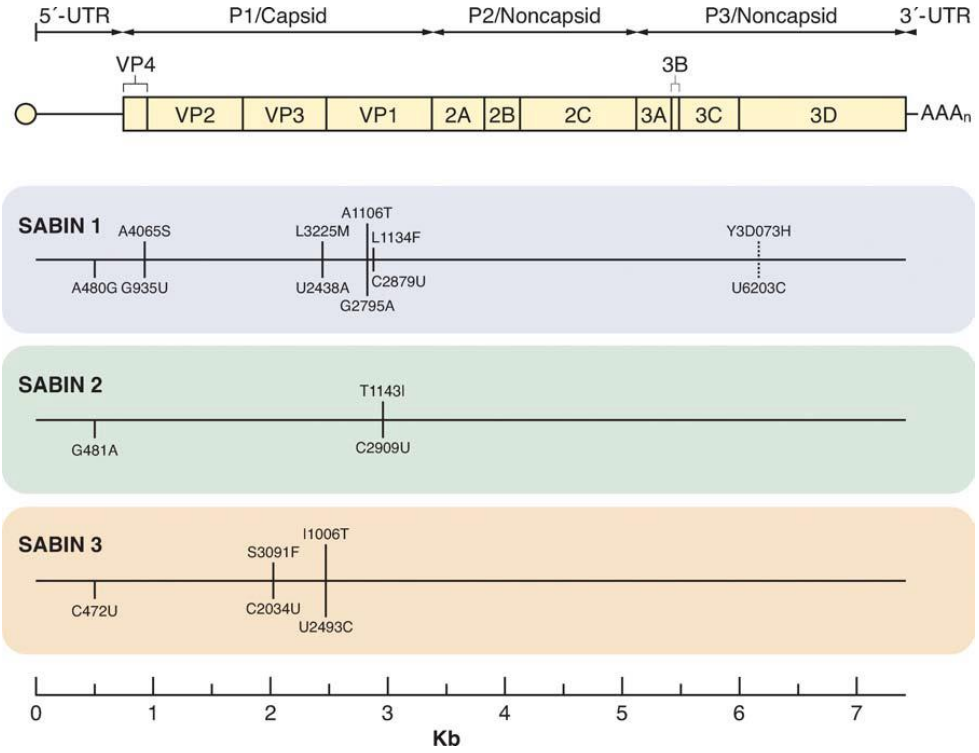

Figure 2. Location of principal attenuating nucleotide (lower bars)and amino acid (upper bars)substitutions in each of the three Sabin OPV strains. Abbreviations of nucleotide residues: A, adenine; C, cytosine; G, guanine; U, uracil. Abbreviations of amino acid residues: A, alanine; C, cysteine; F, phenylalanine; H, histidine; I, isoleucine; L, leucine; M, methionine; S, serine; T, threonine; Y, tyrosine. Substitutions are shown as nonattenuated parent–position–Sabin strain; nucleotide positions are numbered consecutively from residue 1 of the RNA genome; amino acid positions are indicated by the abbreviated name of the viral protein (4, VP4; 2, VP2; 3, VP3; 1, VP1; 3D, 3D-polymerase) and numbered consecutively from residue 1 of each protein. For example, a guanine (Mahoney)→uracil (Sabin 1)substitution at RNA position 935 (G935U) encodes an alanine (Mahoney)→serine (Sabin 1)replacement at residue 65 of VP4 (A4065S). The Y3D073H substitution in Sabin 1 and S3091F substitution in Sabin 3 are important determinants of temperature sensitivity.

In most cases the infection is asymptomatic or causes only mild flu-like symptoms and occasionally transient meningitis. The paralysis for which the disease is so notorious occurs in <1% of poliovirus infections. (1) Moreover, the 3 polio types differ greatly in their ability to cause paralysis, with type 1 causing much more paralytic cases in comparison to type 3 and type 2, the latter being the most sub-clinical type (table 1). (9)

Table 1. Ratio of Paralytic Poliomyelitis Cases to Number of Infections for the 3 Serotypes of Poliovirus, Assuming an Overall Ratio of 1:150[a]

Poliovirus Serotype	% of Paralytic Cases	No. of Paralytic Cases per 100 Infections	No. of Infections per Paralytic Case
1	79	0.526	190
2	8	0.053	1,886
3	13	0.087	1,149
Total	100	0.667	150

a The distribution of paralytic cases by serotype was based on unpublished laboratory studies on typing of poliovirus isolates for the United States for 1955, as reported by the Centers for Disease Control and Prevention. The ratio of 0.667 paralytic cases per 100 infections is the median of values from the 15 studies cited in (10). The breakdown by serotype was computed from these data. Table 1. (9) Reproduced with permission from (9), American Journal of Epidemiology

The incubation period for paralysis is on average 10 to 14 days (range 3 -35d). Paralytic illness often shows 2 phases, starting with a few days of mild disease and a symptom-free period of 1 to 3 days, followed by fever and intense muscle pain. The onset of flaccid paralysis starts suddenly and progresses rapidly to the maximum extent of the paralysis. The paralysis is usually asymmetric, descending (from proximal to more distal muscles), affecting more often lower than upper limbs and is associated with diminished or complete loss of tendon reflexes and an intact sensory system. The anatomic location of motor neuron damage in the spinal cord (anterior horn) or in the brainstem (medulla) defines which muscles will be affected: skeletal muscles (spinal paralysis), respiratory muscles (bulbar paralysis) or a combination of both muscle regions (bulbo-spinal) may occur. This paralysis is usually permanent although partial or total recovery can be achieved because of compensation by other, still functioning muscles. (3) However, bulbar paralysis, had previously a high mortality rate because of respiratory failure before modern methods of assisted ventilation became available. (2)

No real cure for polio exists. The treatment consists mainly of supportive therapy with physiotherapy to stimulate and strengthen the muscles and prevent contractions together with antispasmodic medicines. In case of respiratory impairment intubation and intermittent positive pressure ventilation have replaced the iron lung support which saved many polio patients in the outbreaks of 1940s and 1950s.

1.3 Post-polio syndrome

The acute poliomyelitis infection is followed by a period of partial functional recovery, to a greater or lesser extent, which then remains stable for many years. However, 15 to 60 years later 20 to 75% of polio survivors, depending on the diagnostic criteria applied, experience new gradual onset of progressive and persistent neurological deficits such as muscle weakness and atrophy, fasciculations, myalgia and arthralgia, often accompanied by generalized fatigue. (4) New respiratory or swallow problems are less common. Sudden onset of these symptoms is rare and may follow a period of inactivity, trauma or surgery. New symptoms occur mostly in previous affected regions but can also become apparent in former sub-clinical affected muscle groups. Typically, muscle strength declines slowly with a rate of 1-3% per year, with a decline of 9-15% over an 8 year-period. (11) The impact on the functional capabilities depends mostly on the residual degree of impairment after the polio infection many years ago. (12) This new weakness has to be distinguished from long-term effects of poliomyelitis which are mainly caused by the biomechanical alterations in mobility due to chronic musculoskeletal deformities and weakness induced by the polio infection many years before. (13) Although already recognized in late 1800 post-polio syndrome (PPS) became only accepted as a new medical condition in 1980s because of the high number of cases being reported since the 1970s. PPS is primarily a clinical diagnose based on new progressive muscle weakness persistent for at least 1 year and supported by all kinds of laboratory, electrophysical and other technical examinations to exclude possible other causes. (13)

The pathophysiology of PPS is not yet completely understood. Late attrition of oversized motor neuron units that developed during the recovery process of paralytic polio have been demonstrated and is the most likely etiology. (4) After the acute poliomyelitis infection, reinnervation of the affected muscles occurs through collateral axonal sprouting in response to denervation, resulting in gradually enlarging motor units. When they become too large to be metabolic sustainable these axonal sprouts can no longer be maintained, resulting in distal degeneration and new dysfunction. In addition, physiologic aging and overuse of functioning muscle units can contribute to the gradual motor unit failure. In some PPS patients poliovirus genome fragments in the cerebrospinal fluid (CSF) and peripheral leucocytes have been found together with high immunoglobulin M (IgM) antibodies against poliovirus, suggesting persistence or reactivation of poliovirus. However, it is not yet clear if and to what extent this persistent virus

contributes to the development of PPS. (4) No specific anti-muscle or antineuronal autoantibodies have been detected so far which makes autoimmunity as possible cause less plausible but persistent inflammation can play a role. Increased levels of tumor necrosis factor alpha (TNF-a) and Interferon gamma (IFN-γ) have been observed in serum and CSF of some PPS patients which respond to intravenous (IV) human immunoglobulin (Ig) therapy regarding pain and vitality and will be further investigated. (14)

Treatment of PPS needs to be individualized and multidisciplinary, aiming for quality of life improvement by training activities for muscle strength and endurance without further muscle unit degeneration. Research is exploring the benefits of divers pharmacological products of which IV human immunoglobulin therapy is most promising, showing positive effects on severe pain, fatigue and paresis of the lower limbs below 65 years of age. (4)

1.4 Epidemiology

1.4.1 Pre-vaccine era

In ancient times, poliomyelitis was an endemic disease and polioviruses infected nearly every person but only sporadically caused paralytic symptoms. Only a few historical cases have been reported in literature with probably one of the most earliest records being an Egyptian stele (1580-1350 BCE) showing a man with a flaccid paralytic leg, characteristically for polio paralysis of the lower limbs as we know it nowadays. (9) This changed by the end of the 19th Century when suddenly large epidemics of paralytic poliomyelitis were reported in the United States, Norway and Sweden, rapidly followed by several other European countries. (9) Outbreaks occurred annually and although incidence of paralytic cases varied highly between regions and countries, the burden of disease increased gradually with a peak incidence of 57.879 cases reported in the US in 1952. (2)

This shift towards an epidemic form was mainly due to improvements in sanitation and hygiene delaying poliovirus exposure from infancy, where protection was given by maternal antibodies, to later in life when these maternal antibodies already waned. In the presence of these passively acquired maternal antibodies, intestinal infection still occurred but invasion of the central nervous system was avoided by averting the viremia phase. As such, active natural immunity was obtained while infants were still protected by passive immunity. (9) So, with improvement of personal hygiene and sanitation, transmission of enteric

infections occurred at a later age when children were less protected against paralysis.

In Low and middle income countries (LMIC), the endemic pattern remained much longer and change occurred more gradually. In these poor areas cases were often underreported and epidemics less circumscribed due to the continuing underlying high endemic infection rate. Therefore, it remained for a long time unrecognized that polio prevalence was similar in LMIC countries compared to the industrialized ones. (15) Poor and crowded living conditions support virus circulation and in these communities most children became infected at a very young age and very low incidence of paralysis was seen. Several studies showed the presence of polio specific antibodies in ≥ 80% of children less than 4 years old in areas with endemic circulation, presumable similar to the global seroprevalence in the pre-epidemic era. If paralysis emerged, it occurred in 90% of the cases in children below the age of 5 years. (16) This overall community immunity was illustrated in several cases during World War II when American and British soldiers contracted polio in tropical countries while the disease didn't seem to attack the native soldiers and only few cases in the indigenous people occurred in little children. (17) However, following improvement of public health, outbreaks started also in these areas and soon it became clear that these outbreaks involved a similar shift towards the age of 5-9 years and even adolescents and young adults. As the risk of more severe disease increases with the age of first infection this was reflected in the number of paralytic cases arising in the population. (15)

In the absence of vaccination, paralysis occurs in 1 out of 200 infected children during an epidemic, accounting for approximately 650,000 children becoming paralyzed globally each year. Case fatality rate of paralytic polio is commonly 5-10% but can be much higher depending of the age of onset of the disease. During the epidemics, it became apparent that a higher age at contracting the disease is an important risk factor for severe disease, affecting more limbs or inducing bulbar forms of paralysis requiring respiratory support which frequently occurred in adolescents and adults. (16)

Muscle injuries, fractures and strenuous exercise around the time of polio infection are also known to enhance the risk for paralysis. Paralytic polio occurring within 30 days after a series of multiple injections is known as 'provocation poliomyelitis', whereby the virus uses the injured nerves to reach the CNS by fast retrograde axonal transport. This provocation poliomyelitis contributed to the polio public health problem in countries with a preference for intramuscular

preventive and therapeutic administrations as was seen in Romania and Cameroon. (18) (19)In addition, evidence of epidemics showed that tonsillectomy and adenoidectomy preceding polio infection within one month increases the risk to bulbar poliomyelitis substantially, demonstrating the important role of the mucosal immunity system of the nasopharynx.((20), (21))

Incidence and case fatality rate variations are also determined by the circulating poliovirus serotype. The paralysis attack rate is known to be highest for poliovirus type 1 (0.5%) and lowest for serotype 2 (<0,05%). (3) In addition, immunologically identical virus strains can highly differ in virulence and strains of low virulence can spread longtime unnoticed in the community until a more virulent one emerges and causes a new epidemic in the children that were not yet immunized by the less virulent strains. (22)

In temperate areas, the disease shows a seasonal pattern, predominantly in summer and fall while incidence is more continuous in tropical zones indicating that relative humidity plays a role in transmission. (9) (2) After the development of polio vaccines, this seasonality has been used to its advantage by planning annual mass vaccination campaigns during the low transmission season in many LMIC countries. (23)

1.4.2 Vaccine-era

After the development of inactivated poliovirus vaccine (IPV) by Jonas Salk in 1955, the incidence of paralytic polio decreased very rapidly in the countries where it was implemented. In the US, a decline was shown from 18,308 reported cases in 1954 to 2,499 cases in 1957. Later, the oral poliovirus vaccine (OPV) was developed by Albert Sabin in 1961. The availability and widespread use of both vaccines reduced the number of reported cases even further (in the US: 988 cases in 1961 to 61 cases in 1965) leading to elimination of wild type poliovirus in a gradual increasing number of countries. (24)

In many LMIC countries, national vaccination programs started much later (1970-1980) under the impulse of the World Health organization (WHO)'s Expanded Program on immunization (EPI) and therefore global OPV coverage with 3 OPV vaccines before the age of 1 year only reached 75% coverage by 1990. (25)Poor sanitation, tropical setting, large birth cohorts and high population density are major risk factors for poliovirus transmission. Failure of cold chain, reduced vaccine immunogenicity, use of low-potent vaccines and periods of vaccine shortages are often additional problems to be dealt with in these settings. Hence, these countries had to undertake substantial efforts to reach high OPV coverage

and population immunity, often through annual mass campaigns in addition to strengthening of the routine immunization programs. (see Figure 3)

While cases in LMIC countries in the pre-vaccine era mostly occurred in younger children due to endemic exposure, the decrease in wild poliovirus circulation after vaccine introduction also diminished after a few years natural immunity and outbreaks now typically started to affect also older unvaccinated children and adults. Presence of under-or unvaccinated subpopulations creating a large number of susceptible persons exposed to circulating polioviruses is in any case the most critical factor leading to the emergence of outbreaks. The cause for not being vaccinated is very diverse and varies between countries. Religious communities, refugees or migrant groups such as Roma populations or unregistered children are often representing large groups of people difficult to reach for routine immunization.((15), (26)) In unsecure areas or regions difficult to access e.g. Pakistan and Afghanistan, transmission is more difficult to interrupt, in particular complicated by regular cross-border population movement and civil war. (9) In tropical regions, vaccination strategies cannot benefit from seasonality and concurrent enterovirus infections often reduce vaccine efficacy. Inadequate sanitation and hygiene create ideal conditions for poliovirus circulation and outbreaks have occurred in LMIC countries with coverage levels of ≥ 87%. Root cause analysis for these outbreaks indicated that the usual serological antibody levels can be insufficient in case of large inoculum of wild poliovirus. Therefore, the figure for national immunization coverage can be misleading for the true population immunity. (27)

Adding supplemental OPV doses to routine OPV vaccination schedule to overcome vaccine failure or implementation of annual mass vaccination and door-to-door campaigns to reach all younger children proved to be efficient tools to reach herd immunity as was shown in Cuba. (23) Poliovirus surveillance however is very important to rapidly detect and contain poliovirus circulation. As paralysis only occurs in one of 200 infections, countries can't rely solely on acute flaccid paralysis (AFP) monitoring but need environmental surveillance to detect timely poliovirus circulation. Since poliovirus genome sequencing was realized in the early 1980s and RNA viruses are known for rapid evolution over short time periods by stepwise accumulation of nucleotide substitutions, sequence comparison became a key element in polio control. (28) Genetic and phylogenetic typing of lineages gave insight to geographic distribution of different genotypes over time. (29) This contributed greatly to reconstruction of transmission pathways, detecting sources of imported viruses and identifying pockets of sustained virus circulation within

overall highly vaccinated regions. (28) Environmental surveillance in Israel proved the presence of silence circulation of wild polioviruses despite the high vaccine coverage of more than 90% with IPV in 2013. The overall high population immunity prevented the outbreak of cases but lack of mucosal immunity sustained virus circulation and potential transmission to unprotected individuals. (30)

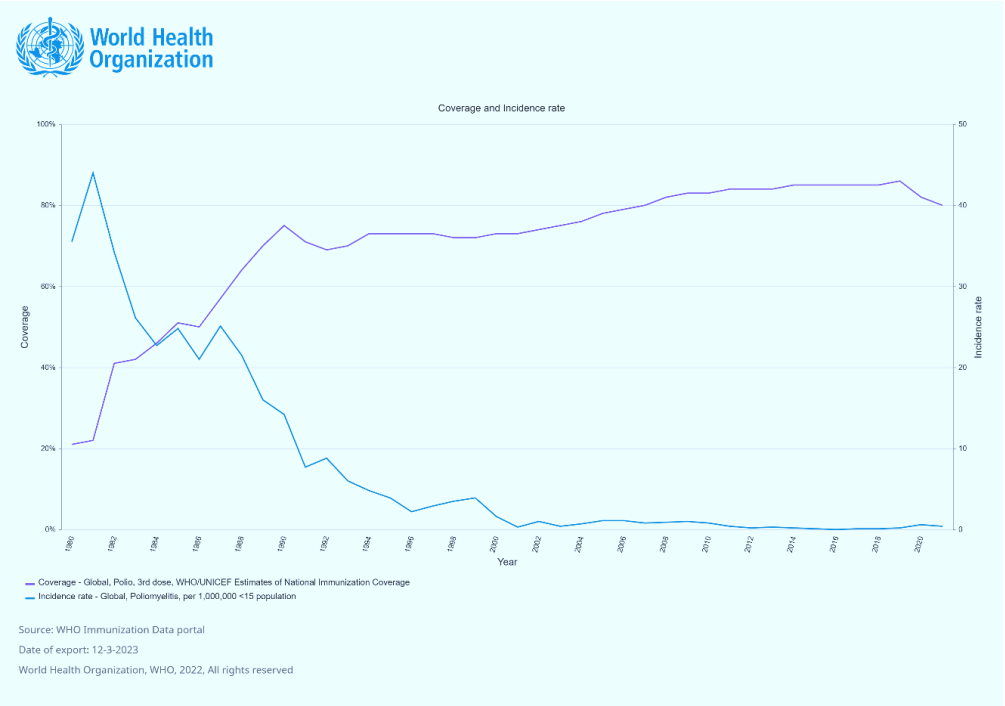

Figure 3: Global polio 3rd dose versus incidence rate. (31)

1.5 Immune responses to poliovirus infection

The innate immune system is the first line of defense and a major contributor to the control of poliovirus infection and prevention of neuronal attack. (6) In particular type 1 interferon (IFN-1), autophagy and apoptosis appear to have key roles in control of viremia and as such in the protection of motor neuron invasion. After cell entry, the virus blocks the host cell replication system and starts its own replication process which triggers an apoptosis mechanism of the host cell. Early cell death before the virus has completed his life cycle and can spread to neighboring cells is an effective strategy of the innate immune system to reduce further spread in the host. (32) Type 1 Interferons produced by infected cells activate in adjacent cells IFN-stimulated genes and certain enzymes like Protein kinase R to block virus replication and cell damage. However, the efficiency of this response depends on timing and magnitude as non-structural proteins 2A and 3C of the poliovirus (figure 1) are able to interfere in this process and deactivate these enzymes. (4) Probably most infections remain subclinical because of this effective innate immune response whereas people with genetic variants in their immune system might not be able to contain the viremia in extra-neural tissues. (33)

After 3 to 4 days of infection the humoral response becomes activated with first Immunoglobulin M (IgM) and Immunoglobulin G (IgG) neutralizing antibodies appearing, followed by secretory Immunoglobulin A (IgA) limiting mucosal virus replication 1 to 3 weeks later. Neutralizing serum antibodies(nAB) are serotype specific, persist lifelong and are protective against paralytic disease but not to reinfection. If reinfection occurs no viremia takes place and shedding duration is reduced. ((2), (4))

The role of the cellular immunity is less clear but presence of CD4+ helper T cells and CD8+ cytotoxic and memory T cells that recognize viral capsid epitopes has been demonstrated. (4) All 4 capsid proteins contain T-cell epitopes but most frequent detected human T cell responses are directed to VP1 and most of its T cell epitopes are located near neutralizing antigenic sites. (34) Polio-specific CD4+ cells not only support B cells for antibody production but also produce gamma interferon (IFN-γ) similar to the CD8+ T cells. Both CD4+ and CD8+ responses are cytotoxic to poliovirus infected cells and their long-term persistence has been demonstrated in OPV vaccinated subjects, contributing as such to the long-term protection seen after PV infection or vaccination. (35)

1.6 Vaccines

Since their development, the IPV and OPV vaccines have been used successful worldwide, with the specific advantages and disadvantages of both vaccines largely determining the choice of a vaccine in the national immunization schedule.

1.6.1 IPV

1.6.1.1 General

The Salk vaccine has undergone considerable development since it was first marketed. It contains three wild reference virus strains, Mahoney (type 1), MEF-1 (type 2) and Saukett (type 3), inactivated by formalin. (3) Shortly after licensure in the US, a failure in the manufacturing process of the vaccine at the Cutter plant led to 260 polio cases with PV type 1 in vaccinated people of which 10 were fatal. (36) After investigation, stringent safety control measures were applied and the production process was modified, leading to a decrease of immunogenicity of the vaccine and thus providing less protection to vaccinated children. Nevertheless, the vaccine was still sufficiently effective to realize a rapid and significant decline of annual polio cases in the US. (37) It was not until the 1980s that a new cell culture technique with Vero cells was developed allowing to increase the immunogenicity of the vaccine again to optimal standards. (38) The potency of the inactivated vaccine is expressed in D-antigens, which are the native antigens of the three serotypes inducing protective antibodies in both infected or vaccinated individuals. While previous IPV formulations differed between manufacturers and countries, the enhanced IPV contains usually 40, 8 and 32 D-antigen units of virus types 1, 2 and 3 respectively and is since then the most commonly used formulation of the vaccine.((36) (3))

After the development of OPV in 1961, many countries switched to the OPV vaccine because of higher immunogenicity in comparison with the IPV available at that time next to lower cost, easy administration and the ability to induce intestinal mucosal immunity. However, polio has been successfully eradicated in Sweden, Finland, Iceland and The Netherlands by the use of IPV only. Since 2014, as part of the global polio eradication plan, all countries are gradually switching to IPV and currently most developed countries only use IPV while other countries are using mixed schedules with OPV and IPV to close the gap in OPV immunogenicity. In some LMIC countries OPV immunogenicity remains low despite multiple doses because of multiple interacting factors. In these countries

a supplementary IPV dose can improve the immune response more effectively compared to another additional dose of OPV. (37)

The IPV vaccine is available as a monovalent vaccine or as part of combination vaccines for primary as well as booster immunization and dosing schedules vary greatly between countries. The vaccines most commonly co-administered with IPV in combination vaccines are, Hemophilus influenzae type b (Hib), hepatitis B, diphtheria+ tetanus+ acellular pertussis (DTaP), while pneumococcal conjugate and rotavirus vaccines can be administrated concomitantly. (39)The addition of IPV to the combination vaccines doesn't interfere with the immune response to the other antigens, nor has a consequence on the safety profile. (38)

The most common route of administration of IPV vaccines is intramuscular. Subcutaneous administration is also possible and e.g. applied in Japan while intradermal administration is increasingly gaining interest in the context of the global eradication plan and the associated current IPV shortages.

1.6.1.2 Safety

The Cutter incident led to highly improved production and testing standards to ensure inactivation is complete and since then no manufacturing failure has been reported. (37) IPV has proven to be a very safe vaccine with little reactogenicity. When used alone IPV is well tolerated causing mostly local reactions such as limited erythema (0.7-2.4%), swelling (0.4%) and pain (0.7-34%) at the injection site. Mild fever is the most frequent systemic reaction (7-18%). (40) In association or combination with other vaccines (e.g. DTP), reactogenicity remains similar to that attributed to those other vaccines alone. No serious adverse events have been causally related to IPV. In addition, adding a dose of IPV prior to OPV immunization has proven to reduce the risk of vaccine associated paralytic poliomyelitis (VAPP) compared to OPV-only regimens and therefore this schedule has been introduced in countries which had previously a history of high VAPP incidence.((38), (39), (41)) (see further)

1.6.1.3 Immune responses

Humoral Immunogenicity

Post-vaccination serum neutralizing antibody titers above the 1:4 to 1:8 dilution threshold have been defined as the correlate of protection against paralytic poliomyelitis by preventing viremia and a ≥ 4-fold rise in neutralizing antibody titer is widely accepted as the standard for seroconversion. (42) Field studies demonstrated a clinical efficacy to poliomyelitis of 36% for 1 dose, increasing to 89% for 2 doses. (43) A common infant immunization schedule of 3 doses leads to seroconversion rates of 85-100% against each serotype and >90% after 2 doses when initiated after 8 weeks of age. However, seroconversion rate might be lower when starting the vaccination schedule earlier due to the presence of high levels of maternally derived antibodies. ((44), (45))

In 2016, all trivalent OPV (tOPV) using countries switched to a bivalent OPV (bOPV) schedule (containing type 1 and 3 types) with minimal 1 dose of IPV preferably at 14 weeks following the WHO recommendation. With this schedule type 1 and 3 seroconversion rates remain high (>85% across different regimens) while type 2 seroconversion rates vary depending on timing of first IPV dosing and the number of IPV doses . One dose of IPV at the age of 14 weeks following 3 doses of bOPV in a 6-10-14 week regimen elicits an average type 2 seroconversion rate of 75%. Adding a second dose of IPV can increase this up to ≥96% (46) These higher responses compared to prior assessments can be explained by the later timepoint of IPV administration when no more impact of maternally derived antibodies is expected. The lower type 2 seroconversion rate (51.3%) noticed in Pakistan with a regimen of bOPV at birth-6-10-14 + IPV at 14w can be possibly explained by high levels of circulating maternal antibodies at baseline due to widespread (19%) passive exposure to circulating type 2 vaccine viruses. This reduced impact of IPV dose at 14 weeks was also seen in India. Literature review of 8 randomized controlled clinical trials between 2015 and 2018 reporting bOPV and IPV regimens (mixed or sequential administration) showed that after 1 dose of IPV >90% of the participants either seroconverted or was primed for type 2 poliovirus. In the latter case rapid seroconversion occurs within 1 week after additional vaccination among those who didn't seroconvert before. However, the correlate of protection against paralysis for priming is not yet clear and will need further investigation. (46)

Mucosal immunogenicity

The ability to induce robust pharyngeal and intestinal mucosal immunity is one of the greatest advantages of OPV because it limits enteric replication and thus shedding at a next exposure to wild or vaccine viruses. Pharyngeal immunogenicity of IPV is similar or even slightly better than that of OPV and has contributed to polio-eradication in the IPV-only using northern European Countries where the oral-oral route of transmission is likely to be predominant. (47) In regions where the faeco-oral route is the most likely way of transmission however, the role of IPV is more complex and limited.

Clinical assessment of intestinal mucosal immunity to poliovirus can be assessed in an individual by measuring vaccine poliovirus shedding after a challenge dose. (48) Although IPV generates high titers of neutralizing antibodies and protects against paralytic disease, several studies have demonstrated the limited effect of IPV on induction of intestinal immunity.((44), (49))This makes the vaccine less useful in outbreak containment and also creates a risk for populations vaccinated IPV-only in case of re-introduction of poliovirus. (50) Vaccinated people will become infected and shed the viruses in their stools for several weeks while being unaware of it because of absence of disease. These circulating viruses can eventually cause outbreaks in subpopulations with low vaccine coverage as occurred in the Dutch religious communities in 1978 and 1992 (in the bible belt in the Netherlands). (51) Isolation of Wild type 1 poliovirus in Israelian sewage also demonstrated that even with a national IPV coverage of 95% for 3 doses such poliovirus circulation can exist unnoticed for very long time.((30), (52))

While the proportion of IPV vaccinees who are shedding after OPV vaccination is similar or mildly reduced in comparison with unvaccinated people, the duration of shedding and titers of excreted viruses are less. Nevertheless, this remains highly inferior to shedding reduction in OPV vaccinees. (44) (50) Also, combined bOPV-IPV schedules show limited overall impact on virus type 2 shedding especially around the day 7 peak of excretion post-challenge. (53) Administration of high dose IPV generates significantly higher serum neutralization titers but doesn't influence protection against shedding after poliovirus exposure.((47), (54)) This was also confirmed by regression model analyses of 2 Latin-American clinical studies in infants primed with mixed or sequential bOPV-IPV schedules. Only weak negative relationship was indicated between type 2 neutralizing antibody titers at the time of mOPV2 challenge and the Shedding Index endpoint, the mean $\log_{10}$ stool type 2 viral titer during the 4 subsequent weeks. Even very

high pre-challenging titers were insufficient to substantially reduce viral shedding. (53)

The reduction of intestinal poliovirus shedding after OPV immunization is especially related to the secretory IgA response induced at the mucosal surfaces. In addition, serum IgA can provide a second line of defense against pathogens that have breached the mucosal surface. (55) IPV administration alone isn't able to induce this response. Infants primed with IPV-only schedule only showed minimal enteric IgA before challenge with mOPV2 but polio-specific IgA in stool and virus neutralizing activity increased rapidly in the 2 weeks post-vaccination indicating that replication of live virus in the intestine is essential for this immune mechanism. However, IPV can boost the poliovirus specific IgA present in individuals who previously have been exposed to wild-type or live-attenuated (OPV) poliovirus and induce gut-homing lymphocytes. (48) This effect is most clearly seen in older children and young adults who had a longer interval between OPV priming and the age of the IPV administration. But also a study in elderly Dutch persons showed that remaining serum IgA titers due to IPV vaccination after natural infection protected against viral excretion after additional OPV challenge. (55), (56)) The most probable explanation is that IPV reactivates polio-specific memory responses after waning of the intestinal response established after live virus exposure. Also, while serial administration of OPV and IPV as primary series didn't show much additional effect on intestinal immunity, one single dose of IPV several months after priming with OPV was much more successful. This boosting of intestinal immunity by IPV is, similar to OPV, temporary and wanes over the next 2 years. Thus, in the absence of additional vaccine doses after primary immunization, mucosal immunity to poliovirus replication declines and might create an immunological gap in older children and adults. ((57) (46))

1.6.1.4 New IPV vaccines and administration routes

Sabin-IPV

In Japan and China, IPV vaccines based on Sabin strains currently have been licensed and in several laboratories in other countries Sabin-IPV vaccines are under development. (58) Because virulent poliovirus strains are required in the manufacturing process production of Salk-IPV has not been allowed in low and middle-income countries with limited resources and weak biosafety regulatory environments. An accidental containment breach in the manufactory would pose

a serious risk to communities with a large number of susceptible people. In view of the global polio eradication process with gradual switch from OPV to IPV, production cost of IPV could be substantially reduced for LMIC countries if they would be able to manufacture the vaccine themselves. (59) Use of Sabin-IPV in that respect would be safer. However, due to antigenic epitope differences between wild and Sabin viruses, their sensitivity to formalin inactivation is not similar and results in different immunogenicity. Typically, type 1 Sabin-IPV is much more immunogenic while type 2 Sabin-IPV is much less immunogenic than the conventional Salk vaccine. ((60) (61)) Therefore, optimal dosing for Sabin-IPV is different from Salk-IPV and due to the methodological difference in D-antigen assays among labs antigen contents of Sabin-IPV from different manufacturers couldn't be directly compared by Sabin-D antigens. Harmonizing of potency assays and establishment of international reference reagents for Sabin-IPV therefore became paramount. (58) In 2018 a new WHO international standard for sIPV products was established with definition of the Sabin D-Antigen unit (SDU) to express potency of sIPV. From then on DU has been used to express the D-antigen content of Salk-IPV and SDU for that of Sabin-IPV. (62) (63) Sabin-IPV is now added to the routine immunization in China (bOPV at 2M, 3M, 4M + Sabin-IPV at 4M) based on large phase 2 and 3 trials which proved similar immunity profile of Sabin and Salk-IPV (64) (65) Following the establishment of a technology transfer hub in the Netherlands (Intravacc) by the WHO and a similar program in Japan (Biken), a growing number of manufacturers are starting up development of Sabin-IPV in order to address the long-standing problem of global IPV shortage. (66)

Intradermal route of administration

To overcome the global shortage of IPV due to upscaling problems after the switch in 2016, intradermal fractional dosing with one fifth of the intramuscular dose has been investigated. Seroconversion rates after 2 intradermal doses were equal to or higher than a single full intramuscular dose for type 1 and 2. Therefore, this way of administration has already been implemented in routine immunization schedules in Asia in response to local IPV supply constraints. (45) No additional benefit can be expected from this alternative administration on primary intestinal immunity. (46). However, studies have shown that similar to intramuscular IPV administration a single fractional IPV (fIPV)dose is able to boost mucosal immunity in previously OPV vaccinated children. (67) (68) In addition, studies have

shown that 3 fractional doses of IPV intradermally administered are protective to all 3 polio serotypes. (69)

Also, promising results have been shown in one Cuban study in which fractional dose administration by intramuscular route given to polio vaccine naïve infants at 4 and 8 months of age was as immunogenic as via intradermal administration. (70) (71)) Further studies will be needed to assess the impact of maternal antibodies to the seroconversion rate if fractional intramuscular doses are administered at an earlier age (e g. at 6 and 14 weeks of age).

Adjuvanted IPV

Neither Salk- or Sabin-IPV contain any adjuvant. (72) (73))Therefore, dose-sparing can also be obtained by adding this to the vaccine. Phase 2 trials in the Dominican Republic and phase 3 trials in the Philippines with IPV adjuvanted with aluminum (IPV-Al), containing one 10^{th} of the standard IPV dose showed a robust immune response with seroconversion similar to conventional IPV administration when used in accordance with the WHO 3-dose EPI schedule. (74) The observed seroconversion rates of IPV-Al versus IPV in the phase 3 trials were 97.1% versus 99.0% for type 1, 94.2% versus 99.0% for type 2 and 98.3% versus 99.6% for type 3. Seroprotection defined as an antibody level of ≥8 was reached by a large majority of infants (≥ 97.9% versus ≥99.6%). Also booster vaccination 6 months later induced a clear immune response proving immunological priming after primary vaccinations. (75)

Clinical trials with Double Mutant Heat-Labile Toxin (dmLT), a potent adjuvant derived from Escherichia coli have been conducted based on promising animal data regarding humoral and mucosal immune responses. Another phase 1 study investigating IPV adjuvanted with dmLT administrated intradermally as $1/5^{th}$ of a standard IPV dose induced higher serum neutralizing antibodies compared to standard IPV in young adults without any effect on intestinal neutralization antibodies. (76)

1.6.2 OPV

1.6.2.1 General

Since 1961, live attenuated oral polio vaccine developed by Sabin has been marketed. First as monovalent oral polio vaccine for each type (mOPV) and followed in 1963 by trivalent OPV (tOPV) which contained the three types of polioviruses. tOPV quickly became globally the most used polio vaccine thanks to benefits of lower cost, easy use, induction of mucosal immunity, secondary vaccination of susceptible individuals by spread in the environment and a humoral immunity superior to that of IPV at that time (before the development of the enhanced IPV). (3) The vaccine strains were originally derived by passage of polioviruses in cultured primate cells, followed by selection of mutants with low virulence for primates. Currently, they are yielded by passages on Vero cells which ensures better bacterial and viral sterility and selected by genetic sequencing. (77) Polioviruses are RNA viruses which are known for their high mutation rate (in contrast to DNA viruses) because of lack of proofreading mechanisms of RNA polymerases. Therefore, RNA virus populations do not consist of a single genotype but are a heterogenous collection of related sequence variants, also called 'quasispecies'. This presence of high variety in the virus population enables the quasispecies to evolve and adapt to challenging environmental conditions rapidly because of cooperation of different subpopulations. (78) Because OPV strains are also quasispecies, stringent measures and test procedures are applied during the production process of the vaccine to keep the number of revertant variants as low as possible (<0.1%) to ensure a uniformly highly safe product. (77) OPV strains are derived from the original Mahony strain (type 1), P712 strain (type 2) and Leon strain (type 3). (3) The low degree of neurotropism of OPV is acquired by specific nucleotide substitutions of which the most critical ones are located in domain V of the IRES (internal ribosome entry site) in the 5'-UTR region of the Sabin strains. This RNA stem-loop structure domain V is a highly preserved region among polioviruses and assumed to be important for efficient translation initiation. (79) The attenuation mechanism is not yet completely understood but it results in a decreased fitness for replication in all tissues which might enable the immune response to prevent sufficient virus reach the CNS. (80) While Sabin 1 is characterized by 57 nucleotide substitutions, Sabin 2 and 3 contain far less nucleotide constitutions with for each only 2 main substitutions in combination with stabilizing mutations in the capsid region. (3) This explains some of the features of the Sabin strains: Sabin 1 appears to be highly genetically stable which is important because of the high paralytic ability of WPV1. Sabin 2 is less stable

but is also low neurovirulent and highly immunogenetic while Sabin 3 has low stability and immunogenicity with an intermediate attack rate, hence the high VAPP (Vaccine associated polio paralysis) involvement. The applied genetic alterations have not been isolated in wild poliovirus specimens, however, these genetic manipulations are not stable in the human intestine and reversion to the original structure or to variants with higher replicative fitness happens frequently. Nevertheless, neurovirulence of Sabin OPV strains is much lower than of wild strains as shown by the incidence of VAPP (2-4 cases/ million births in tOPV using countries) compared to incidence of paralytic poliomyelitis in areas with wild poliovirus circulation and contributed highly to the rapid decrease of polio epidemics worldwide. (3)

Throughout history, OPV has been produced as mOPV (for each type), tOPV (3 types) and bOPV (type 1 and 3). The vaccine is available in multidose vials that contain doses of 0.1 ml to be administrated as 2 drops in the mouth while tOPV also exists in single dose vials (0.5 ml). (81) Generally, OPV contains 10^5median tissue culture infective dose ($TCID_{50}$). Because lower seroconversions were seen for type 1 and 3 with tOPV administration, a more balanced dosing was applied to the trivalent vaccine which formulation should contain at least 10^6 $TCID_{50}$, $10^5 TCID_{50}$ and $10^{5.8}$ $TCID_{50}$ for Sabin serotypes 1, 2 and 3 respectively. Especially in tropical countries, this balanced dosing was necessary to reach a better immunogenicity with tOPV for serotype 3. ((82), (83)) After withdrawal of type 2 in 2016, mOPV is only used for a stockpile of vaccine available for potential outbreak control and bOPV became the most used oral polio vaccine in combination with one or more doses of IPV in routine immunization. No combination products containing OPV have been licensed. (81)

Thermal instability is inherent to live vaccines, so OPV has to be stored frozen and can be used after thawing for 30 days if kept refrigerated. Since 1996, the vaccines are equipped with vaccine vial monitors that change in color in response to heat exposure. This development facilitated the use in tropical areas as it enables to take the vaccine beyond the cold chain to reach remote locations and reassures the vaccinator of the potency of the vaccine. (84)

1.6.2.2 Immune responses

The immune response after OPV administration mimics natural infection and is characterized by induction of humoral as well as mucosal and cellular immunity with complex interaction.

Humoral response

IgM antibodies are temporary present from the first days after vaccination until 2 or 3 months, while IgG antibodies remain and may persist for life. Any titer of homologous neutralizing antibodies is sufficient to prevent viremia after infection, a necessary step for invasion of CNS, and thus protective against paralytic polio. (44)

a) Trivalent OPV

In industrialized countries, seroconversion rate with 3 doses of balanced[1] tOPV is >95% for the three types of polioviruses. However, in LMIC countries the immunogenicity of OPV is generally much lower and although immune responses to the specific serotypes vary greatly between countries, in particular for serotype 1 and 3 these are often low. A review of 32 studies in LMIC countries showed that the mean seroprotection rate after 3 doses of tOPV in infants was only 73% (36-99%) for type 1, 90% (71-100%) for type 2 and 70% (40-99%)for type 3. (85) In a study in Brazil and Gambia with different tOPV dosages, only 57-70% of infants had detectable neutralizing antibody to all 3 serotypes after 4 doses. (86) This oral vaccine failure in low income countries is also known for other oral viral and bacterial vaccines such as the live attenuated rotavirus and cholera vaccines and is due to several factors, such as malnutrition, concurrent infection, diarrhea and tropical enteropathy. (87) The mechanism of interference of concurrent enteric pathogens is complex. Some enteroviruses use the same cell receptor as polio or compete after cell entry for other cellular factors required for viral replication and others intervene by causing diarrhea with higher intestine transit. The extent of interference varies among serotypes, serotype 1 seroconversion mostly affected in the presence of other enteroviruses while diarrhea diminishes mostly seroconversion of serotypes 2 and 3. (88) Impact of diarrhea on seroconversion is highest when present at the moment of the vaccination around 6 weeks of age or when a diarrhea episode occurred in the 2 weeks before vaccination and this might have long-term implications. In a study in Brazil, it was noticed that

[1] To reach a better immunogenicity for serotypes 1 and 3 balanced tOPV contains higher doses than the original tOPV for these 2 types.

seroconversion could remain inhibited when diarrhea occurred around the second OPV dose, even after normal further completion of the immunization schedule. Because of this high impact of diarrhea on immune response, WHO recommends an additional dose of OPV in children having diarrhea at the time of vaccination. (89) Concurrent pathogens might also impact immune responses to OPV by induction of non-specific innate immunity. Type I, II and III interferons raised by these enteropathogens may inhibit also vaccine virus replication and immunogenicity. (87) Several studies investigated possible associations between the microbiota composition and the OPV immune response but results vary between geographic areas. In Bangladesh, a study on 48 infants showed a positive association between an abundance of Actinobacteria, such as Bifidobacterium, at the moment of OPV administration and $CD4^+$ T-cell and IgG serum response unlike the negative association of a high abundance of Clostridiales, Enterobacteriales and Pseudomonales. (90) On the other hand, a study in India evaluating the effect of Azithromycin administration on the immune response of mOPV3 vaccination 2 weeks later, showed no improved immunogenicity although prevalence of bacterial pathogens was reduced. In this study a high prevalence of non-polio enteroviruses (NPEV) was associated with reduced seroconversion, seen in symptomatic as well as asymptomatic subjects. The effect was independent of the subspecies and recently acquired infections appeared to have a larger impact, possibly due to a peak of innate immune response shortly after exposure. (91) In a review of 25 trials meta-analysis evaluating the impact of concomitant enteric infections on OPV immune response indicated a significant negative effect of NPEV presence on the odds of serotype 1 shedding and seroconversion. Noteworthy, after administration of mOPV of any serotype the immune response appeared to be more subject to this negative interference than after tOPV. (88)

Additionally, in poor areas with limited sanitation and hygiene people are continuous challenged by pathogens in the gut often leading to chronic inflammation of the small intestine. This environmental enteropathy (EE) is a gut disorder typically characterized by structural changes resulting in malabsorption and impaired gut immune function leading to oral vaccine failure and is independent of presence of diarrhea. (92) One hypothesizes that the chronic inflammation of the small intestine keeps the innate immune system in a continuous pro-inflammatory state and generally hostile to any incoming pathogen. As such, attenuated polio vaccine strains will not induce specific adaptive immune responses but be destroyed by an already highly activated generally innate immune response. Another explanation could be that alterations

in regulatory T-cell or dendritic-cell function lead to suppression of immune responses. (87)

Maternal antibodies passively acquired via the placenta are known to interfere with seroconversion of several vaccines, are an important aspect of IPV failure in neonates and can affect OPV immune responses in infants, especially for serotype 3. In particular, titers of ≥ 1:360 can suppress antibody response after vaccination completely during the first 2 months after birth but the effect declines rapidly thereafter. (93) Polio-specific IgA titers in breastmilk may also play a role for OPV performance in LMIC countries where breastfeeding generally raises higher titers and is given for a longer period than in industrialized countries. The effect on oral vaccine immunogenicity is not yet completely understood as conflicting findings in studies demonstrate. The presence of IgA and innate immune factors in breastmilk might have a beneficial impact on the maturation of the infant immune system and provide resistance to enteric infection but can be inhibitory by impairing the replication of vaccine viruses in the intestine. (87) Several studies, however, have shown the benefit of an additional dose of OPV at birth, raising the immune responses with each successive dose with increased protection rate at the end of the primary immunization. As the incidence of enteropathogens is rather rare in neonates this may explain the better immune responses with a birth dose in tropical countries and has led to the recommendation of the WHO to add a birth dose of OPV in countries with lower OPV performance. (94)

Another factor contributing to the lower immunogenicity of OPV in LMIC countries is inherent to the vaccine itself. Because the type 2 vaccine poliovirus is more able to replicate in the intestine than the other types this type is immunodominant and contributes further to the lower seroconversion rates of the other types which might be impaired already by concurrent pathogens and enteropathy. (87)

b) Monovalent OPV

mOPV is more immunogenic than tOPV and therefore useful in LMIC countries to target specific needs for additional immunization against a specific serotype. Literature review showed median seroconversion rates in low income countries as 81% (range 53-89), 89% (77-93) and 72% (52-80) for types 1,2 and 3, respectively. However, immune responses can vary considerably between countries or even geographic regions within countries depending on poverty and lack of sanitation (95) Nevertheless, in regions where tOPV vaccine failure for a certain serotype occurs, targeted mass campaigns with mOPV have shown to induce a significant higher efficacy per dose and an important tool in interruption

of transmission and circulation of polioviruses.((96), (45)) Particularly mOPV1 and 3 have been used in such supplemental mass campaigns in the period 2005 and 2009, contributing successfully to the gradual eradication of these wild serotypes in settings difficult to control with tOPV alone. A stockpile of mOPV2 is maintained for outbreak control of cVDPV2 after cessation of OPV2. (45)

c) Bivalent OPV

Since 2010, mOPV mostly has been replaced by bOPV as this vaccine showed a superior vaccine response to tOPV and not inferior to mOPV. The success of this vaccine in the supplemental vaccine campaigns and certification of wild type poliovirus 2 in 2015 lead to the global switch in 2016 from tOPV to bOPV in the routine immunization. Currently, tOPV is not available anymore for routine immunization and mOPV2 remains only in stockpile to be released by WHO for outbreak control of vaccine derived poliovirus type 2 (cVDPV2) outbreaks. (45)

Mucosal immune response

OPV has the ability to induce mucosal immunity at the level of the pharynx as well as the intestine. Limiting replication of polioviruses in the intestine is reflected in reduction of amount and duration of shedding after contact with live or vaccine polioviruses and is an important feature of OPV for LMIC countries where the faeco-oral route is the most important route for transmission. This immune mechanism is primarily driven by neutralizing antibody responses mediated by IgA. Trials in infants have shown a strong type-specific intestinal immunity after vaccination with OPV which reaches peak levels after 2 weeks and limits type-specific virus shedding after a next exposure to live polioviruses. This mucosal immunity however seems to wane over time as lower fecal IgA responses and longer shedding have been reported after exposure of OPV to older children and adults. Inhibition of shedding after OPV challenge may already start to wane within a year after OPV priming and can remain incomplete even after multiple doses. Age-related studies in children aged 1 to 4 years demonstrated that the odds of virus excretion significantly increases with the time since last vaccination In addition, studies in older children and adolescents previously primed with IPV show very limited IgA responses after OPV challenge despite enteric replication of the virus and strong type- specific serum antibodies suggesting an age-related diminishing mucosal immunity. (49) Also, investigation of intestinal immunity of IPV-primed adults challenged with nOPV candidates in Belgium showed similar findings of very low or absent virus neutralizing titers in stool with otherwise high serum neutralizing responses. (54) Microbial competition due to infection with

other enteroviruses and environmental enteropathy in LMIC countries are possible factors that might contribute to this reduced polio-specific IgA in the intestine and need to be further explored. (49)

Interestingly, while IPV is not able to induce mucosal immunity after a priming series with OPV or IPV in infants, supplementary dosing of IPV in older children has a clear added value in seroconversion and intestinal immunity after a series of OPV. In regions with lack of good sanitation and hygiene often poor seroconversion rates remain even after multiple rounds of mass OPV campaigns. In Uttar Pradesh, India the efficacy of tOPV reached only 9% and 21% throughout the rest of India despite the fact that in some parts of the country children had received on average 15 doses through national immunization rounds. (97) Adding IPV to the vaccination regimen in these circumstances can boost the intestinal immunity by reactivation of polio-specific memory responses established after the live vaccine exposure during infancy. (49)

Cellular immune response

Studies in poliovirus receptor-transgenic mice revealed the synergic action between T cells and B cells necessary to elicit an adequate immune response. Because protection is predominantly acquired through polio-specific neutralizing antibodies (nAB), CD4+ T cells were expected to belong to the T helper class Th2 which are known for their support to antibody production by B cells. However, research showed that poliovirus-specific murine CD4+ T cells secreted interleukin 2(IL-2) and gamma Interferon (IFN-γ) typically for cell-mediated immunity of the Th1 class of T cells. Yet, IFN-γ secreted by polio-specific Th1 clones clearly also increases the AB response of primed B cells. This was demonstrated in murine challenge experiments in which transfer of primed B cells without T cells only resulted in a low and insufficient antibody response. Similarly, transfer of CD4+ cells alone didn't protect the transgenic mice from paralysis in case of poliovirus challenge. (98) Research on whole blood of OPV-primed subjects confirmed further the role of antigen presenting cells to elicit T cell proliferation after exposure to poliovirus or its isolated viral capsid proteins. All 4 capsid proteins contain T cell epitopes but strongest human T cell responses are seen to VP1 and most of its T cell epitopes are located near neutralizing antigenic sites. (34) The importance of adjacent B and T cell epitope locations to induce a high neutralizing antibody response is also shown for other viruses, such as influenza A. (99) In addition, viral clearance by cytotoxic CD8+ has been demonstrated in PBMCs of OPV vaccinated adult subjects. Similar to CD4+T cells IFN-γ is secreted by CD8+ T

cells in response to poliovirus antigen presented by dendritic cells or macrophages and enables the CD8+ T cells to lyse infected cells. Furthermore, as this immune response could be triggered from T cells of subjects vaccinated more than 20 years earlier evidence has been demonstrated for long-term persistence of poliovirus specific memory CD4 + and CD8 + T cells. (35). Infants however, show remarkably high nAB responses when vaccinated with 4 doses (birth, 1,2 and 3 M) while T cells produce only poor IFN-γ responses and also low or no cytokines known for the Th2 pathway. Therefore, it remains unclear which T cells support the neonate B cell in antibody production and more research will be needed to increase our understanding of the role of the cellular immune system. (100)

1.6.2. 3 Safety

Vaccine associated paralytic poliomyelitis (VAPP)

The most important adverse event that can occur only after administration of OPV is vaccine associated paralytic poliomyelitis (VAPP). (45) Vaccine polio strains replicate in the intestine and can in rare cases mutate and revert to a neurovirulent state, able to enter CNS and cause polio paralysis similar to wild polio viruses. This can occur in a recently vaccinated individual (recipient-VAPP), with manifestation of paralysis within 7 to 30 days as well as in a susceptible contact of a recent (7-60 days) OPV vaccinated person (contact-VAPP).((101), (102))

Typically, poliovirus isolates of immunocompetent VAPP cases show only limited genetic divergence from the parental OPV strains except for reversion of some key attenuations. (3)Type 3 VAPP mostly occurs in healthy recipients or household contacts whereas type 2 is mostly observed in immunodeficient people or in non-household contact cases. VAPP due to serotype 1 is less frequent, presumably because it contains more attenuating nucleotide substitutions than the vaccine viruses type 2 and 3. (103)

The risk of contracting VAPP varies between different countries depending on environmental circumstances and country-specific immunization policies. In industrialized countries, the highest risk is with first dosing in a recipient (mostly infants) or unvaccinated contacts or in immunocompromised people with B-cell immune deficiency (e.g. agammaglobulinemia or hypogammaglobulinemia) affecting the humoral response. (103) Multiple muscular injections within 30 days before administration of OPV also increases the risk of VAPP, as was seen in

Romania and Hungary, 2 countries that suffered in particular from a high VAPP burden.((36), (41)) The kind of serotype administered in mono-bi-or trivalent dose also appears to influence the risk. In Hungary, annual mOPV3 mass campaigns targeting children at the age of 2 months to 3 years being in use for more than 20 years in absence of routine infant immunization were probably also a contributing factor to a high number of annual VAPP cases. Data from this country suggest that administration of tOPV gives a lower risk than mOPV3 administration and a higher one than with mOPV1 administration. (41)

In low income countries, highest risk is observed in children >1y (e.g.1-4 years in India) and can occur even after multiple previous doses of OPV. Possible reason for the continuing VAPP risk at later age is the lower immunogenicity of OPV in LMIC countries. This is mainly due to concurrent intestinal infections inhibiting a good immune response to the vaccine by limiting the replication of polioviruses in the intestine and the longer protection by maternal antibodies. In LMIC countries, maternal antibodies show higher titers and thus longer prevalence and in addition OPV immunization starts early, often at birth with the second dose administered at 6 weeks of age. Therefore, maternal antibodies might prevent infection of the gut by OPV in the first months of life and thus possible development of VAPP while this risk rises with the first subsequent dose given after waning of these maternal antibodies. In high income countries, the first dose is given only at 2 months of age when maternal antibody titer is already lower with higher risk of 1th dose occurrence of VAPP. ((103), (104))

The global risk for VAPP before 2014 was estimated to be 2-4 cases per million births with an annual burden of 250 to 500 cases in tOPV using countries. In endemic countries, the risk of VAPP was much lower than the risk of paralysis by circulating WPV (approximately 1/200 for WP1, 1/1000 for WP3). Since wild type cases declined drastically thanks to the global efforts in the polio eradication program, VAPP cases have become an important reason to gradually withdraw all OPV worldwide. (103) Global removal of OPV type 2 in 2016 and addition of 1 or more doses of IPV to the infant immunization schedule has been a first step in this direction and reduced the estimated VAPP incidence below 2 cases per million births. (45)

Vaccine derived polioviruses (VDPVs)

Another important safety aspect of Sabin OPV is due to the low genetic stability of the attenuations and the ability to spread to contact persons. By analyzing clinical or environmental poliovirus isolates through genetic sequencing 3

categories (wild, vaccine-related and vaccine-derived polioviruses) can be distinguished based on the extent to which they genetically differ from the Sabin strains. For this purpose, the 900-nucleotide region encoding the major poliovirus surface protein VP1 is systematically checked during routine surveillance. (105) Current wild polioviruses are genetically unrelated to any vaccine strain and show more than 15% difference in VP1 nucleotides. (3) The difference between vaccine-related and vaccine-derived isolates is based on the natural mutation rate over time. Nucleotide substitutions accumulate naturally in polioviruses at an overall rate of 1% per year. After infection of a susceptible person, the duration of replication in the gut lasts in general 4 to 6 weeks. (106) During this timeframe normally less than 5 VP1 substitutions occur. Therefore, poliovirus isolates are classified as vaccine-related if they differ from the respective parental Sabin strains by <1% of VP1 nucleotides for type 1 and 3 or <0.6% for type 2, whereas isolates with differences above these limits are classified as VDPVs and indicate prolonged replication or circulation through transmission. (105)

VDPVs can be further divided in **immunodeficiency-associated VDPVs (iVDPVs),** derived from immunodeficient persons with prolonged excretion after OPV, **circulating vaccine derived polioviruses (cVDPVs)** when person to person transmission can be proven and **ambiguous VDPVs (aVDPVs)** when no classification in one of these categories can be done because of absence of any link to immunodeficiency or transmission.

a) iVDPVs

iVDPVs develop in individuals with B-cell immunodeficiency, such as common variable immunodeficiency disorder (CVID), agammaglobulinemia or hypergammaglobulinemia and whose limited immune response doesn't succeed to overcome the intestinal poliovirus infection. This results in long term and sometimes even chronic (>5y) intestinal replication and shedding. The immunodeficiency is previously not always diagnosed and prolonged shedding after OPV can occur for several months or years before causing iVAPP after which the immunodeficiency is diagnosed. Between the marketing of OPV in 1961 and 2019 only 149 iVDPVs have been identified, mostly in high income countries because of more difficult survival of immunodeficient people in LMIC countries. Typically, the iVDPV isolates frequently have highly divergent and variable neutralizing antigenic sites in contrast to cVDPVs. After vaccination, the most important (gate-keeper) substitutions of the Sabin strains are rapidly lost enhancing replication and molecular evolution fitness at the initial stages of

replication before slowing down to the usual rate of 1 to 2% mutations per year. Most iVDPVs are type 2 infections (56%), followed by type 3 (22%) and type 1 (18%) with a few mixed infections (4%). Since the tOPV to bOPV switch in 2016, incidence of iVDPV2 has declined and type 3 and 1 iVDPVs became most prevalent. (107) At this moment, cVDPV2 outbreaks derived from iVDPV2 excretors have not been identified yet, probably because of a tight transmission bottleneck for the rapidly acquired gatekeeper mutations in the OPV2 recipient. (108) Nevertheless, chronic iVDPV2 excretors remain a threat for polio eradication because the risk of seeding cVDPV2 transmission will rise in communities with lowered intestinal immunity against type 2 poliovirus. (109) No standard treatment is available for iVDPV. Often IV and oral immunoglobulin replacement therapies are administered with variable outcome. Case reports describe some successful hematopoietic stem cell transplantations and recently poliovirus clearance was established following a therapy with the antiviral remdesivir for chronic SARS-CoV-2 infection. ((110), (111)). Further research is going on, including investigation of antivirals and the effect of monoclonal antibodies. (112) Therefore, as long as treatment of primary immunodeficiency is still limited, surveillance among persons with primary immunodeficiency is essential in low income countries for early detection and follow-up of iVDPV excretions to mitigate the risk for iVDPV spread. (107)

b) cVDPVs

Up to 2000, it was generally believed that in contrast to the neurovirulence attenuations in OPV strains the genetic determinants of limited transmissibility are much more stable. (113) During a polio outbreak in Haiti and Dominican Republic due to circulation of a derivative of OPV1 strain however, it was discovered that vaccine derived viruses certainly could be transmitted from person to person and be responsible for outbreaks. Increasing sensitivity of detection of cVDPVs quickly led to identification of cVDPV in other countries e.g. Philippines, Egypt, Nigeria, but also in middle income countries, such as Belarus and Romania. (114) Common denominators in these outbreaks always are areas with low background immunity due to low OPV coverage and previous eradication of WPV increasing the number of susceptible people, often combined with lack of adequate surveillance systems. Tropical conditions and lack of sanitation and hygiene are contributing factors but cVDPVs can thus also occur in industrialized countries if OPV coverage is not sufficient. (115) In Ukraine an outbreak of cVDPV1 emerged in 2015 due to an insufficient polio vaccination coverage of 50%.

Vaccination coverage increased in the following years but was still insufficient in 2021 (84%) causing a new outbreak in October 2021, this time of cVDPV2. (116).

Most cVDPVs are type 2, followed by type 1 and rather uncommon type 3, mostly due to transmissibility differences inherent to the 3 serotypes. ((3), (117)) Estimated needed duration of sustained transmission before VDPV emergence are 210, 300 and 390 days for types 2,1 and 3 respectively. (117) As type 2 poliovirus however, is low neurovirulent (1 paralytic case for approximately 2000 infections) the virus can circulate undetected for a long time. (115)

Research of OPV2 isolates in weekly stools of infants after OPV dosing in Bangladesh revealed that the 3 gatekeeper mutations of OPV2 rapidly occur (in the first weeks) but are not preferentially transmitted. Possibly, mutations must rise to an appreciable frequency early enough in a donor population to be frequently transmitted through a narrow bottleneck. As transmission usually occurs in the first 2 weeks after vaccination, mutations that tend to occur later are less likely transmitted. Additional mutations in the capsid region improve the within-host replication and thus cause greater shedding and transmission. This appears to be the main function of these mutations rather than antigenic escape as outbreaks of cVDPV always can become controlled by use of OPV campaigns. (108)

iVDPVs as well as cVDPVs show reversion or recombination at the critical determinants for attenuation. Recombination can occur between different vaccine variants deriving from the same initial OPV dose or with concurrent non-polio enteroviruses (NPEV), often with C species which is closely related to the polio viruses. Recombination vaccine/non-vaccine is usually not seen in iVDPVs. (3)

c) aVDPVs

Environmental ambiguous VDPVs most likely are iVDPVs or cVDPVs whose origin has not yet been discovered. The typical properties of both VDPVs sometimes can give an indication in the search for identification. Absence of non-vaccine recombinants and highly divergent and variable neutralizing antigenic sites point in the direction of iVDPV. aVDPVs discovered in areas with low OPV coverage are more likely indication of circulating VDPVS and gaps in surveillance. (105)

1.7 Polio endgame

To date, smallpox is the only disease that has been globally eradicated. The success of eliminating this disease by widespread immunization and case surveillance led to the establishment of the Expanded Program on Immunization (EPI) by the WHO in 1974. This program supported the global development and expansion of immunization programs with polio as one of the diseases of interest. At that time, fewer than 5% of children in LMIC countries received a third priming dose of polio vaccine in their first year of life. ((118), (119)) In 1985, the Pan American Health Organization (PAHO) set up a strategy for polio eradication including achievement of high infant tOPV immunization coverage, effective AFP surveillance and diagnosis under the age of 15 years, with additionally area-wide vaccination around any new cases. Combining enhanced infant immunization with national immunization days (NIDs) and mop-up campaigns if applicable resulted in a rapid decline of polio cases, even with a higher number of reported AFP cases which confirmed the improved surveillance system. (120)

This success encouraged the World Health Assembly in 1988 to target global polio eradication by 2000. To achieve this goal, the Global Polio Eradication Initiative (GPEI) was established, consisting of Rotary International, United Nations Children's fund (UNICEF), Centers for Disease Control and Prevention (CDC), Bill & Melinda Gates Foundation (BMGF), Global Alliance for Vaccines and Immunizations (GAVI) and the WHO. At that time, around 350,000 polio cases in more than 125 countries were reported annually. However, by implementing the triple strategy of PAHO in other countries, each country adapting it to their specific needs, the global incidence of polio cases decreased rapidly and by 1999 no more indigenous WPV cases were detected in the Americas, the Western Pacific Region, China and Europe. (45)

By 2000, global polio eradication was not yet achieved but significant progress was made as shown by the drastic drop in number of polio-endemic countries that declined to 20, further diminishing to 10 by 2004, and in the number of polio cases which declined with >99% (estimated global number of 1253 cases). Declining genetic diversity of WPV genotypes and lineages confirmed the success of the global efforts and in 1999 the last global indigenous WPV2 was reported, which occurred in northern India. Certification of wild poliovirus eradication can only be declared when any isolation of wild poliovirus from AFP patients, healthy individuals or the environment can be detected for at least three years in the presence of high quality surveillance next to containment of wild poliovirus laboratory stocks. (3)

By 2004, three WHO regions were certified polio-free: the Region of the Americas (1994), the Western Pacific Region (2000) and the European region (2002). (45) For the three remaining WHO regions enhanced efforts were needed to prevent regular import from endemic regions and to improve immunization as lower immunogenicity of tOPV in tropical countries was recognized. The basic principle is to achieve sufficient high rate of routine OPV immunization to block poliovirus transmission. However, in areas with high population density, lower sanitary and hygiene conditions and the presence of concurrent enteroviruses even OPV coverage rates above 90% might not be sufficient to achieve adequate immunity to all 3 serotypes. Supplementary immunization campaigns are needed such as NIDs targeting all children below a certain age and mop-up campaigns when polio surveillance indicates reservoir areas. (3), (45))The intertypic interference of tOPV itself and the interference in the intestine with other enteropathogens are the underlying cause of the diminished immunogenicity of tOPV in tropical conditions and especially type 1 was very difficult to get under control. In some regions of India e.g. protective efficacy per dose tOPV was estimated to be 11%. (96) Because of the higher immunogenicity of the mono- and bivalent vaccines mOPV1 and mOPV3 were re-introduced in 2005 and bOPV in 2009 to be deployed in the supplementary immunization campaigns. (45) The use of the different OPV formulations was complementary: tOPV was part of the routine immunization and national immunization days and was used in cVDPV outbreak control, bOPV was primarily administered in supplementary mass campaigns in endemic WPV1 and WPV3 regions, mOPV1 or mOPV3 were used in mass campaigns or mop-up campaigns in response to cases. (9) This successful strategy further diminished the number of polio cases to a few hundred in 2010 and only remaining in 4 countries Afghanistan, Pakistan, India and Nigeria. Eventually, also WPV1 in India became eradicated which allowed in 2014 the South East Asian Region to be certified polio-free (Fig.4). (45)

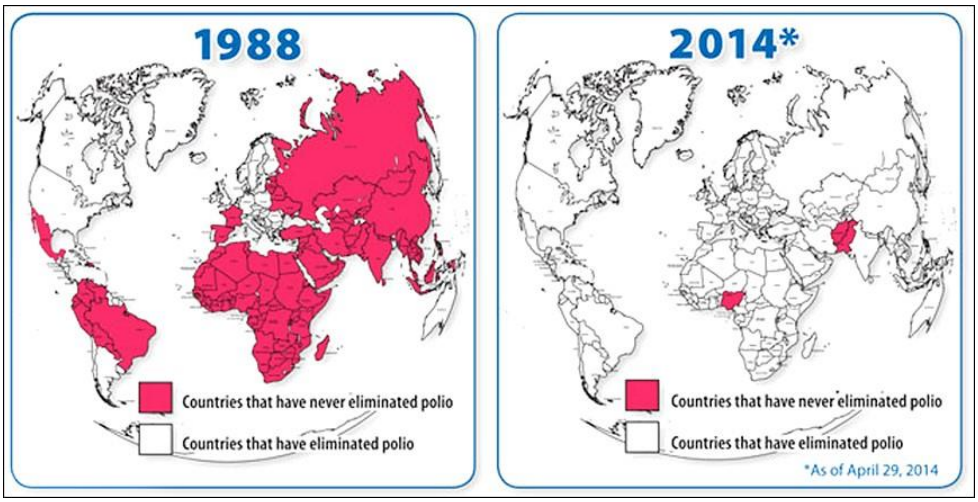

Figure 4. Map of the world comparing countries with polio cases in 1988 and 2014. Centers for Disease Control and Prevention, CDC (2014) via This Week in Global Health_ Blog Archives_ consulted 4Mar2023 (121).

In 2015, a major milestone was reached by global certification of wild type 2 poliovirus eradication. (122)

As it was recognized however that Sabin strains have the ability to revert and reacquire neurovirulence, causing VAPP and cVDPV it became apparent that a transition from OPV to IPV would be necessary to achieve a global polio eradication. The Polio Endgame Strategy, based on modeling for estimation of the risk of increased cVDPV outbreaks was endorsed by the World Health Assembly in 2012. (123) Sabin strains would be sequentially removed, in accordance with the certified eradication of the types, while at the same time one or more doses of IPV would be added to the routine immunization program to prevent immunity gaps that would arise from waning population immunity. (45)

Following the certification of WPV2 eradication in September 2015, a global switch from trivalent to bivalent OPV in 125 countries was coordinated in April-May 2016. A stockpile of mOPV2 remained at the WHO only to be used for outbreak control of cVDPV2. The impact on VAPP incidence was drastically reduced after this switch, preventing 150 to 300 cases annually. (45) However cVDPV2 circulation remained and caused an increasing number of outbreaks year after year. Contributing factors were multiple: cVDPVs already circulating pre-switch continued to spread, mucosal immunity for type 2 in young children post-

switch was waning and vaccine coverage was inadequate, partly due to supply constraints for IPV. Manufactures experienced major problems in the upscale of the production resulting in delay of IPV introduction or stock out in countries that used IPV already in their routine vaccination programs, affecting more than 43 countries until late 2019. Also, supplementary vaccination campaigns for outbreak control often started too late in regions with insufficient AFP and environmental surveillance. In addition, outbreak control with mOPV2 induced new seeds of strains with the ability to revert and to start circulating. (122) WPV cases declined further and since 2016 WPV1 is only endemic in 2 countries Afghanistan and Pakistan, except for 1 imported case of WPV1 from Pakistan to Malawi. (Feb 2022) and a few WPV1 cases in the neighboring country Mozambique (May 2022). The latter WP1 isolates were similarly genetically linked to a strain detected in 2019 in Pakistan and demonstrated the continuous risk of international spread as long as populations remain under-immunized. (124) In August 2020 Africa has been declared free of wild poliovirus by the WHO as no wild poliovirus was detected for more than 3 years in any African country. The last WPV type 3 case was detected in 2012 in Nigeria and global WPV3 eradication has been certified in 2019, which means that only WPV1 is left to be eradicated. By contrast, after the switch of tOPV to bOPV cVDPV cases were spreading rapidly to 24 countries and the number increased gradually from 2 in 2016 to 1074 in 2020 (Fig.6). Most cVDPV outbreaks are caused by type 2 and some by type 1, almost none by type 3. (125)

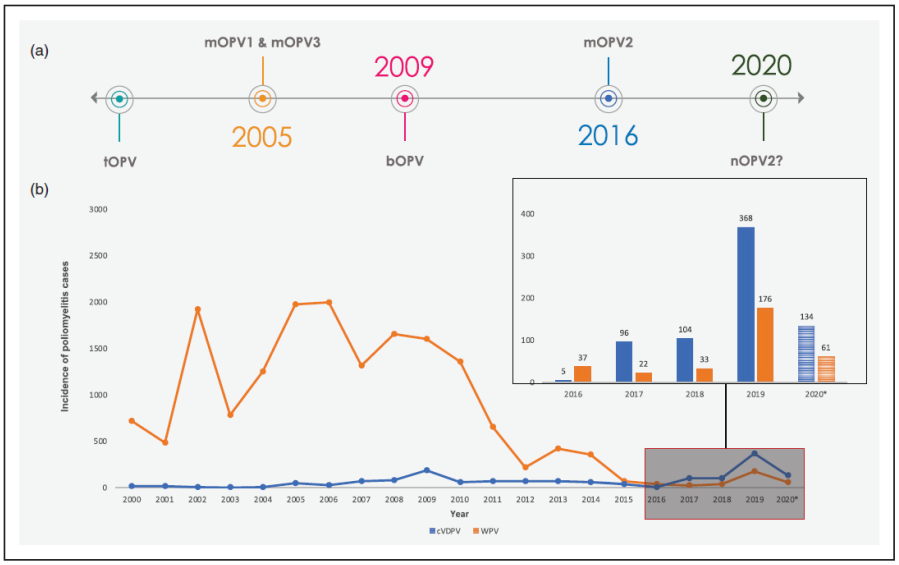

Fig.5 a) Use of different oral poliovirus vaccines in outbreak response over time. (b) Incidence of poliomyelitis cases from wild poliovirus and circulating vaccine-derived poliovirus, January 2000 – June 2020. _Cases for 2020 are those in the period 01 January to 01 June only. Data as of 03 June 2020. bOPV, bivalent oral polio vaccines; mOPV, monovalent OPV; nOPV, novel OPV; tOPV, trivalent OPV. Reproduced with permission from *Final frontiers of the polio eradication endgame* (70).

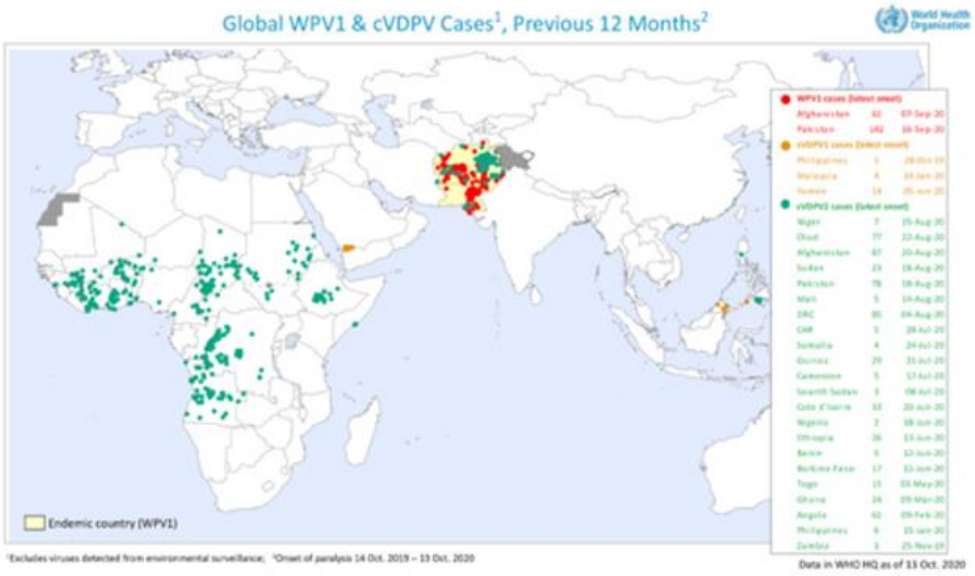

Fig.6 Overview of global WPV1 & cVDPV Cases of the previous 12 months: status at 13Oct2020, website GPEI Polio Now, https://polioeradication.org/polio-today/polio-now/ , consulted Oct2020. (126)

It is clear that OPV will gradually need to be replaced by IPV in order to ever achieve global polio eradication. However, this huge process is hampered particularly in LMIC countries by lack of manpower, cost and supply constraints of IPV on the one hand and the limited ability of IPV to induce mucosal immunity on the other which means continuous need of (instable) OPV for outbreak control.

Antigen sparing by fractional dosing of IPV (fIPV) via intradermal route can be a solution to limit costs and create supplemental doses. The WHO's Strategic Advisory Group of Experts on Immunization (SAGE) SAGE recommended in 2016 that countries should start preparing to implement 2 fIPV doses (at 6 and 14 weeks of age) instead of 1 full dose IPV at 14weeks. Since then several South Asian countries (e.g. India, Nepal, Sri Lanka and Bangladesh) and some Latin American countries (e.g. Cuba and Ecuador) adopted this strategy. (127) To overcome the more difficult administration via the intradermal route, several new devices are developed and some of them already approved by the WHO for fIPV use in outbreak control campaigns. (123)

Fractional dosing will also be an important aspect for the immunization strategy in the post-global polio eradication era. The 2-dose schedule with IPV will have to be continued for several years after global OPV withdrawal and therefore any kind of fractional dosing would help global IPV supply management.((127), (71))

Adding of adjuvants to the vaccine can reduce the needed amount of antigen to reach the same immunogenicity as with unadjuvanted IPV. However, no adjuvanted IPV is already in use in LMIC and currently IPV adjuvanted with aluminum is only licensed in Denmark. ((45), (70)) Research on other adjuvants continues. Double-mutant labile toxin (dmLT) showed promising results in animal studies, not only enhancing serum neutralizing antibodies but also inducing intestinal IgA secretion indicating enhanced mucosal immunity. However, this effect on the mucosal immunity could not be demonstrated in human adult studies with intradermal administration. (76) .

Production of IPV requires virus growth and currently mostly wild viruses are used to be treated with formalin for deactivation. Upscaling of IPV production needed pre- as well as post-eradication period involves maintaining of large laboratory virus stocks that have to be contained at a high level. IPV based on Sabin strains has been developed and licensed in Japan and China. (128) Although these strains are much safer than the wild ones, Sabin strains are unstable and the risk for reversion remains. An alternative could be the development of VLP particle vaccine derived from empty viral particles which could be engineered by recombinant technology and which process doesn't involve live viruses anymore at any stage. In early studies on transgenic mice these VLPs appear to be comparable with IPV for stability, immunogenicity and presence of antigenic structures. (129)

Research for novel oral polio vaccines (nOPV) that are genetically more stable than the Sabin strains has been supported since 2011 by the Bill and Melinda Gates Foundation and became more urgent because of the rapid increase of cVDPVs since 2017. Priority has been given to nOPV2 with the objective of outbreak control. Two candidate vaccines were developed with stabilized key attenuations in the 5´-nontranslated region and ready to be studied in phase 1 human trials in 2017. (130)

Chapter 2

Research questions and aim of the thesis

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2.1 Research questions:

The overall aim of the thesis is to explore if new polio vaccine candidates can fill the gap between IPV (high humoral immunogenicity but limited mucosal immunity) and OPV (high mucosal immunity but genetically unstable) regarding outbreak response and thus contribute to global polio eradication. The first (main) part of the thesis covers the assessment of 2 novel oral polio vaccine candidates (nOPV2-c1 and nOPV2-c2) and the following research questions are studied:

- What is needed infrastructurally to make the conduct of a first in human study in contained conditions possible? (Chapter 1)
- Are the 2 nOPV2 candidates safe for human use? (Chapter 2)
- What are the characteristics of the viral shedding: duration of shedding, mutation of shed viruses, neurovirulence? (Chapter 2)
- What are the immunogenicity characteristics of these nOPV2 candidates? (Chapter 2)
- What are the safety and immunogenicity characteristics of the current mOPV2 vaccine, so that they can be used as data for historical control in studies with new oral polio vaccines after global withdrawal of OPV2 in 2016? For, from then on the 3th WHO Global Action Plan (GAPIII) will be in place and mOPV2 use will be restricted to outbreak control only(Chapter 3)
- Has each nOPV2 candidate an acceptable safety profile in adults? For OPV primed subjects: in comparison with the mOPV2 data of the historical phase 4 study UAM1 conducted in 2016 at CEV, for IPV subjects: compared with placebo (Chapter 3)
- Does each nOPV2 candidate show non-inferior immunogenicity in comparison with the mOPV2 data collected in the UAM1 study? (Chapter 3)

In the second part of the thesis a new inactivated vaccine candidate has been studied and we address the next research question:

- What are the effects of the addition of dmLT adjuvant to IPV in respect to safety, immunogenicity and in particular: does it enhance mucosal immunogenicity? (Chapter 4)

2.2 Aim of the thesis:

A scientific consortium has been working since 2011 on research and development of novel poliovirus strains engineered to be more genetically stable with less likelihood of reversion to neurovirulence while retaining the benefits of Sabin OPV. The main aim of this PhD thesis is to investigate these 2 novel oral polio vaccine candidates, novel oral polio vaccine type 2 candidate 1 (nOPV2-c1) and novel oral polio vaccine type 2 candidate 2 (nOPV2-c2) for safety, immunogenicity, shedding characteristics and neurovirulence in healthy adults and to explore if at least one of these candidates could be of added value to the Global Polio Eradication Initiative.

In Chapter 3 we describe the necessary infrastructure that had to be developed to make conduct of the FIH study possible in 2017. Because WPV2 was declared eradicated in 2015 all OPV containing PV type 2 was withdrawn globally with only a limited stock of mOPV2 to be used in outbreak response. In addition, WHO installed the 3th Global Action Plan (GAPIII) with specific containment requirements for all facilities that process samples or retain materials that contain or potentially contain polioviruses. The aim of GAPIII is to minimize poliovirus facility-associated risk to re-introduce PV2 into the environment and the community. As the facility of the Center for the Evaluation of Vaccination at that time was only equipped for conduct of ambulatory trials and because of the urgent need for starting up a phase 1 trial in humans a temporary quarantine facility had to be built completely conform the biosafety restrictions of GAPIII. The design of the facility had to allow a 28-day stay for 2 groups of 15 volunteers and ensure that the vaccine strains could not enter the environment through excretion of the virus in the feces or other body fluids of the vaccinated participants or through transmission by study personnel. All biological samples that might contain polioviruses had to be captured and contained for shipment to central labs or subsequent decontamination and destruction.

- *Van Damme P*, De Coster I*, Bandyopadhyay A S, Suykens L, Rudelsheim P, Neels P et all. Poliopolis: pushing boundaries of scientific innovations for disease eradication. Future Microbiol. 2019; 14(15), 1321-1330*
  **joined lead authors*

In Chapter 4 the safety, immunogenicity and viral shedding of the 2 candidate vaccines have been investigated in a phase 1 study (UAM4a) with 2 cohorts of 15 participants. Healthy adults (aged 18-50 years) were clinically and psychologically screened for eligibility. Participants had to be IPV primed and never received OPV to ensure absence of mucosal immunity before receiving the study vaccine and to enable sufficient shedding for studying all shedding characteristics. Because no Belgian IPV-primed adults were available in 2017 (Belgium switched from OPV to IPV use in 2001) we recruited volunteers from Dutch origin with IPV only history. Volunteers were enrolled sequentially in 2 cohorts and in each cohort all participants were vaccinated with the same vaccine candidate which was determined by randomization of the first participant of the first cohort. After vaccination subjects had to remain in the contained facility until all participants of that cohort reached shedding cessation (PCR-negative viral shedding on 3 consecutive stool samples) or until day 28 post-vaccination was reached for the whole cohort, whichever occurred first. If shedding persisted after 28 days subjects were allowed to leave the facility but had to remain in Belgium and comply with restrictive measurements until they reached shedding cessation. Type 2 specific neutralizing antibodies were assessed at D0 and D28 and safety was followed up until D42.

- *Van Damme P*, De Coster I*, Bandyopadhyay, Revets H, Withanage K De Smedt P et al. The safety and immunogenicity of two novel live attenuated monovalent (serotype 2) oral poliovirus vaccines in healthy adults: a double-blind, single-centre phase 1 study. Lancet 2019; 394: 148-58*
  **joined lead authors*

In Chapter 5 we compare data of a phase 2 study (UAM4) with a historical control study (UAM1).

After eradication of WP2 and because of increasing circulation of vaccine derived polio viruses type 2 (cVDPV2) global removal of type 2 polio vaccine was planned to start in April 2016. At that time 2 novel oral vaccine candidates were developed

but not yet ready to be tested in human trials. To allow for comparison between the novel vaccines and the original Sabin vaccines at a later timepoint 2 trials have been conducted to serve as historical controls, one study (UAT1) with the trivalent polio vaccine (tOPV) and one study (UAM1) with the monovalent type 2 polio vaccine (mOPV2).

The UAM1 trial has been conducted in 1 center (CEV, Antwerp) in 2016 before the global switch. One hundred OPV primed healthy adults have been randomized to receive 1 or 2 doses of mOPV2. In 2018 the UAM4 trial was conducted in which 200 OPV-primed subjects were randomly assigned to receive 1 or 2 doses of nOPV2-c1 or 1 or 2 doses of nOPV2-c2 and 50 IPV-primed subjects were randomized to receive 2 doses of nOPV2-c1 or nOPV2-c2 or placebo. The study was carried out in 2 centers in Belgium (CEV, Antwerp and CEVAC, Ghent), each center to enroll half of the participants. Both studies were specifically designed to enable comparison between both nOPV2 candidates and Sabin mOPV2, primarily regarding safety and immunogenicity and exploratory in regards to viral shedding and genetic stability.

- *De Coster I*, Leroux-Roels I*, Bandyopadhyay A, Gast C, Withanage K, Steenackers K et al. Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in healthy adults: two clinical trials. Lancet 2021; 397: 39-50*
  **joined lead authors*

In Chapter 6 the effect on intestinal immunity of adding the adjuvant double mutant labile Toxin (dmLT) to inactivated trivalent poliovirus vaccine (IPV) has been studied in a phase 1 clinical trial. IPV is known to induce very good humoral immunity but only limited mucosal immunity. As such, IPV has little effect on virus transmission and cannot replace OPV in outbreak response. In addition, in 2016 trivalent OPV has been replaced in routine immunizations by bivalent OPV (containing serotypes 1 and 3) with at least 1 dose of IPV. However, since the global switch many countries suffered from insufficient IPV supplies due to global IPV shortage. Adding an adjuvant to IPV that can reduce the amount of antigen required to induce a proper humoral response while enhancing mucosal immunity could address these constrains. In this study we investigated in 80 healthy IPV-

primed adults safety, humoral and intestinal responses of 1 dose of IPV or 1 dose of IPV+ dmLT compared to 1 dose of bOPV (bivalent OPV containing serotypes 1 and 3) at D1 and the impact on viral shedding following challenge with bOPV at D29.

- *Erdem R*, De Coster I*, Withanage K, Mercer L D, Marchant A, Taton M, et al. Safety, tolerability, and immunogenicity of inactivated poliovirus vaccine with or without E.coli double mutant heat-labile toxin (dmLT) adjuvant in healthy adults; a phase 1 randomized study. Vaccine 2023; 41:1657-1667*
  **joined lead authors*

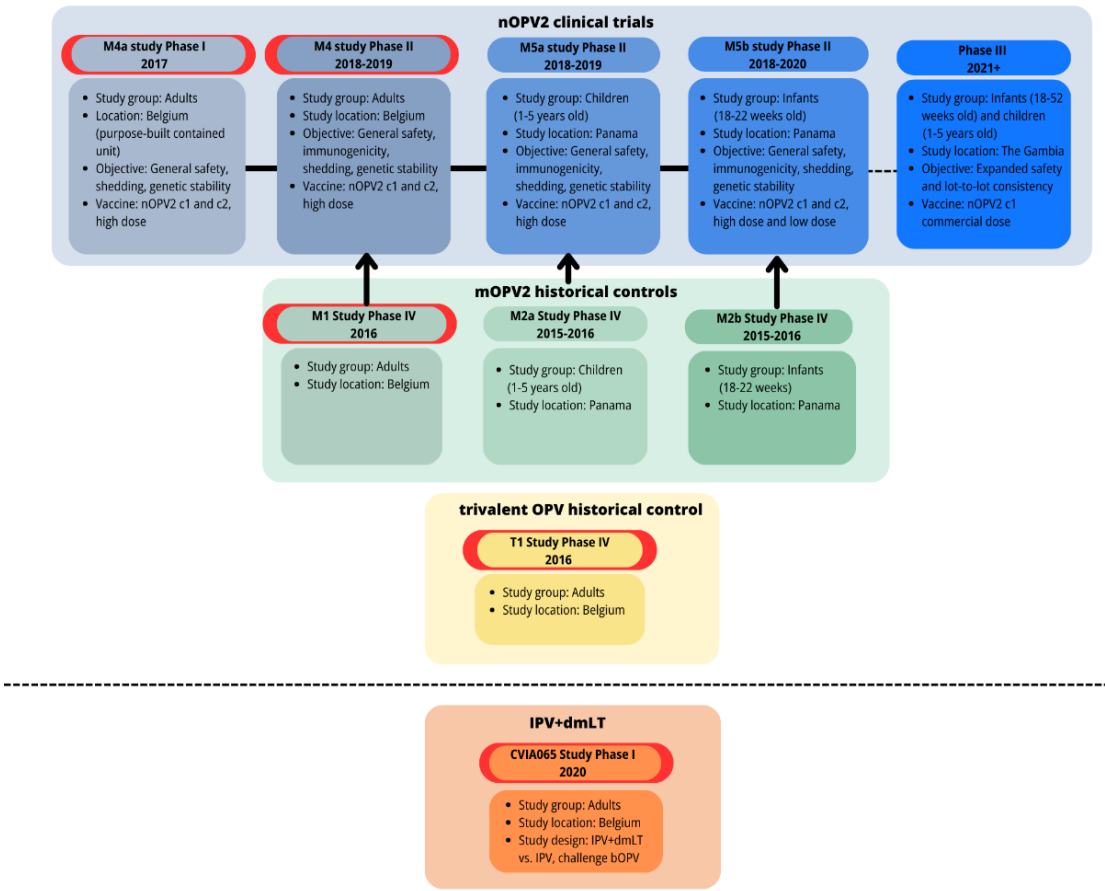
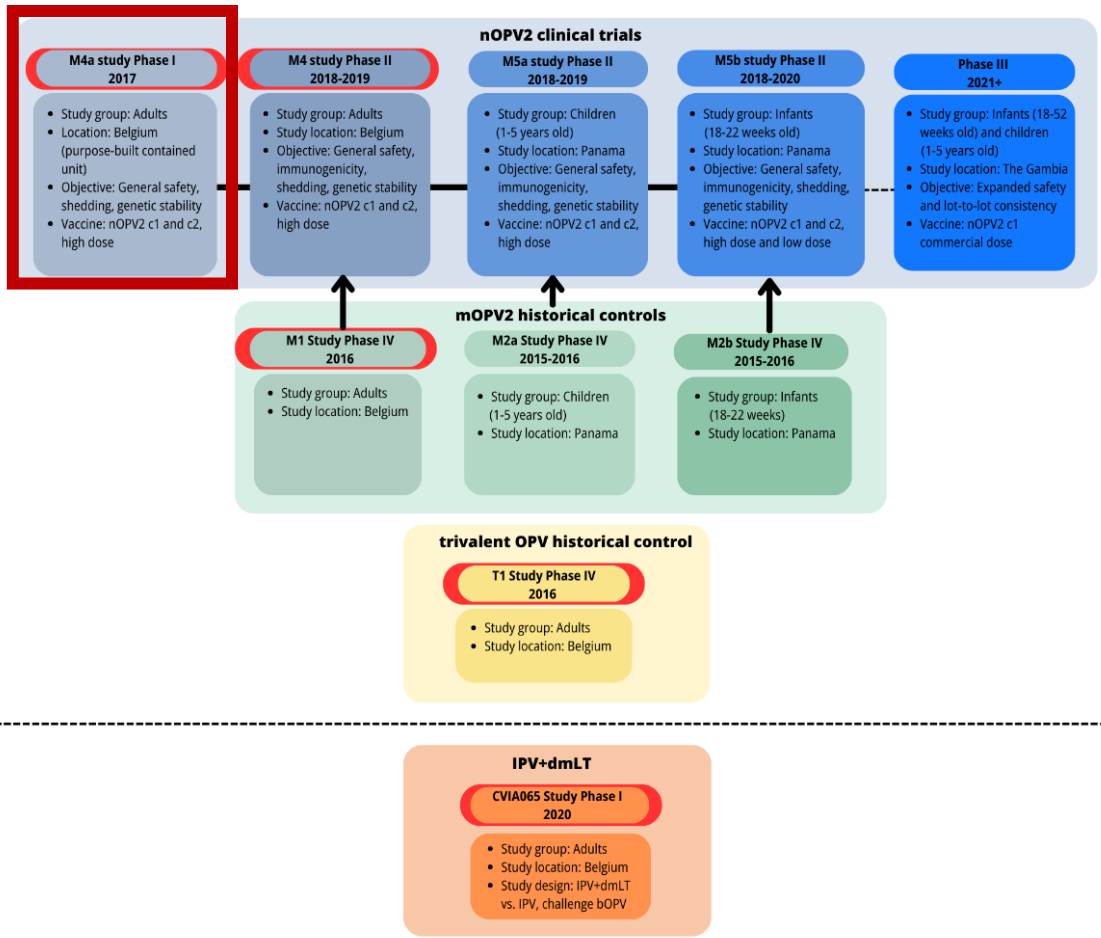


Fig. 1. Overview of studies discussed in this thesis. Titles in red are the studies that are subject of this PhD. Inspired by (131)

Chapter 3:
Poliopolis: pushing boundaries of scientific innovations for disease eradication

This chapter is published: "Van Damme P. & De Coster I., Bandyopadhyay A.S., Suykens L., Rudelsheim P., Neels P., Oberste M.S. et al. Poliopolis: pushing boundaries of scientific innovations for disease eradication. Future Microbiol. 2019;14(15):1321-1330." doi:10.2217/fmb-2019-0196


nOPV2 clinical trials
M4a study Phase I
2017
Study group: Adults
Location: Belgium (purpose-built contained unit)
Objective: General safety, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M4 study Phase II
2018-2019
Study group: Adults
Study location: Belgium
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5a study Phase II
2018-2019
Study group: Children (1-5 years old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5b study Phase II
2018-2020
Study group: Infants (18-22 weeks old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose and low dose
Phase III
2021+
Study group: Infants (18-52 weeks old) and children (1-5 years old)
Study location: The Gambia
Objective: Expanded safety and lot-to-lot consistency
Vaccine: nOPV2 c1 commercial dose
mOPV2 historical controls
M1 Study Phase IV
2016
Study group: Adults
Study location: Belgium
M2a Study Phase IV
2015-2016
Study group: Children (1-5 years old)
Study location: Panama
M2b Study Phase IV
2015-2016
Study group: Infants (18-22 weeks)
Study location: Panama
trivalent OPV historical control
T1 Study Phase IV
2016
Study group: Adults
Study location: Belgium
IPV+dmLT
CVIA065 Study Phase I
2020
Study group: Adults
Study location: Belgium
Study design: IPV+dmLT vs. IPV, challenge bOPV

3.1 Abstract

Although global polio eradication is within reach, sustained eradication of all polioviruses requires cessation of oral poliovirus vaccine use to mitigate against vaccine-derived poliovirus circulation and vaccine associated paralytic poliomyelitis. The first step in this direction was the WHO-recommended global withdrawal of live attenuated type 2 Sabin poliovirus from routine immunization in May 2016, with future use restricted to outbreak response, and handling controlled by strict containment provisions (GAPIII). This creates unique challenges for development and testing of novel type 2 poliovirus vaccines. We describe the creation of a novel purpose-built containment facility, Poliopolis, to study new monovalent OPV2 vaccine candidates in healthy adult volunteers, which may be a model for future endeavours in vaccine development for emergency use.

3.2 Background

Normal Poliomyelitis is close to becoming the second vaccine-preventable disease, after smallpox, ever to be eradicated. In 2015, wild type 2 poliovirus (WPV2) was certified eradicated after the last naturally occurring case was reported in 1999. Wild type 3 poliovirus (WPV3) transmission has also likely been interrupted with no case or environmental isolation since 2012 ((132), (133)). Three countries, Nigeria, Pakistan and Afghanistan remain endemic for transmission of wild type 1 poliovirus (WPV1) (133), although a silent outbreak of WPV1 was detected in Israel in 2013–2014, but no clinical cases were reported (134). The Sabin live attenuated trivalent oral poliovirus vaccine (OPV) has played a major role in interrupting polio transmission globally, in rare circumstances it can revert to neurovirulence resulting in vaccine-associated paralytic poliomyelitis in vaccinees or their close contacts. (135) In settings of low population immunity due to poor immunization coverage, excreted OPV strains can also acquire neurovirulence and transmissibility, leading to circulating vaccine-derived polioviruses (cVDPV). The risk of cVDPV spread in polio-free countries has been illustrated in the past two decades, with outbreaks reported from the Caribbean, Asia and Africa and most recently, in situations of social breakdown, security and conflict issues such as in Ukraine, Syria and Iraq, and Nigeria and Somalia (136) - (144)) and most recently in Papua New Guinea (145). A particular concern is the transmission of polioviruses including cVDPV from outbreaks in such regions into neighboring countries and beyond (146) (147).

3.3 The need for novel polio vaccines

Polio eradication will not be complete unless the risks of spread and transmission from all types of polioviruses, including VDPV, are adequately mitigated. The endgame of polio eradication, therefore, has complex vaccine choices. Trivalent inactivated polio vaccine (IPV) induces excellent humoral immunogenicity and thereby prevents paralysis, but its impact on intestinal immunity – and as such on transmission – is limited compared with OPV. ((148), (149), (46)) In settings of poor sanitation and hygiene where the fecal–oral route of transmission predominates, OPV is a more effective vaccine to interrupt person-to-person transmission, and thus has typically been the vaccine-of-choice for outbreak response. However, the risks of vaccine-associated paralytic poliomyelitis and VDPV arising from OPV usage, although rare (150), are an important consideration in the context of achieving and sustaining complete eradication of polio. With the global cessation of all Sabin type 2 vaccine use, intestinal immunity to type 2 is also on the decline. Under these circumstances, in the event of a vaccine-derived type 2 polio outbreak, the use of the current stockpiled monovalent OPV2 is the only option for outbreak control but brings with it its own risk of generating new type 2 cVDPVs.

To minimize this risk and to ensure complete eradication, a scientific consortium supported by the Bill & Melinda Gates Foundation in coordination with vaccine developers and global agencies (WHO, PATH) has developed novel OPV vaccine strains with the intent of stockpiling such vaccines for emergency use, if and when needed for outbreak response (44), (151)). The genetic sites of attenuation have been identified and the genetic sequence can be manipulated to stabilize these attenuations and minimize the frequency of reversion. Two novel OPV type 2 (nOPV2) candidates based on attenuated serotype 2 polioviruses derived from a modified Sabin 2 infectious cDNA clone have been genetically designed, engineered, produced and tested in a series of preclinical studies.((44), (130)) In these preclinical models, the two candidates have been proven to be less transmissible and genetically more stable than the Sabin OPV, and so less likely to revert to neurovirulent strains that are shed in vaccinees' stools. The natural consequence of these preclinical studies was the proposal to test the two candidates in human volunteers in a Phase I study. Given the current unique situation of global certification of WPV2 eradication, the subsequent global cessation of all elective use of OPV containing type 2 from May 2016, and the

recommendations of the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of OPV use (GAPIII) (152), studies with new live polio vaccines require specific containment measures (153).

In this paper we describe the implementation of a unique project that allowed the first-in-human Phase I clinical study with two attenuated nOPV2 candidates to evaluate these novel vaccines in healthy adult volunteers in these unprecedented circumstances of containment. The study was performed with extensive monitoring and with the time pressure in the current context of multiple and increasing type 2 cVDPV outbreaks taking place in different parts of the world. The encouraging results of that study have recently been reported (130).

3.4 The rationale for containment

The objectives of this first-in-human Phase I, blinded, single-center trial were the assessment of the safety and the immunogenicity of two nOPV2 candidates in healthy adult volunteers. The two nOPV2 candidates (S2/cre5/S15domV/rec1/hifi3 and S2/S15domV/CpG40) are live-attenuated serotype-2 polioviruses derived from a modified Sabin type-2 infectious cDNA clone propagated in Vero cells. The viral nucleotide sequences in parts of the 5'-untranslated region of both candidates are modified to improve the genetic stability of this major attenuating determinant of Sabin type-2, with various other modifications made to further improve stability of the attenuation, inhibit recombination and reduce replicative fitness to improve stability of the attenuated phenotype while also reducing transmission (130).

The trial also included extensive assessment of viral shedding in stool samples and testing of shed virus for genetic stability and neurovirulence following oral receipt of one of the two nOPV2 candidates. In addition to containment recommendations (GAPIII), which currently apply to all type 2 polioviruses, the novelty of the genetically modified nOPV2 viruses necessitated performing the study in a fully contained environment with maximal effort to avoid any accidental release into the environment by ensuring that all biological samples that could potentially contain vaccine virus were captured and contained for subsequent decontamination. Previous quarantine and human challenge studies reported isolation of clinical trial volunteers for 9–14 days ((154), (155)). In view of the

nature of orally administered polioviruses and available shedding data, a longer quarantine period of 28 days was recommended for the study on nOPV2 candidates. Shedding data reported in a study in Panama of previously IPV-vaccinated children challenged with licensed OPV2 vaccine indicates that 1 week after challenge, 78.3% experience shedding with a median fecal titer of 4.45 log $CCID_{50}$ (50% of the cell culture infectious dose), which dropped to 46% and 2.75 log $CCID_{50}$, respectively after 3 weeks (149). Across studies, 63–100% of IPV-vaccinated children demonstrate fecal excretion at 7–10 days ((46), (156), (157)). One recent study in 144 IPV-primed adults challenged with OPV1, showed that 98% were infected, at a peak stool titer of $10^{6.0}$ $CCID_{50}$/gram and shed the challenge virus for a mean of 13 days (154). Thus, a novel purpose-built facility was created for sufficient numbers of study volunteers to be accommodated in isolation from the external environment for a period of 28 days, a unique situation for a vaccine trial.

3.5 Planning

The Center for the Evaluation of Vaccination (University of Antwerp, Belgium) was contacted by the Bill &Melinda Gates Foundation in December 2016 with a request to perform a Phase I study with the nOPV2 candidates. To ensure the vaccine strains did not get into the environment through excretion of virus in stools from the vaccinated volunteers and potential manual transmission, the capture of all excreted fluids from the vaccinated volunteers and standard collection and disposal of clothing and all other materials handled by them, for example, towels, disposable eating utensils, uneaten food and all waste would have to be strictly enforced. As standard Phase I facilities are not designed to meet these specific requirements and after having screened alternative containment facilities like isolated holiday accommodations, unoccupied buildings (such as unused or empty closed centers for asylum seekers), together with the specificity of the study and the biosafety requirements, it was decided that the only option was to construct a new purpose-built quarantine facility. A geographical requirement was that the facility had to be located close to a hospital in case of any medical emergency, and in view of the participation of the personnel of the Center for the Evaluation of Vaccination, the Antwerp University Hospital was readily identified as the site-of-choice. Acknowledging that the facility would not be a permanent structure, the original intention being for a duration of 2 years, it was agreed that the University of Antwerp would construct a temporary self-

contained unit. The transitory nature of the facility immediately suggested building it using purpose-built modular 'containers'.

As soon as conceptual plans for the construction were available the interactions with technical support services, biosafety experts and the local municipality were initiated. Other expert groups and authorities including the local police and fire brigade became involved later in the planning. In the subsequent 5 months, the environmental and building applications were submitted and approved, approvals from the Ethics Committee and Belgian Regulatory authorities were obtained and an intervention dossier for the fire brigade was finalized. One of the key challenges was to find technical and balanced solutions to the diverse and sometimes conflicting concerns of these groups, for example, easy access for the fire brigade while the police wanted limited access to ensure external safety and easy control of the area (particularly with the externally located effluent containment tanks). Further considerations were the requirements for the 'contained use' of genetically modified organisms (158) and the stringent restrictions for OPV2, when developing entry and exit procedures, emergency plans and waste and effluent treatment processes. The close collaboration that developed between the clinical trial team, the facility manager and the respective university and hospital (bio)safety officers to find workable technical solutions and procedures also formed the basis for submitting the necessary applications and obtaining the approvals from the competent authorities (including the specific regional biosafety notification for a contained use of a GMO, reference SBB 219 2017/0209K).

3.6 Building the infrastructure, 'Poliopolis'

Due to the proactive planning and early off-site manufacture of the prefabricated modules by the company who designed them, the facility, now named Poliopolis, was constructed over the course of 3 days in April 2017 and finalized within 1 month. This was exactly 5 months after the decision was taken to set up this study, during which period all necessary local and national building and scientific approvals were obtained. The final construction was a one-store building composed of 66 specially designed and constructed linked modules that housed all facilities in a contained environment (Figure 1). Facilities included private, individual bedrooms for a maximum of 17 volunteers, a common kitchen and dining room, a recreation room with TV and library, a fitness room with

gymnasium equipment, shower rooms and toilets (including separate toilets for study staff) and a laboratory facility for on-site testing and sample preparation. There were also offices for the clinical staff and the study psychologist to examine and interview participants and collect samples. In addition there was a room for decontamination of materials, and four separate entrances and exits (Figure 2) – namely, an entrance for staff where they put on protective clothing (overall, overshoes and gloves) and a separate exit for removal of said clothing – the two rooms being connected by pass-through lockers where their outdoor clothing and personal belongings, laptops or cell phones were stored during their visit to the facility. The staff exit room also had a shower facility. In case clinical staff had been contaminated, for example, disruption of the overshoes, they had to take a shower of at least 5 min and redress with clean clothes that were kept available in that room. When the volunteers left the facility after 28 days, a similar procedure was followed for decontamination.

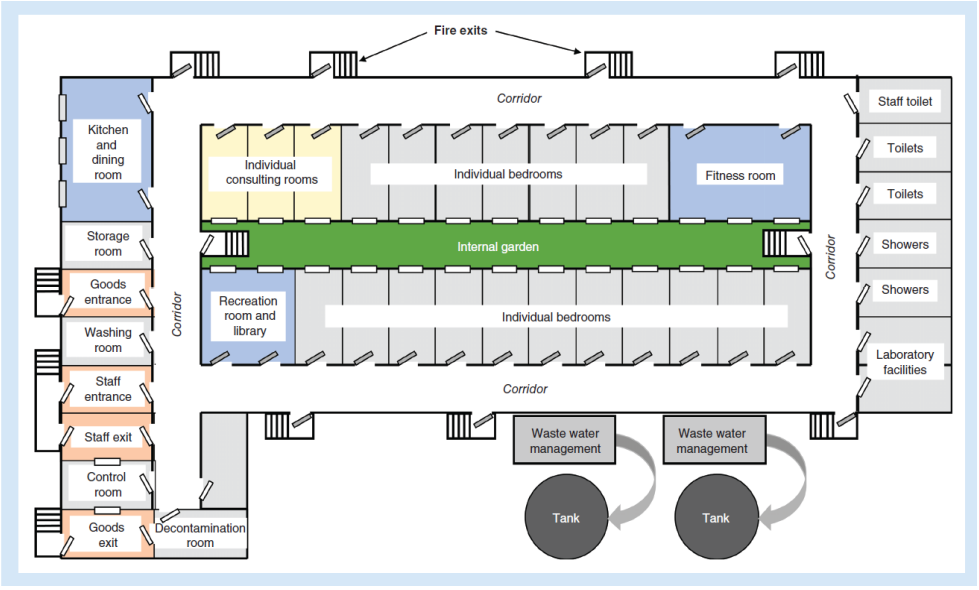


Figure 1. Diagrammatic representation of the modular design of the Poliopolis facility

There was a separate entrance for receipt of goods (food, laboratory supplies, etc.) and an exit for the materials and goods leaving the facility after external decontamination (e.g., waste materials for incineration, stool and other samples from the participants). These were the only means of entry into the facility, although fire exits that could only be unlocked from the inside were also present.

All entrances and exits were linked with alarm systems to ensure no unauthorized access in or out of the facility once the trial was underway. These entrances and exits worked as 'air locks', when the outside door was opened with a magnetic key, the inner door could not be opened until the outside door was closed again. This prevented anyone from entering or exiting the facility without being identified or dressed as required.

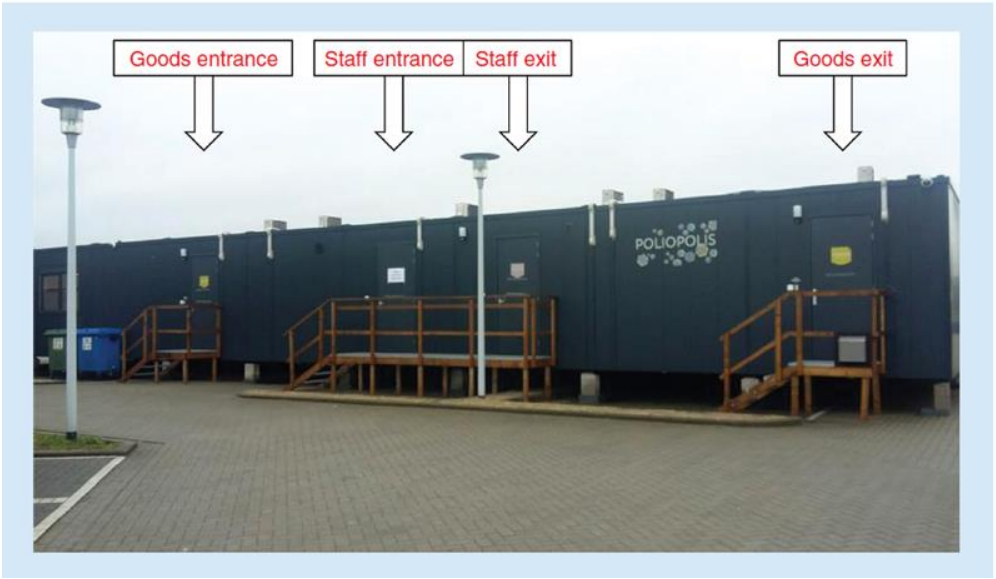


Figure 2. External appearance of Poliopolis showing the personnel entrance (left hand door) and exit (middle door) and the goods entrance and exit

The kitchen was equipped with one-way glass windows allowing volunteers to look outside but preventing the public from seeing into the facility. Individual bedrooms and clinical offices had windows onto a central atrium which was open to the sky. This area was set up with garden furniture to allow participants to be outside, together with a barbecue facility for social events during the trial. Power and water were supplied directly from the hospital building, while the capture and containment of all waste meant that no sewage services were necessary.

All study personnel involved in direct interaction with the volunteers had to avoid contamination or accidental release of study vaccines or samples into the environment through use of body coverings (gowning), but not masks or eye protection. Since the risk of spread of vaccine virus through aerosols or droplets in the study population was considered negligible, air filtration of the facility and

masking of the study personnel was not considered necessary, although masking and eye protection were recommended during medical visits when oropharyngeal swabs were taken.

The importance of wearing a gown was implicit to avoid any potential release of vaccine virus particles into the external environment on clothing of personnel leaving the contained facility, so training of personnel was critical to success. Hence, competency of gowning/de-gowning procedures in a 'green zone' was clearly documented, and periodic gowning certification conducted to confirm personnel maintained consistent practices. As with study personnel, all people who entered the facility were administered an IPV booster dose at least 14 days before study start and were trained in the gowning/de-gowning procedure by a qualified person.

A dedicated emergency team and vehicle was on constant stand-by, although never used to provide transport between the facility and the emergency room of the university hospital in the case of any medical emergency. As decontamination of the vehicle following transport of a vaccinated volunteer would take 3–4 h, this precluded use of a standard emergency vehicle.

3.7 External decontamination

A special decontamination team was established and trained for a rapid, effective decontamination response according to a dedicated standard operating procedure (SOP) describing an emergency response plan, including an incident command system for emergency responses. As noted, all wastewater was collected for subsequent decontamination, which included not only water from toilets, but also from showers, wash basins in bathrooms and kitchens and clothes-washing facilities. Two double walled 20,000 l capacity tanks were set up the outside of the facility (Figure 3) for collection and storage of wastewater, for subsequent collection and decontamination by a specialist company. A two-pronged approach was chosen to ensure adequate disinfection of the collected wastewater, initially using chlorine dioxide treatment followed by a pH increase through addition of sodium hydroxide prior to discharge in a municipal wastewater treatment plant. Chlorine dioxide was selected as disinfectant based on several scientific studies showing high efficacy of the active substance in killing poliovirus in wastewater. ((159) - (161)) One of these studies indicated that

approximately 5 $\log_{10}$ killing (i.e. >99.999% reduction) was reached with a dose of 5 mg/l (161). To ensure equivalent or more disinfection efficacy, a dose of 90 mg/l was used for decontamination of the wastewater.

For decontamination of the facility, chlorine dioxide gas was used. In addition to its high efficacy in killing poliovirus, chlorine dioxide gas has the added benefit of being smaller than all microorganisms, with a molecular size of 0·124 nm, so no organism can be concealed from the gas. Chlorine dioxide gas can be accurately measured in real time from multiple points within the area being decontaminated, guaranteeing that the correct dosage needed for an effective decontamination is being met before the decontamination is deemed complete and aeration is started. The chlorine dioxide product used was Diox Forte 0·75%, which contains 7·5 g/l chlorine dioxide and is registered as a sterilant capable of eliminating all viruses, bacteria, fungi and spores. This product, among others, is approved for water disinfection in Belgium. A WHO report on the thermostability of vaccines reports a strong decrease in the stability of OPV as the pH increases above 8. (162) Thus, increasing the pH was added as another disinfection step to prevent contamination by contact with the wastewater outside the contained facility.

Discharge of the decontaminated wastewater into a municipal wastewater treatment plant introduced another geographic requirement for the facility – to be located near a road that allowed easy access to the containment tanks designed to capture all the wastewater for transport to a specialist decontamination facility.

Food waste, together with the surgical gowns worn by study staff when within the facility, was collected into medical waste containers. These containers were incinerated according to the local hospital protocol. Clothing worn by the volunteers was decontaminated the day before their departure from the facility using the chlorine dioxide gas, and on departure they wore the clothes they arrived in, which had been kept in the 'green zone'. In addition, tailored procedures needed to be established for the decontamination of the entire facility upon departure of all volunteers, intermittent decontamination of waste containers and of the belongings of the volunteers upon departure in a dedicated room within the facility. Decontamination with chlorine dioxide was again chosen for its characteristics as described above, but also as it leaves no post-decontamination residue, so all equipment (including sensitive electronics) could be left inside the facility during a decontamination cycle without risk of corrosion or other damage.

Figure 3. External appearance of Poliopolis showing the two external waste water tanks.

3.8 Quality control & assessment

Operating within a contained environment necessitates preparations to prevent circumstances that might result in occupational injury, ill health or adverse environmental impact. In order to anticipate and prevent such circumstances, a structured approach was needed to identify hazards or forms of public health concern. Extensive health and environmental risk assessments were performed before the start of the study to determine the appropriate protective measures needed. Based on this assessment, SOPs were written including information about the hazards identified and how these can be mitigated. Personal safety measures, gowning procedures, waste management and decontamination procedures, accident prevention and contingency plans were among the most important potential hazards identified. In addition, a comprehensive communication plan was drafted with the support of the communication team of the University of Antwerp. Clearly, dealing with any incidents of potential virus escape via accidental release or need for emergency medical care of a vaccinated volunteer were among the most important risks to address and necessitated an emergency preparedness plan.

A specific concern of a clinical trial in a quarantine situation is that one must be prepared to adjust and adapt in case a volunteer has to leave a clinical trial at any point of time. The critical requirement in this context was the agreement to abide by a specially prepared SOP for anyone who left early so that they can be monitored with stool collection and testing until negative for viral shedding. In this case, arrangements were made for any potential early leavers to be accommodated in a local hotel in Antwerp where they would be provided with a chemical toilet to contain all stools and additional tailored guidance on hygienic measures, travel and contact restrictions. They were expected to continue to report to the study team with submission of stool samples on a daily basis. This only applied to one participant, who left on the last evening of their scheduled confinement but remained in the local area and participated in all final assessments on day 28.

3.9 Lessons learned

Planning and building a Phase I quarantine infrastructure like Poliopolis is a very challenging activity, considering the timing, global urgency for early data generation and specifications of containment. The whole concept allows collection of high-quality data and samples daily but requires total dedication and commitment for the duration of the study, from the study team as well as the volunteers. The study ran from 22 May to 22 August 2017, partly coinciding with the summer holiday period, creating an additional challenge to guarantee the permanent availability of nurses, coordinators and doctors by switching and shifting weekends and holidays. Volunteers went through a two-stage screening process, with medical and psychological assessments. The psychologists selected participants who would be able to cope with the constraints on an individual level, as well as to ensure each group of 15 volunteers, who did not know each other in advance, could function as a group. The psychological proofing appeared to be effective as there was a good spirit maintained in both groups throughout the study and any issues which arose were resolved through effective communication.

An inspection by the Regulatory Authorities in week 3 after the start of the Phase I study confirmed the high quality of the planning, preparedness, building facility and SOPs. The report of the clinical outcomes describes how the study was successfully completed by all enrolled individuals, with no dropouts, no issues with contamination and no evidence of any leaks of the candidate viruses. (130) Both vaccine candidates were well tolerated and immunogenic, and both were

detected in the stools of the majority of participants, 100% after the first candidate and 87% after the second candidate. The median for cessation of shedding was 23 days (interquartile range [IQR]: 15–36) after the first candidate and 12 days (1–23) for the second candidate. More virus was shed by recipients of the first candidate than the second candidate, both as higher titers of and a longer duration of shedding. As reported, shed viruses from both candidates were found to be stable in terms of genetic sequence and reversion to neurovirulence (130).

This detailed description of the novel purpose-built contained facility illustrates the major effort by all concerned in planning and rapid implementation that were needed to achieve success in the Phase I novel polio vaccine study, allowing the analysis of the immunogenicity, safety and shedding. The clinical trial conducted in the facility described here was the first major step in the development of new OPVs in more than five decades. The steps followed in envisioning, planning and implementing the operational aspects of this study might be a model for future quarantine and human challenge vaccine experiments, and an important example for other projects performed as part of emergency vaccine development programs. New and evolving global initiatives such as the Coalition for Epidemic Preparedness Innovations (CEPI) recognize the urgency to respond to pandemic threats with rapid implementation of vaccine trials under containment (163).

3.10 Summary

The Poliopolis experience is the first of its kind, established in an unprecedented manner under the WHO containment recommendations (GAPIII) and with severe time constraints to implement an operationally challenging clinical trial with vaccine candidates that cannot yet be used under deliberate release conditions. The successful planning and implementation of this study should not only pave the way for rapid clinical development of the safer OPV formulations but should also provide a planning and contextual framework for future studies under containment to support global health initiatives such as those funded by CEPI in pandemic preparedness planning.

3.11 Future perspective

Although the global eradication of poliovirus type 2 was officially declared in September 2015, outbreaks of cVDPV2 still occur around the globe due to viral shedding from recipients of live OPVs. The development of the Poliopolis facility has been driven by the necessity to develop and test more genetically stable type

2 OPV for use in containing such outbreaks. Global withdrawal of live polioviruses has already started, with trivalent OPV being replaced by bivalent OPV which do not contain type 2 in May 2016. As the last case of type 3 poliovirus infection was reported in November 2012, one can anticipate the future declaration of eradication and withdrawal of type 3 poliovirus from bivalent OPV, leaving only type 1, which is currently still being transmitted in three countries – Afghanistan, Pakistan and Nigeria. Once type 1 is eradicated, the use of OPV and global replacement with IPV would remove the risk of cVDPV and hence the requirement for genetically stable monovalent OPV (for outbreak use) and the Poliopolis facility (to test them).

Practice points

Need for novel vaccines

- Although wild poliovirus cases have virtually been eradicated there is an ongoing medical need for live vaccines to manage circulating vaccine-derived polioviruses (cVDPV) which are responsible for outbreaks in many countries.
- Current live vaccines are the source of cVDPV so new more genetically stable vaccines are required, particularly for type 2 poliovirus which has been declared to be globally eradicated.
- All oral poliovirus vaccines are shed in the stools of vaccinees to enter in the local environment, this being the source of cVDPV.

Rationale for containment

• Development of novel monovalent type 2 oral poliovirus vaccine in the current era of containment required a new approach to clinical testing.

• Following the global withdrawal of all live type 2 poliovirus vaccines (apart from stockpiles to manage cVDPV2 outbreaks) the GAPIII imposes stringent containment conditions for clinical testing of new vaccines to ensure minimal risks of environmental release by vaccine researchers and manufacturers.

• This Phase I study is the first to evaluate the stability of the novel genetically modified type 2 oral poliovirus vaccine candidates when administered to humans, so all precautions must be taken.

Planning

• The successful completion of the novel facility, Poliopolis, was the outcome of collaborative team effort involving medical, local and national government, and global health and regulatory authorities.

Building the infrastructure, 'Poliopolis'

• The facility was designed to accommodate 15 volunteers at any one time, with free access to cooking, entertainment and exercise facilities.

• An 'air lock' system allowed entry of study staff without risking release of study vaccine polioviruses.

External decontamination

• All potentially contaminated materials – waste washing and toilet water, food and clothing – were decontaminated using chlorine dioxide.

Quality control & assessment

• Extensive health and environmental risk assessments were performed before the start of the study to determine the appropriate protective measures needed.

• As participants were to be confined for more than 28 days, psychological assessment of their suitability and compatibility with each other was essential.

• Regular monitoring of participants ensured they were happy to remain in the facility, helped by providing different forms of entertainment during the study.

Acknowledgments

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Chapter 4:
The safety and immunogenicity of two novel live attenuated monovalent (serotype2) oral poliovirus vaccines in healthy adults: a double-blind, single-centre phase 1 study

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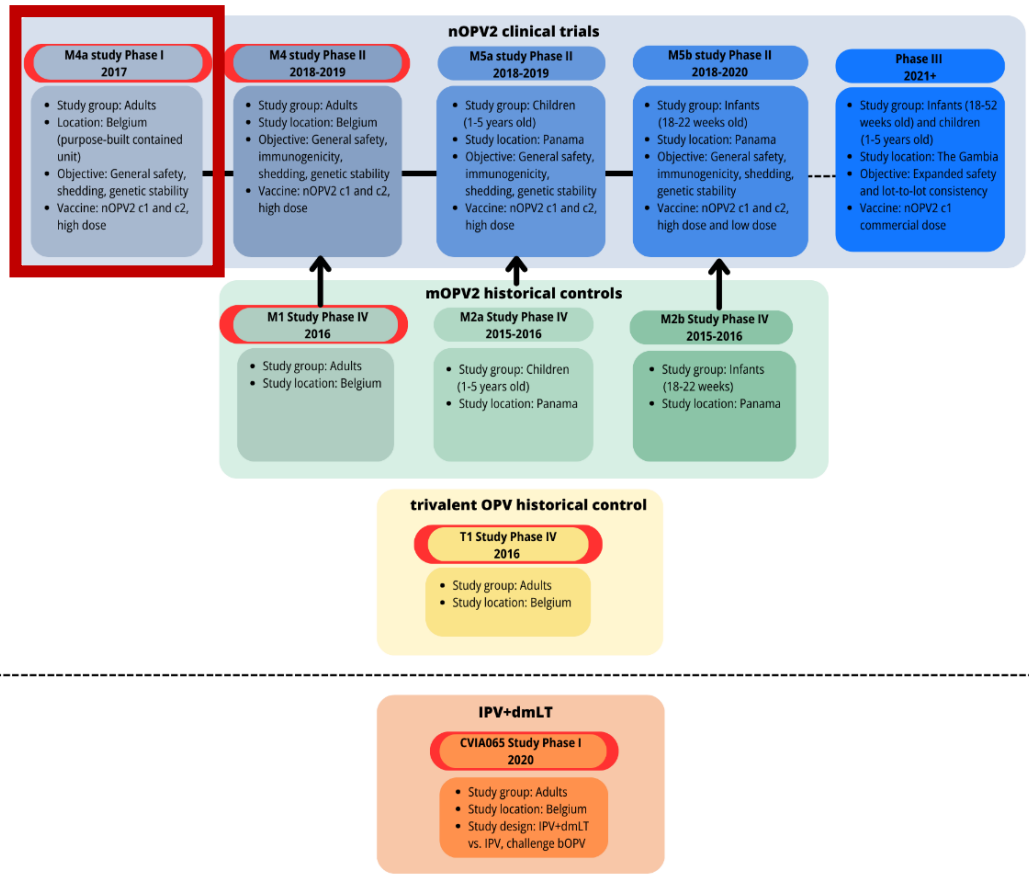
nOPV2 clinical trials
M4a study Phase I 2017
Study group: Adults
Location: Belgium (purpose-built contained unit)
Objective: General safety, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M4 study Phase II 2018-2019
Study group: Adults
Study location: Belgium
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5a study Phase II 2018-2019
Study group: Children (1-5 years old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5b study Phase II 2018-2020
Study group: Infants (18-22 weeks old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose and low dose
Phase III 2021+
Study group: Infants (18-52 weeks old) and children (1-5 years old)
Study location: The Gambia
Objective: Expanded safety and lot-to-lot consistency
Vaccine: nOPV2 c1 commercial dose
mOPV2 historical controls
M1 Study Phase IV 2016
Study group: Adults
Study location: Belgium
M2a Study Phase IV 2015-2016
Study group: Children (1-5 years old)
Study location: Panama
M2b Study Phase IV 2015-2016
Study group: Infants (18-22 weeks)
Study location: Panama
trivalent OPV historical control
T1 Study Phase IV 2016
Study group: Adults
Study location: Belgium
IPV+dmLT
CVIA065 Study Phase I 2020
Study group: Adults
Study location: Belgium
Study design: IPV+dmLT vs. IPV, challenge bOPV

4.1 Summary

Background

Use of oral live-attenuated polio vaccines (OPV), and injected inactivated polio vaccines (IPV) has almost achieved global eradication of wild polio viruses. To address the goals of achieving and maintaining global eradication and minimising the risk of outbreaks of vaccine-derived polioviruses, we tested novel monovalent oral type-2 poliovirus (OPV2) vaccine candidates that are genetically more stable than existing OPVs, with a lower risk of reversion to neurovirulence. Our study represents the first in-human testing of these two novel OPV2 candidates. We aimed to evaluate the safety and immunogenicity of these vaccines, the presence and extent of faecal shedding, and the neurovirulence of shed virus.

Methods

In this double-blind, single-centre phase 1 trial, we isolated participants in a purpose-built containment facility at the University of Antwerp Hospital (Antwerp, Belgium), to minimise the risk of environmental release of the novel OPV2 candidates. Participants, who were recruited by local advertising, were adults (aged 18–50 years) in good health who had previously been vaccinated with IPV, and who would not have any contact with immunosuppressed or unvaccinated people for the duration of faecal shedding at the end of the study. The first participant randomly chose an envelope containing the name of a vaccine candidate, and this determined their allocation; the next 14 participants to be enrolled in the study were sequentially allocated to this group and received the same vaccine. The subsequent 15 participants enrolled after this group were allocated to receive the other vaccine. Participants and the study staff were masked to vaccine groups until the end of the study period. Participants each received a single dose of one vaccine candidate (candidate 1, S2/cre5/S15domV/rec1/hifi3; or candidate 2, S2/S15domV/CpG40), and they were monitored for adverse events, immune responses, and faecal shedding of the vaccine virus for 28 days. Shed virus isolates were tested for the genetic stability of attenuation. The primary outcomes were the incidence and type of serious and severe adverse events, the proportion of participants showing viral shedding in their stools, the time to cessation of viral shedding, the cell culture infective dose of shed virus in virus-positive stools, and a combined index of the prevalence, duration, and quantity of viral shedding in all participants. This study is registered with EudraCT, number 2017-000908-21 and ClinicalTrials.gov, number NCT03430349.

Findings

Between May 22 and Aug 22, 2017, 48 volunteers were screened, of whom 15 (31%) volunteers were excluded for reasons relating to the inclusion or exclusion criteria, three (6%) volunteers were not treated because of restrictions to the number of participants in each group, and 30 (63%) volunteers were sequentially allocated to groups (15 participants per group). Both novel OPV2 candidates were immunogenic and increased the median blood titre of serum neutralising antibodies; all participants were seroprotected after vaccination. Both candidates had acceptable tolerability, and no serious adverse events occurred during the study. However, severe events were reported in six (40%) participants receiving candidate 1 (eight events) and nine (60%) participants receiving candidate 2 (12 events); most of these events were increased blood creatinine phosphokinase but were not accompanied by clinical signs or symptoms. Vaccine virus was detected in the stools of 15 (100%) participants receiving vaccine candidate 1 and 13 (87%) participants receiving vaccine candidate 2. Vaccine poliovirus shedding stopped at a median of 23 days (IQR 15–36) after candidate 1 administration and 12 days (1–23) after candidate 2 administration. Total shedding, described by the estimated median shedding index (50% cell culture infective dose/g), was observed to be greater with candidate 1 than candidate 2 across all participants (2·8 [95% CI 1·8–3·5] vs 1·0 [0·7–1·6]). Reversion to neurovirulence, assessed as paralysis of transgenic mice, was low in isolates from those vaccinated with both candidates, and sequencing of shed virus indicated that there was no loss of attenuation in domain V of the 5’-untranslated region, the primary site of reversion in Sabin OPV.

Interpretation

We found that the novel OPV2 candidates were safe and immunogenic in IPV-immunised adults, and our data support the further development of these vaccines to potentially be used for maintaining global eradication of neurovirulent type-2 polioviruses.

4.2 Introduction

The world is on the threshold of achieving the 1988 World Health Assembly's goal to eradicate poliovirus. Eradication of wild type-2 polioviruses was confirmed in September, 2015, leading the WHO Strategic Advisory Group of Experts to recommend global cessation of use of vaccines containing live Sabin type-2 poliovirus. ((164), (165)) By May, 2016, trivalent oral poliovirus vaccines (OPV) were replaced with bivalent OPV that only contained poliovirus type 1 and type 3, and they were supplemented with at least one dose of trivalent inactivated poliovirus vaccine (IPV) in an unprecedented globally synchronized effort. (166) Although OPV use has interrupted poliovirus transmission in most of the world, on rare occasions, live-attenuated vaccine polioviruses can produce a neurological disease, termed vaccine-associated paralytic poliomyelitis, or they can acquire neurovirulence and transmissibility, creating the infectious circulating vaccine-derived polioviruses (cVDPVs). ((167), (168)) For instance, in 2018, type-1 cVDPV outbreaks in Papua New Guinea (169) and Indonesia and other type-2 cVDPV outbreaks (170) occurred.

Since global cessation of OPV2 use in May, 2016, distinct circulating vaccine-derived type-2 poliovirus (cVDPV2) outbreaks have occurred in seven countries, with more than 150 cases of vaccine-derived poliovirus reported. cVDPVs have been designated a Public Health Emergency of International Concern, for which the only effective control is use of stockpiled monovalent OPV2. Use of this vaccine carries the inherent risk of seeding new cVDPVs, particularly with waning mucosal immunity of the population following OPV2 cessation. ((171), (150)) IPV use cannot generate cVDPVs but these vaccines do not induce primary intestinal mucosal immunity, so IPV use is ineffective in interrupting transmission in settings where transmission is predominantly via the fecal–oral route. (48), (46) Therefore novel OPV2 candidates have been developed with improved genetic stability, which decreases the likelihood of reversion to neurovirulence, thereby minimizing the risk of generating new cVDPVs. (46)

Given the unique context of certified wild type-2 poliovirus eradication, OPV2 withdrawal, and WHO GAPIII containment guidelines, (152) additional measures were needed to study these novel OPV2 candidates in humans. In the first clinical trial of a new polio vaccine in more than 50 years, we report the first in-human phase 1 trial of two novel OPV2 candidates (S2/cre5/S15domV/ rec1/hifi3 and

S2/S15domV/CpG40). Our primary aims were to evaluate the safety and shedding of these vaccines in IPV-immunized adults in a purpose-built, contained research unit designed to prevent release into the general population, as a further step toward the permanent global eradication of poliomyelitis.

4.3 Methods

Study design and participants

In this double-blind, single-center phase 1 trial, we used local advertising to recruit volunteers at the University of Antwerp Hospital (Antwerp, Belgium). In addition to the restrictions imposed by GAPIII (152) that apply to all type-2 polioviruses, the novelty of the genetically modified novel OPV2 viruses necessitated performing the study in a fully contained, purpose-built facility (which we named Poliopolis), to avoid any accidental environmental release by ensuring that all biological samples that could potentially contain vaccine virus could be captured and contained for subsequent decontamination.

Screening for inclusion included a medical examination, laboratory testing (serology, chemistry, coagulation, and hematology), and interviews by two psychologists to assess the volunteers' mental fitness to cope with the confinement and restrictions of containment for 28 days and their compatibility with each other. Eligible volunteers were healthy men or women (aged 18–50 years), with complete IPV-only polio vaccination histories. Inclusion criteria included a willingness to adhere to all prohibitions and restrictions necessary for full containment for the study duration, no intended travel to polio-endemic countries or the Netherlands (because of low vaccination coverage in the so-called Bible belt), and no professional food handling activity or household or professional contact with immunosuppressed individuals or people without a full poliovirus vaccination (such as infants under 6 months of age). These restrictions were to be enforced until viral shedding ceased after participants left Poliopolis. Principal exclusion criteria included any condition that the investigator believed could compromise the participant's wellbeing, any gastrointestinal condition (e.g. Crohn's disease or ulcerative colitis), receipt of immunosuppressive medication within 6 months preceding the start of the study, previous receipt of OPV, any polio vaccination within 12 months of the start of the study, or any other vaccinations within 28 days of the study or planned within the study period. Women of childbearing age were required to have a negative urine pregnancy test

on day 0, not to be breastfeeding, and to use an approved contraceptive method until 3 months after vaccine administration. Volunteers were enrolled sequentially in two groups, with each group receiving one of the two vaccine candidates, to avoid cross-(contamination, after which they were confined to Poliopolis for 28 days, with further monitoring until end of shedding.

All participants provided written informed consent at enrolment, and the study was overseen by an independent Data and Safety Monitoring Board. Ethical approval was received from university and hospital institutional review boards and the study was done according to prevailing Declaration of Helsinki and ICH Good Clinical Practice guidelines. The study protocol was reviewed by the US Centers for Disease Control and Prevention (CDC) Human Research Protection Office and determined that CDC was not-engaged.

Randomization and masking

After a screening visit, in which baseline blood and stool samples were collected, we enrolled the first three volunteers in the first group sequentially. Participants and study staff assessing shedding were masked to vaccine group allocations until the end of the study period by labelling of the vaccines with the letters A and B (labelling done by CSM, Belgium). The first participant opened one of two envelopes to select vaccine A or B for that group and received the corresponding vaccine (candidate 2). If no serious or severe adverse events were reported within 48 h, volunteer 2 was enrolled and vaccinated, and this method was repeated for volunteer 3. The Data and Safety Monitoring Board assessed safety data from these first three participants within 24 h, before approving enrolment of the remaining 12 volunteers for that group. The subsequent 15 participants enrolled after this group were allocated to receive the other vaccine.

Vaccines

The novel OPV2 candidates are live-attenuated serotype-2 polioviruses that were derived from a modified Sabin type-2 infectious cDNA clone and propagated in Vero cells; candidate 1 (S2/cre5/S15domV/rec1/hifi3) was given to the second group to be sequentially allocated (participants 16 to 30) and candidate 2 (S2/S15domV/ CpG40) was given to the first 15 participants allocated to groups. We modified the viral nucleotide sequences in part of the 5’-untranslated region in both candidates, to improve the genetic stability of this major attenuating determinant of Sabin type-2. In candidate 1, this alteration was augmented with two modifications in the polymerase 3D to further improve stability of the

attenuation, and relocation of a key replication element from the 2C coding region to the 5'-untranslated region, to inhibit recombination. In candidate 2, silent non-coding modifications engineered within the capsid (VP1–4) were designed to reduce replicative fitness and, potentially, to improve stability of the attenuated phenotype while also reducing transmission.

Clinical trial lots of both novel OPV2 candidates underwent manufacturing release testing, including standard WHO monkey neurovirulence testing, and vaccine formulation by use of methods employed for current Sabin-based OPV products by P T Bio Farma (Bandung, Indonesia). (172) To establish safety in our phase 1 study, a high dose of approximately 10^6 50% cell culture infectious dose units ($CCID_{50}$) was administered orally as six drops (totaling 0·3 mL), which were given with a supplied dropper to guarantee the dose.

Procedures

After vaccination on day 0, we monitored volunteers for adverse events, and we assessed them with safety laboratory tests, including evaluations of viral shedding in stools, nasopharyngeal secretions, and humoral immunity. Containment was intended to last until three consecutive stool samples were virus-free, determined by PCR, for all participants in a group or until the 28th day after vaccination, whichever occurred first, meaning that the first three volunteers remained in the containment facility for 35 (first participant), 33 (second participant), and 31 days (third participant). If shedding persisted after 28 days, participants were allowed to leave but they were requested to remain in Belgium and to continue providing stool samples in an ambulatory manner by use of provided chemical toilets (with a stool storage capacity of 3–4 days) and mandatory containers for infectious waste disposal. A final safety follow-up call (for those no longer shedding) or visit (for those still shedding) was made 42 days after vaccine administration. After completion of the study by the first group and cleaning and decontamination of the facility, this procedure was repeated in an identical manner for the second group, who were given novel OPV2 candidate 1.

During containment, we gave participants physical examinations daily, starting on day 0, and particularly on days 7 and 28, and further examinations were made as required after presentation of symptoms of adverse events. We took blood samples for laboratory assessments at screening and on days 7, 14, and 28 for standard hematology analyses and blood chemistry measurements; we used the

Common Terminology Criteria for Adverse Events version 4.03 (toxicity grades) to record clinical events or the US Food and Drug Administration manual for clinical laboratory measurements. A medical team consisting of four doctors conducted daily consultations with all participants throughout the containment period, and they questioned participants on any mental or physical issues.

Adverse events were solicited from the participants by the medical team during the 7 days after vaccination. Solicited events comprised signs and symptoms that were reported with a predefined checklist (headache, fatigue, myalgia, arthralgia, paresthesia, anesthesia, paralysis, nausea, vomiting, diarrhea and abdominal pain) in a diary card. Unsolicited events comprised other signs and symptoms that participants recorded in their diary card. Adverse events were graded as mild (easily tolerated), moderate (sufficiently discomforting to interfere with normal everyday activities), or severe (preventing normal everyday activities), and they were also assessed by the investigator for causality (unrelated, unlikely, possibly, or probably related to the vaccination). Oral temperature was also measured by participants during days 0–7; body temperatures of 37·5°C or more were defined as a fever, and temperatures of more than 39·0°C were defined as a severe fever.

Strict procedures were imposed to collect daily stool samples, which were partly processed on-site to prepare for shipping at regular intervals to the CDC (Atlanta, GA, USA). Samples were stored at –20°C until analysis. We detected the type-2 poliovirus genome with a Sabin multiplex real-time RT-PCR assay of total nucleic acid extracted from stool suspensions (50% weight to volume in cell culture medium). (173) Nucleic acid extraction was done with a Thermo Fisher Scientific KingFisher Flex 96-deep-well analyzer. (174) Before extraction, stool suspensions were spiked with an extraction control (Qβ bacteriophage; Attostar) and detected with Qβ- specific real-time RT-PCR; stool suspensions with a negative extraction control (Ct>40, indicating inefficient extraction) were re-extracted. In samples that were positive for type-2 poliovirus, infectious virus was measured as $CCID_{50}$ per g of stool by use of a modification of the WHO cell sensitivity assay, as described previously. (175) Nasopharyngeal swabs that were obtained from each participant on days 0, 3, 7, and the final day of containment were processed and shipped to the CDC laboratory, where they were stored at –20°C until real-time RT-PCR evaluation for the presence of poliovirus type-2 with the same procedure as the stool suspensions.

Humoral immunogenicity was assessed as poliovirus type-2-specific serum neutralizing antibodies at days 0 and 28 with a standard protocol.(175) We calculated the median and geometric mean titers, seroprotection (the proportion of the groups with poliovirus type-2-specific antibody reciprocal titers ≥8), and seroconversion at day 28 from these samples. Seroconversion was defined as a change from seronegative to seropositive (poliovirus type-2- specific antibody reciprocal titers ≥8) or, in seropositive participants, an antibody titer increase of at least four- fold more than baseline.

The specimen tested for neurovirulence, the exploratory endpoint specimen, was the last stool sample provided by each participant that had adequate concentrations of virus for the neurovirulence and deep sequencing assays (≥4 $\log_{10}$[$CCID_{50}$/g]). The WHO poliovirus receptor transgenic mouse (Tg-PVR21) neurovirulence test that characterizes the potential for neurovirulence of shed virus (176) was modified and we used this modified test to evaluate exploratory endpoint specimen samples. Detected polioviruses in exploratory endpoint specimen samples were amplified in HEp2-C cells for 3 days at 33°C to achieve sufficient virus for each mouse inoculation. Briefly, for each exploratory endpoint specimen ten Tg-PVR21 mice were each administered intraspinal inoculations of 4 $\log_{10}$($CCID_{50}$) amplified virus in 5 µL volumes, and each exploratory endpoint specimen was tested in triplicate. Candidate 1 and 2 clinical trial bulk preparations of viruses were also tested at the 4 $\log_{10}$($CCID_{50}$) dose. As controls ten mice were each inoculated with SO+2/II at 5·0 $\log_{10}$($CCID_{50}$) and 6·0 $\log_{10}$($CCID_{50}$) doses (176) and a sample of shed Sabin 2 virus collected 7 days after vaccination with monovalent OPV2 in a previous clinical trial. (177) Inoculated mice were monitored for paralysis for a 14-day observation period as per established guidance. (176) At the end of the observation period, a final outcome of paralyzed or non-paralyzed was assigned to each mouse, to determine the paralysis frequency per exploratory endpoint specimen We also examined exploratory endpoint specimens by deep sequencing, to assess their genetic stability by demonstrating the retention of key genetic regions engineered in the vaccine candidates. Deep sequencing was performed on the cell culture-amplified virus and on viral RNA isolated directly from a 10% suspension of the exploratory endpoint specimen of each participant, to assess retention of these genetic modifications. Viral RNA was isolated from amplified viral stock from HEp2-C cells or stool by use of a QIAamp Viral RNA Mini kit (Qiagen), followed by cDNA synthesis and full-length poliovirus genome amplification (KOD Xtreme Hot Start

DNA Polymerase kit; Millipore). We did tagmentation and library preparation with the Nextera XT kit (Illumina), followed by 300-cycle paired-end sequencing with MiSeq reagent kit version 3 reagents on a MiSeq sequencer and MiSeq analysis software version 1.8.46 (all Illumina) to generate FASTQ files. (178)

Outcomes

The primary outcomes, which were assessed in both groups, were the safety of the novel OPV2 candidates by assessment of the incidence and type of serious and severe adverse events, the proportion of participants showing viral shedding in their stools, the time to cessation of viral shedding, the cell culture infective dose of shed virus in virus-positive stools, and a combined index of the prevalence, duration, and quantity of viral shedding in all participants.

The secondary outcomes, assessed in both groups, were the incidence, severity, and type of adverse events (solicited and unsolicited, for the first 7 days and throughout the study period), deviations from reference laboratory results, the median titers of type-2 poliovirus antibodies in participants' serum at days 0 and 28, the proportion of participants with seroprotection at days 0 and 28, the proportion of participants showing seroconversion at day 28, and the neurovirulence of shed virus from exploratory endpoint specimens in a mouse model.

The exploratory outcomes were the geometric mean titer of type-2 poliovirus in all participants' serum at days 0 and 28, the genetic stability of shed virus in a subset of stool samples, and nasopharyngeal viral shedding in swabs from all participants.

Statistical analysis

The sample size of 15 participants per group was considered reasonable and sufficient for a first-in-human contained study of investigational vaccines, as agreed by the stakeholders from the Global Polio Eradication Initiative, to gain a preliminary assessment of safety and the incidence, quantity, and characteristics of shed virus. There was no hypothesis testing, since all analyses were descriptive.

For binary variables, which included safety endpoints, seropositivity and seroconversion, viral shedding, and mouse paralysis, numbers and percentages are shown with two-sided 95% CIs, which were computed by the exact or score method. Antibody geometric mean titers were calculated with 95% CIs with

asymptotic methods on the log scale, and they were back-transformed with the upper limit of quantitation (1448 or 10·5 $\log_2$) as an observed value where necessary. Median titers and $\log_{10}$[$CCID_{50}$/g] of shed virus in stools were shown with bootstrap-based 95% CIs with 10 000 replicates. As described previously, (179) a viral shedding index was calculated as the average of $\log_{10}$[$CCID_{50}$/g] of samples collected 7, 14, 21, and 28 days after vaccine administration, with the lower limit of quantitation (2·75 $\log_{10}$) as an observed value, and with real-time RT-PCR-negative values contributing 0 to the mean. Missing values (from missing samples on specific study days) were replaced with values from the days before or after or the average of these two values, as necessary. SAS version 9.3 was used for analyses. This study is registered with EudraCT, number 2017-000908-21 and ClinicalTrials.gov, number NCT03430349.

4.4 Results

Between May 22 and Aug 22, 2017, 48 volunteers were screened, of whom 15 (31%) volunteers were excluded for reasons relating to the inclusion or exclusion criteria (figure 1). The main reasons for not being enrolled were psychological incompatibility with other selected participants and laboratory test abnormalities (e.g., abnormal blood cell counts or liver enzyme concentrations). Three (6%) volunteers were not treated because of restrictions to the number of participants in each group, but they were retained as back-ups in case of early withdrawal by any participants who were enrolled and later excluded.

Participants were predominantly male (25 [83%] of 30 participants), with a mean age of 32·8 years, ranging from 21 to 50 years (table 1). Both groups were similar in terms of sex and age distribution. Vaccination records showed that 24 (80%) participants had received at least six IPV vaccinations, and 28 (93%) participants had received at least five IPV vaccinations; no participants had received OPV previously. There were no early terminations from the study and all participants fully complied with all procedures and sampling requests.

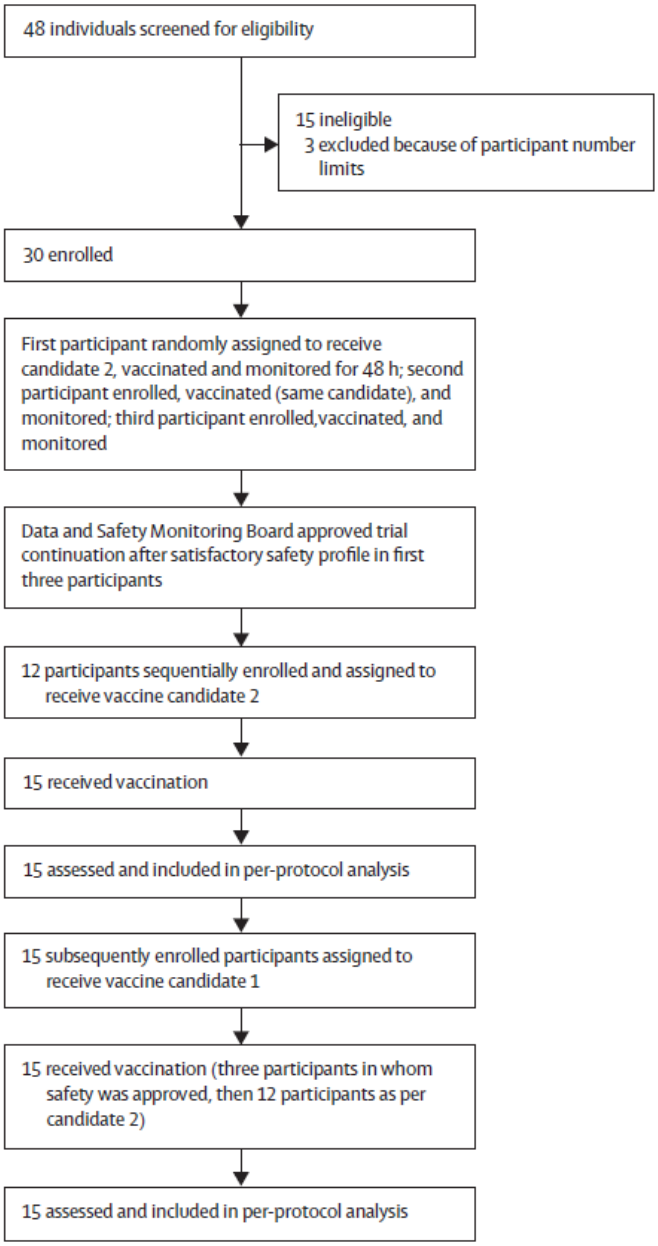

Fig. 1: Trial profile

	Candidate 1 (n=15)	Candidate 2 (n=15)	Total (n=30)
Sex, n (%)			
Male	13 (87%)	12 (80%)	25 (83%)
Female	2 (13%)	3 (20%)	5 (17%)
Age, years			
Mean (SD)	31·1 (7·7)	33·5 (10·9)	32·3 (9·4)
Minimum–maximum	21–50	21–49	21–50
Race, n (%)			
White	14 (93%)	14 (93%)	28 (93%)
Asian	0	0	0
Black	1 (7%)	0	1 (3%)
Other	0	1 (7%)	1 (3%)
Height, cm			
Mean (SD)	182·2 (8·7)	180·0 (10·1)	181·1 (9·3)
Minimum–maximum	163·0–193·0	160·0–198·5	160·0–198·5
Weight, kg			
Mean (SD)	79·1 (15·6)	79·7 (11·8)	79·4 (13·6)
Minimum–maximum	53·6–114·1	64·9–95·4	53·6–114·1
Body-mass index, kg/m²			
Mean (SD)	23·7 (3·9)	24·7 (3·7)	24·2 (3·7)
Minimum–maximum	19·0–31·3	19·0–30·7	19·0–31·3

Table 1: Study group demographics

	Candidate 1		Candidate 2		Total	
	Number of participants (% of n=15)	Number of events	Number of participants (% of n=15)	Number of events	Number of participants (% of n=30)	Number of events
≥1 serious adverse event	0	0	0	0	0	0
≥1 severe adverse event	6 (40%)	8	9 (60%)	12	15 (50%)	20
Probable	0	0	0	0	0	0
Possible	6 (40%)	7	9 (60%)	10	15 (50%)	17
Unlikely	0	1	0	2	0 (3%)	3
Unrelated	0	0	0	0	0	0
Adverse events that led to study withdrawal	0	0	0	0	0	0
≥1 adverse event	15 (100%)	103	15 (100%)	84	30 (100%)	187
Any solicited	13 (87%)	31	9 (60%)	18	22 (73%)	49
Mild solicited	8 (53%)	25	8 (53%)	17	16 (53%)	42
Moderate solicited	5 (33%)	6	1 (7%)	1	6 (20%)	7
Severe solicited	0	0	0	0	0	0
Any unsolicited	15 (100%)	72	15 (100%)	66	30 (100%)	138
Mild unsolicited	3 (20%)	51	3 (20%)	37	6 (20%)	88
Moderate unsolicited	6 (40%)	13	3 (20%)	17	9 (30%)	30
Severe unsolicited	6 (40%)	8	9 (60%)	12	15 (50%)	20

Each participant was counted only once in each category, under the maximum causality or severity. Both solicited and unsolicited events are included, unless otherwise noted.

Table 2. Adverse events in the total vaccinated population

Safety

There were no serious adverse events (table 2). 15 participants (six [40%] participants receiving candidate 1 and nine [60%] participants receiving candidate 2) presented with 20 severe adverse events (eight events with candidate 1 and 12 events with candidate 2). Of these severe adverse events, seven events (in six participants receiving candidate 1) and ten events (in nine participants receiving candidate 2) were judged to possibly be related to the vaccine. Most of these severe adverse events were transient increases in blood creatinine phosphokinase (six events with candidate 1 and nine events with candidate 2), and the other two events were increased aspartate aminotransferase concentrations (one event with each candidate vaccine); none of these transient increases was associated with clinical signs or symptoms. Other severe adverse events (which were

considered to be unlikely to be associated with the vaccine) were individual episodes of diarrhea and gastroenteritis in individuals receiving candidate 2, and severe headache in a participant receiving candidate 1. Most severe adverse events resolved spontaneously or with standard treatments within the study period.

Most participants reported at least one mild or moderate solicited adverse event within 7 days of novel OPV2 administration: 13 (87%) participants receiving candidate 1 reported 31 events, and nine (60%) participants receiving candidate 2 reported 18 events (table 4). After receiving candidate 1, ten (67%) participants reported fatigue and eight (53%) participants reported a headache. No solicited adverse events were markedly common after participants received candidate 2; the most frequent adverse event was diarrhea in four (27%) participants. All solicited adverse events resolved within the study period, without any permanent or long-term consequences.

All 30 participants reported at least one unsolicited adverse event, to a total of 138 events (table 2). Events were reported at similar frequencies by both groups: 72 events were reported with candidate 1 and 66 events were reported with candidate 2. 118 (86%) events were described as mild or moderate, and 67 (49%) events were considered to be either possibly or probably related to the treatment. Of the unsolicited severe adverse events, seven of the eight events (reported in six participants receiving candidate 1), and ten of the 12 events (in nine participants receiving candidate 2) were considered to be possibly related to the vaccines. All possibly related severe adverse events were abnormal laboratory findings, mainly changes in levels of alanine transaminase, aspartate transaminase, and creatine kinase. Transient increases in alanine transaminase (≥41 U/L) were observed in three (20%) participants receiving candidate 1 and six (40%) participants receiving candidate 2. Transient increases in aspartate transaminase (≥37 U/L) were reported in five (33%) participants receiving candidate 1 and six (40%) participants receiving candidate 2. These increases peaked on days 7–14 then gradually recovered to normal values. This finding led to unplanned investigations of γ-glutamyl transferase and creatine kinase; we found no abnormal γ-glutamyl transferase or bilirubin levels, but increased creatine kinase levels in six (40%) participants receiving candidate 1 and 11 (73%) participants receiving candidate 2. Individual abnormal concentrations of creatine kinase above the normal upper limit of 190 U/L peaked at 14 632 U/L in a participant who received candidate 1 and 19 500 U/L in a participant who received

candidate 2, 7 days after vaccine administration. Abnormal concentrations of creatine kinase were still present at day 28 in several participants in both groups, which had returned to normal at the 42-day follow-up. We found no clinically relevant qualitative or quantitative changes in other blood chemistry or blood cell counts.

	Candidate 1		Candidate 2		Total	
	Number of participants (% of n=15)	Number of events	Number of participants (% of n=15)	Number of events	Number of participants (% of n=30)	Number of events
Total						
Solicited adverse events	13 (87%)	31	9 (60%)	18	22 (73%)	49
Abdominal pain						
Any	2 (13%)	3	2 (13%)	2	4 (13%)	5
Mild	2 (13%)	3	2 (13%)	2	4 (13%)	5
Moderate	0	0	0	0	0	0
Arthralgia						
Any	0	0	1 (7%)	1	1 (3%)	1
Mild	0	0	1 (7%)	1	1 (3%)	1
Moderate	0	0	0	0	0	0
Diarrhoea						
Any	1 (7%)	1	4 (27%)	4	5 (17%)	5
Mild	1 (7%)	1	4 (27%)	4	5 (17%)	5
Moderate	0	0	0	0	0	0
Fatigue						
Any	10 (67%)	13	2 (13%)	2	12 (40%)	15
Mild	9 (60%)	12	2 (13%)	2	11 (37%)	14
Moderate	1 (7%)	1	0	0	1 (3%)	1
Headache						
Any	8 (53%)	8	3 (20%)	3	11 (37%)	11
Mild	7 (47%)	7	2 (13%)	2	9 (30%)	9
Moderate	1 (7%)	1	1 (7%)	1	2 (7%)	2
Myalgia						
Any	3 (20%)	3	3 (20%)	3	6 (20%)	6
Mild	1 (7%)	1	3 (20%)	3	4 (13%)	4
Moderate	2 (13%)	2	0	0	2 (7%)	2
Nausea						
Any	2 (13%)	2	3 (20%)	3	5 (17%)	5
Mild	1 (7%)	1	3 (20%)	3	4 (13%)	4
Moderate	1 (7%)	1	0	0	1 (3%)	1
Vomiting						
Any	1 (7%)	1	0	0	1 (3%)	1
Mild	0	0	0	0	0	0
Moderate	1 (7%)	1	0	0	1 (3%)	1

Table 4: Solicited adverse events in the total vaccinated population. Solicited events comprised signs and symptoms that were reported within 7 days of vaccination by use of a predefined checklist in a diary card. Participants graded their adverse events from mild to severe. No severe adverse events are reported.

Viral shedding

Viral RNA was detected in stool samples from most participants within a few days of administration, and was eventually positively identified in 15 (100%) participants who received candidate 1 and 13 (87%) participants receiving candidate 2. There was a gradual decrease in the number of participants shedding virus (as determined by RNA-positive stool samples) during the study period (figure 2). Observed shedding ceased (judged as three consecutive real-time RT-PCR-negative samples) and then resumed in three (20%) participants in each group, for a further 3–6 days. The shedding duration was longer than the containment period in some participants, continuing after day 28 in seven (47%) participants receiving candidate 1 and four (27%) participants receiving candidate 2. The last days of shedding for any of the volunteers, who were housed locally in Belgium until cessation, were day 89 in a participant receiving candidate 1 and day 48 in a participant receiving candidate 2. Shedding cessation occurred more rapidly after use of candidate 2 than candidate 1, which was complicated by the observed cessation and resumption in some participants. Shedding cessation, as defined prospectively, was met at a median of 23 days (IQR 15–36) after receiving candidate 1 and 12 days (1–23) after receiving candidate 2. Total shedding, described by the estimated median shedding index ($\log_{10}$[$CCID_{50}$/g]), was observed to be greater with candidate 1 than candidate 2 across all participants (2·8 [95% CI 1·8–3·5] *vs* 1·0 [0·7–1·6]) and among only those shedding virus at any time (2·8 [1·8–3·5] *vs* 1·3 [0·9–2·0]) (table 3). The maximum $\log_{10}$[$CCID_{50}$/g] observed for any participant at any time was 5·34 (day 3) in a participant receiving candidate 1, and 5·19 (day 8) in a participant receiving candidate 2. Seven participants receiving candidate 1 and one participant receiving candidate 2 had observed $CCID_{50}$ values of more than 5·0 $\log_{10}$, but no participant maintained this concentration for more than 2 days.

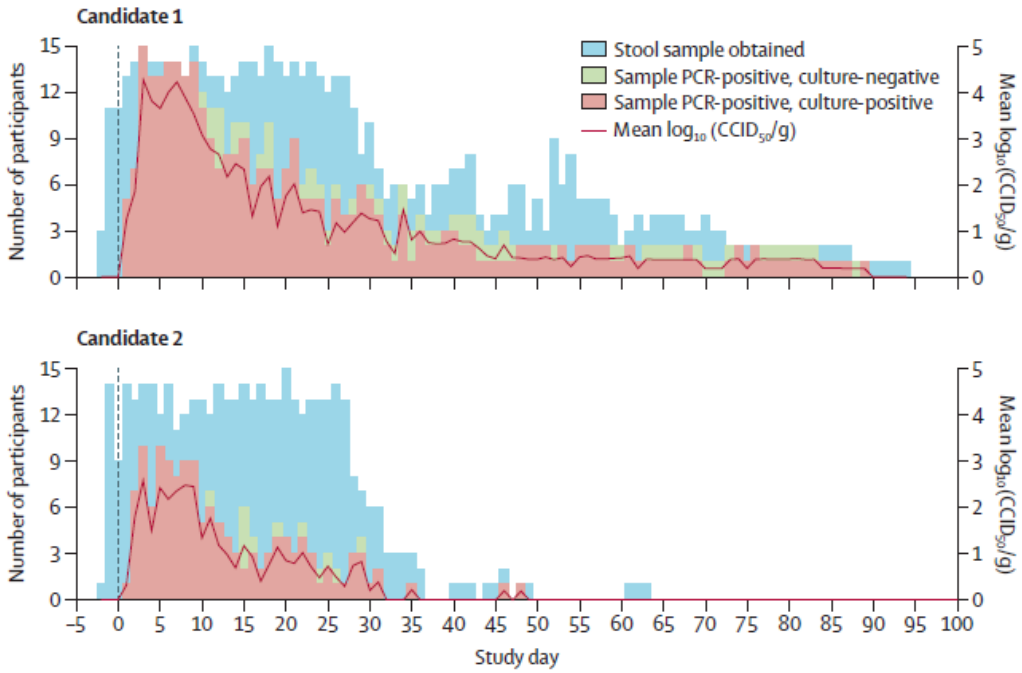

Figure 2: Viral shedding of the two novel oral type-2 poliovirus vaccine candidates

Data are the number of PCR-positive stool samples and the $\log_{10}(CCID_{50}/g)$ after administration on day 0. Participants who had ceased shedding were included, at a value of 0, in the computation of the mean $\log_{10}(CCID_{50}/g)$.

	Candidate 1 (n=15)	Candidate 2 (n=15)
Day 7		
Number of stool samples	14	11
Number of participants shedding (%)	14 (100%)	8 (73%)
Median $log_{10}(CCID_{50}/g)$ (95% CI)	4·1 (3·8–4·5)	3·3 (0·0–3·7)
Median $log_{10}(CCID_{50}/g)$ among those shedding (95% CI)	4·1 (3·8–4·5)	3·4 (2·9–3·8)
Day 14		
Number of stool samples	13	13
Number of participants shedding (%)	10 (77%)	3 (23%)
Median $log_{10}(CCID_{50}/g)$ (95% CI)	2·9 (2·8–3·3)	0·0 (0·0–0·0)
Median $log_{10}(CCID_{50}/g)$ among those shedding (95% CI)	3·1 (2·9–3·6)	2·9 (2·8–4·0)
Day 21		
Number of stool samples	14	13
Number of participants shedding (%)	9 (64%)	3 (23%)
Median $log_{10}(CCID_{50}/g)$ (95% CI)	2·9 (0·0–3·3)	0·0 (0·0–0·0)
Median $log_{10}(CCID_{50}/g)$ among those shedding (95% CI)	3·0 (2·8–3·4)	3·2 (3·1–3·9)
Day 28		
Number of stool samples	11	8
Number of participants shedding (%)	5 (46%)	3 (38%)
Median $log_{10}(CCID_{50}/g)$ (95% CI)	0·0 (0·0–2·9)	0·0 (0·0–3·2)
Median $log_{10}(CCID_{50}/g)$ among those shedding (95% CI)	2·9 (2·8–3·7)	3·2 (3·0–4·1)
Shedding index endpoint (days 7, 14, 21, and 28)		
Number of stool samples	15	15
Median shedding index (95% CI)	2·8 (1·8–3·5)	1·0 (0·7–1·6)
Median shedding index among those shedding (95% CI)	2·8 (1·8–3·5)	1·3 (0·9–2·0)

The viral shedding index endpoint was calculated as the mean $log_{10}(CCID_{50}/g)$ over days 7, 14, 21, and 28, with the lower limit of quantitation (2·75 log_{10}) as an observed value and all titers in stool samples with negative shedding results set to zero. CIs were obtained via the percentile bootstrap method. If a titer was missing at days 7, 14, 21, or 28, it was replaced by a neighboring value. If both neighboring values were available, the titer was replaced by the mean of these values. The median shedding index among those shedding was calculated by excluding subjects that were PCR-negative for shedding at all timepoints. $CCID_{50}$=50% cell culture infectious dose.

Table 3. Stool poliovirus shedding index point

Humoral Immunogenicity

On day 0, both groups had similar titers of serum neutralizing antibodies against poliovirus type-2 and all but one participant, who received candidate 2, had seroprotective titers (table 5). Increases in median titers of neutralizing antibodies were observed 28 days after vaccination, with a median 8·0-fold increase after use of candidate 1 and a 12·7-fold increase after use of candidate 2, indicative of immune response to the two candidates. All participants had seroprotective titers after vaccination, with seroconversion reported in ten (83%) of 12 participants receiving candidate 1 and 11 (85%) of 13 participants receiving candidate 2 (i.e., among those whose baseline titers were sufficiently below the upper limit of quantitation to allow detection of a four-fold rise).

	Candidate 1 (n=15)	Candidate 2 (n=15)
Geometric mean titre (95% CI)		
Day 0	93·3 (55·3–170·0)	52·8 (25·2–123·1)
Day 28	680·9 (367·8–1098)	575·0 (330·1–871·9)
Geometric mean fold change in titre (95% CI)		
Day 28	7·3 (3·8–13·5)	10·9 (4·5–24·6)
Median titre (IQR; Q1–Q3), (95% CI)		
Day 0	56·9 (40·6–147·4; 106·7), (36·0–181·0)	36·0 (22·6–81·3; 58·6), (22·6–90·5)
Day 28	1152 (650–1448; 798), (576·0–1448)	724·1 (408·6–1152·1; 743·5), (362·0–1152)
Median fold change in titre (IQR; Q1–Q3), (95% CI)		
Day 28	8·00 (2·6–22·8; 20·2), (2·00–20·1)	12·73 (4·1–38·2; 34·1), (3·18–25·5)
Seroprotection, n (%), 95% CI		
Day 0	15 (100%), 78·2–100	14 (93%), 68·1–99·8
Day 28	15 (100%), 78·2–100	15 (100%), 78·2–100
Seroconversion, n (%), 95% CI		
Day 28*	10 (83%), 51·6–97·9	11 (85%), 54·6–98·1

95% CIs were calculated with the Clopper-Pearson method. *Three participants given candidate 1 and two participants given candidate 2 had baseline titers close to the upper limit of quantitation so it was not possible to measure a 4-fold increase.

Table 5: Immune responses as poliovirus type-2 neutralizing antibody titers in the total study population

Neurovirulence and genetic stability

When clinical trial bulk material of viruses for candidates 1 and 2 were evaluated in the modified mouse neurovirulence assay, we did not find paralysis in any mouse inoculated with candidate 1. However, we found paralysis in four (13%) of 30 mice inoculated with candidate 2 vaccine bulk material (appendix p 2). All 15 (100%) participants receiving candidate 1 provided the necessary exploratory endpoint specimen of stool for neurovirulence assessment, which arose from day 2 to day 56 after vaccination. However, only six (40%) participants who received candidate 2 provided an exploratory endpoint specimen, of whom only two participants had shed virus samples that could be amplified to adequate concentrations to perform the neurovirulence testing; these specimens were provided on days 2 and 3 after vaccination. Among exploratory endpoint specimens from participants given candidate 1, five (33%) of 15 samples contained virus that paralyzed mice: seven of 446 mice were affected, giving an overall proportion of paralysis of 2% (range 0–10 for the 15 samples). One sample from a participant given candidate 2 contained virus that paralyzed four of 28 mice, giving an overall proportion of paralysis of 6·9% (0–14) across the two samples. By contrast, 70–90% of mice were paralyzed by a Sabin OPV2 sample shed on day 7 in a previous trial (177) in infants (data not shown) in the same assay, across replicates.

Among the 15 samples from participants given candidate 1, all genetic modifications engineered into candidate 1 were retained. Specifically, there were no variants consistent with reversion in domain V, the site of the main attenuation determinant in Sabin OPV2. Similarly, deep sequencing analysis of the six samples from participants given candidate 2 revealed no mutations in domain V. For both candidates, the secondary attenuation site, VP1–143, reverted in a manner consistent with Sabin OPV2, but observed changes were insufficient to cause meaningful observable paralysis in the neurovirulence test. For example, there were three samples from participants given candidate 1 in which the VP1–143 position was mutated in more than 90% of the viruses, two samples at 99%, and one sample at 93%, and none of these samples showed more than 10% paralysis.

All nasopharyngeal swabs taken on days 0, 3, 7, and the last day of containment tested negative for poliovirus by real-time RT-PCR in all participants in both groups.

4.8 Discussion

We evaluated two novel OPV2 candidates that were designed to stabilize the poliovirus genome against acquisition of neurovirulence, to provide safer alternatives for outbreak control of cVDPVs in the era following OPV2 cessation. Our study is the first to generate human clinical data for the development of an OPV with new strains in almost 60 years. Preclinical analysis of both candidates provided strong evidence of increased genetic stability of the viral genome, with a lower risk of reversion to neurovirulence relative to Sabin OPV2 (unpublished). With the global withdrawal of OPV type-2 vaccines in May, 2016, and GAPIII containment requirements, it was determined that this initial study should be performed in fully vaccinated adults residing in a contained environment, to avoid potential environmental contamination. The successful demonstration of the candidates' safety profiles and stability in this study resulted in initiation of a larger phase 2 study in October, 2018, that is not using containment measures but that has an extensive plan for monitoring of stool samples and follow-up. This phase 2 trial in the target population will assess safety, immunogenicity, genetic stability, and neurovirulence as primary criteria for selection of candidates for further development and licensure, with secondary factors such as cost of goods sold and manufacturing yield to be considered, if necessary, to select a candidate for a phase 3 study for full licensure.

The results from our phase 1 trial indicate that both candidates are **safe** and immunogenic in adults. There were no serious adverse events but severe adverse events that were considered possibly to be related to the vaccines were increased blood enzyme concentrations (predominantly creatine kinase, but also alanine transaminase and aspartate transaminase), which were observed in about half the participants 1 week after vaccine administration. These increases were transient and without any abdominal symptoms or other indicators of liver damage; γ-glutamyl transferase and bilirubin concentrations were unaffected. Although we cannot fully explain these findings, they are consistent with the confined participants making unaccustomed and excessive use of the fitness equipment and daily supervised fitness training that was available in the facility. Some volunteers did report training-associated muscular pains but, for some, these reports were independent of liver enzyme increases. As observed in previous studies,((180) - (182)) increased creatine kinase concentrations up to 30-times the upper normal limit without changes in γ-glutamyl transferase are associated with strenuous muscular exercise, often accompanied by increases in alanine

transaminase and aspartate transaminase. To assess the cause for these creatine kinase increases, group 2 (candidate 1) participants were asked to intensify their daily fitness exercises in their last week of containment, on a voluntary basis. Those who agreed were surveyed daily to recall their exercise activities, with an additional blood sample after 3 days (day 24). Results were inconclusive, with similar changes in enzyme concentrations in some but not all participants (data not shown). The subsequent larger phase 2 study with both candidates has been designed with a placebo group and participants who are not confined as in the present study, with the expectation that participants are unlikely to change their daily habits to overexercise. This phase 2 study will provide evidence as to whether the changes in enzymes were associated with the vaccination itself, since enteroviruses can cause liver enzyme increases, or the study circumstances.

Both vaccines were **immunogenic** in the IPV-only vaccinated study population, with substantial increases in type-2 neutralizing antibody titers 4 weeks after vaccination, and showed evidence of **intestinal replication**, evidenced by stool shedding. We did not find any evidence of nasopharyngeal shedding from any participant. This finding is reassuring because procedures for collection and analysis of nasopharyngeal samples were as rigorous as for the stool samples. As discussed by Hird and Grassly, (48) nasopharyngeal shedding has not been extensively studied and previous reports of decreased nasopharyngeal shedding of wild-type virus in IPV- vaccinated children are confounded by variations in age and other factors. We observed differences in stool shedding between the candidates, both in duration and magnitude of fecal titers, with observed shedding greater for candidate 1 than candidate 2. Shedding of both viruses persisted for longer than the study containment period in seven (47%) participants given candidate 1 and four (27%) participants given candidate 2. We observed no laboratory or immunological abnormalities in participants who shed for the longer periods. Although we have no comparative data for monovalent OPV2 in an IPV-only vaccinated adult population, in IPV-only vaccinated infants challenged with monovalent OPV2, the extent of viral shedding measured by the shedding index was similar to, or greater than, the levels we observed; a substantial proportion of infants (around 25%) had a median viral shedding index score of more than 5·0 $\log_{10}$, a value that was rarely observed for individual samples in our study. (179) This result suggests that viral shedding might not be substantially increased and could even be lower with these novel candidates compared with vaccines

containing Sabin-2, which will require additional evaluations in the target population for confirmation.

Although there was evidence of accumulating genetic substitutions in shed virus for both candidates, as would be anticipated for an RNA virus, no variants consistent with increased virulence were detected in domain V of the 5-untranslated region, the site of the primary determinant of attenuation for Sabin OPV2 (nucleotide 481). Notably, in both candidates, the unprotected secondary attenuation site, VP1–143, reverted in a manner consistent with expectations for Sabin OPV2. However, shed virus with variants in this position showed low paralysis (1·6–6·9%), compared with a sample of reverted Sabin monovalent OPV2 (90%) in the same test. These data are consistent with previous values for monovalent OPV2 and provide strong support for the improved genetic and phenotypic stability of the novel OPV2 candidates. ((183), (184))

In one clinical study, (185) all type-2 virus that was shed 7 days after administration contained substantial (33–96%) reversion at the primary attenuation site (nucleotide 481); another study (186) showed similar results, with almost 100% reversion within 14 days after trivalent OPV vaccination. These neurovirulence data provide encouraging evidence for the genetic and phenotypic stability of both candidates relative to the rapid loss of attenuation of Sabin OPV2 vaccines that result in high paralysis rates in transgenic mouse assays. (174) Therefore, irrespective of other variations occurring over time, the excreted viruses were expected to maintain their attenuation to a large degree.

Limitations of this phase 1 study were that it necessarily involved few participants, used what is expected to be the maximal dose of vaccine virus and, because of the global cessation of elective use of monovalent OPV2, there was no direct comparator to test both candidates.

Our study shows that these two novel OPV2 candidates have the potential to provide more genetically stable alternatives to the current Sabin monovalent OPV2 that is stockpiled for control of cVDPV2 outbreaks. With all live type-2 polioviruses withdrawn from routine immunization use, the global population is now reliant on IPV to provide immunity against type-2 polioviruses. The limited primary intestinal immunity provided by IPV requires that an outbreak response must rely on Sabin oral live-attenuated poliovirus vaccines to interrupt person-to-person transmission. ((175), (187)) With the known reversion of Sabin monovalent OPV2 and developing evidence that it might have generated new

cVDPVs in some settings, (170) novel candidate vaccines such as those we have tested could be crucial components of efforts to ensure complete and permanent global eradication of poliovirus type-2.

Acknowledgments

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The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention or other contributing agencies.

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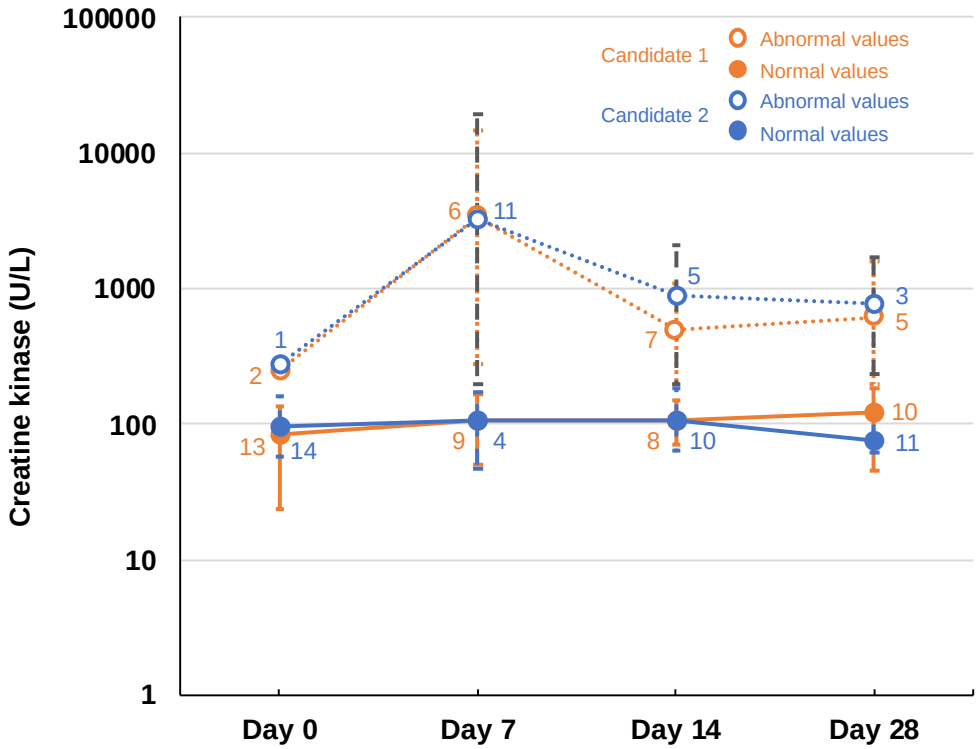
Supplementary Appendices

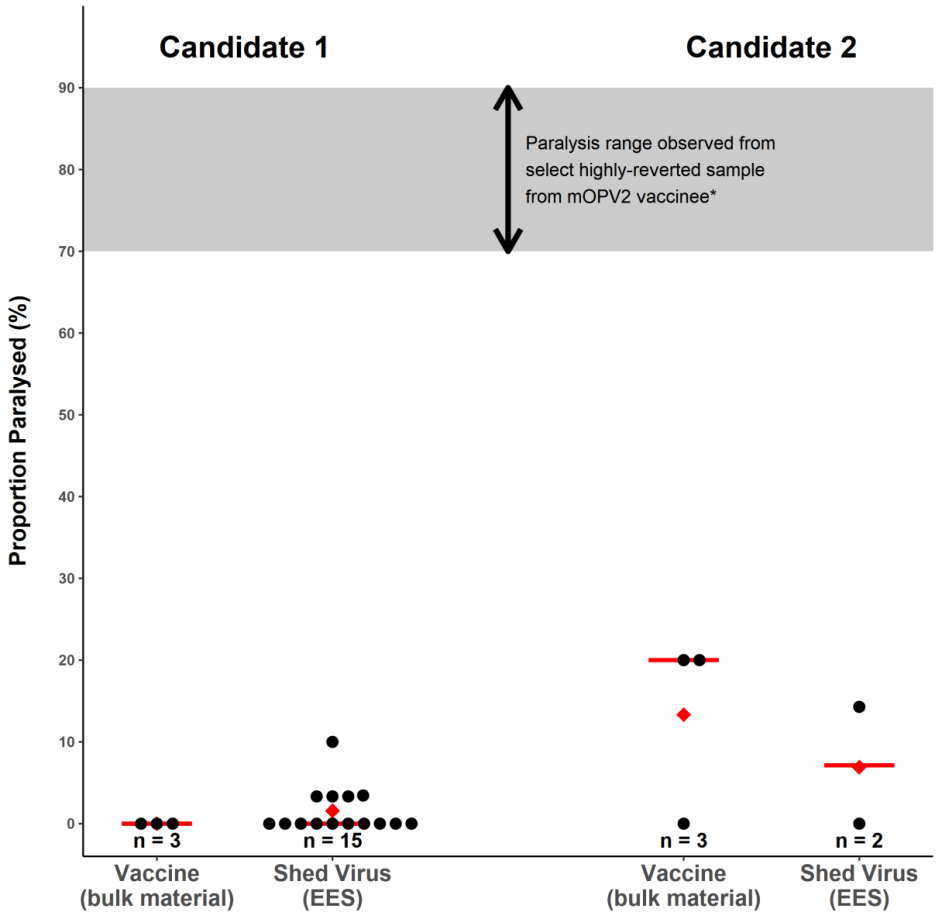
Appendix 1:

Creatine kinase levels. Values show mean levels grouped according to being in the normal range (closed symbols) and abnormal ranges (open symbols) for candidate 1 (orange) and candidate 2 (blue). Error bars show ranges of values for each group.



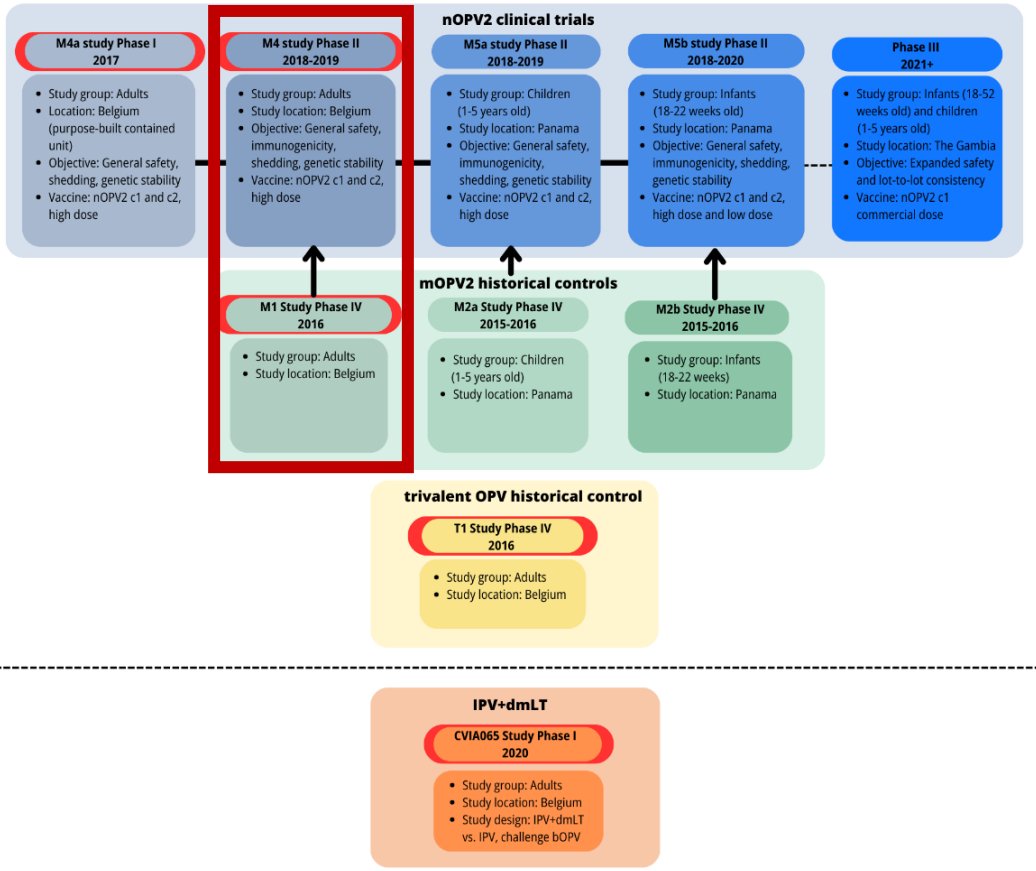
Appendix 2:

Mouse paralysis proportion for Exploratory Endpoint Samples (EES), as well as clinical trial material ("Vaccine") in a single-dose (4.0 $\log_{10}$ [$CCID_{50}$] intraspinal inoculum) transgenic mouse model. Ten mice per sample were assayed, alongside controls, with three replicates each. Points indicate samples/subjects (combined over each replicate), with diamonds indicating the overall means and horizontal lines indicating the median across subjects * Reference range of 70–90% paralysis developed from repeated assay (n=5) of a type-2-containing sample from an infant vaccinee who received mOPV2 at 40 weeks, following bOPV at 6/10/14 weeks and IPV at 14/36 weeks in a prior clinical trial. (179) Sample collected 7 days post-challenge and selected based on high reversion (89% 481G).



Chapter 5:
Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in healthy adults: two clinical trials

This chapter is published: "De Coster I. & Leroux-Roels I., Bandyopadhyay A.S., Gast C., Withanage K., Steenackers K., et al. Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in healthy adults: two clinical trials. The Lancet 2021;397:39-50." doi:10.1016/S0140-6736(20)32541-1


nOPV2 clinical trials
M4a study Phase I
2017
Study group: Adults
Location: Belgium (purpose-built contained unit)
Objective: General safety, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M4 study Phase II
2018-2019
Study group: Adults
Study location: Belgium
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5a study Phase II
2018-2019
Study group: Children (1-5 years old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5b study Phase II
2018-2020
Study group: Infants (18-22 weeks old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose and low dose
Phase III
2021+
Study group: Infants (18-52 weeks old) and children (1-5 years old)
Study location: The Gambia
Objective: Expanded safety and lot-to-lot consistency
Vaccine: nOPV2 c1 commercial dose
mOPV2 historical controls
M1 Study Phase IV
2016
Study group: Adults
Study location: Belgium
M2a Study Phase IV
2015-2016
Study group: Children (1-5 years old)
Study location: Panama
M2b Study Phase IV
2015-2016
Study group: Infants (18-22 weeks)
Study location: Panama
trivalent OPV historical control
T1 Study Phase IV
2016
Study group: Adults
Study location: Belgium
IPV+dmLT
CVIA065 Study Phase I
2020
Study group: Adults
Study location: Belgium
Study design: IPV+dmLT vs. IPV, challenge bOPV

5.1 Summary

Background

Two novel type 2 oral poliovirus vaccine (OPV2) candidates, novel OPV2-c1 and novel OPV2-c2, designed to be more genetically stable than the licensed Sabin monovalent OPV2, have been developed to respond to ongoing polio outbreaks due to circulating vaccine-derived type 2 polioviruses.

Methods

We did two randomized studies at two centers in Belgium. The first was a phase 4 historical control study of monovalent OPV2 in Antwerp, done before global withdrawal of OPV2, and the second was a phase 2 study in Antwerp and Ghent with novel OPV2-c1 and novel OPV2-c2. Eligible participants were healthy adults aged 18–50 years with documented history of at least three polio vaccinations, including OPV in the phase 4 study and either OPV or inactivated poliovirus vaccine (IPV) in the novel OPV2 phase 2 study, with no dose within 12 months of study start. In the historical control trial, participants were randomly assigned to either one dose or two doses of monovalent OPV2. In the novel OPV2 trial, participants with previous OPV vaccinations were randomly assigned to either one or two doses of novel OPV2-c1 or to one or two doses of novel OPV2-c2. IPV-vaccinated participants were randomly assigned to receive two doses of either novel OPV2-c1, novel OPV2-c2, or placebo. Vaccine administrators were unmasked to treatment; medical staff performing safety and reactogenicity assessments or blood draws for immunogenicity assessments were masked. Participants received the first vaccine dose on day 0, and a second dose on day 28 if assigned to receive a second dose. Primary objectives were assessments and comparisons of safety up to 28 days after each dose, including solicited adverse events and serious adverse events, and immunogenicity (seroprotection rates on day 28 after the first vaccine dose) between monovalent OPV2 and the two novel OPV2 candidates. Primary immunogenicity analyses were done in the per-protocol population. Safety was assessed in the total vaccinated population—i.e., all participants who received at least one dose of their assigned vaccine. The phase 4 control study is registered with EudraCT (2015-003325-33) and the phase 2 novel OPV2 study is registered with EudraCT (2018-001684-22) and ClinicalTrials.gov (NCT04544787).

Findings

In the historical control study, between Jan 25 and March 18, 2016, 100 volunteers were enrolled and randomly assigned to receive one or two doses of monovalent OPV2 (n=50 in each group). In the novel OPV2 study, between Oct 15, 2018, and Feb 27, 2019, 200 previously OPV-vaccinated volunteers were assigned to the four groups to receive one or two doses of novel OPV2-c1 or novel OPV2-c2 (n=50 per group); a further 50 participants, previously vaccinated with IPV, were assigned to novel OPV2-c1 (n=17), novel OPV2-c2 (n=16), or placebo (n=17). All participants received the first dose of assigned vaccine or placebo and were included in the total vaccinated population. All vaccines appeared safe; no definitely vaccine-related withdrawals or serious adverse events were reported. After first doses in previously OPV-vaccinated participants, 62 (62%) of 100 monovalent OPV2 recipients, 71 (71%) of 100 recipients of novel OPV2-c1, and 74 (74%) of 100 recipients of novel OPV2-c2 reported solicited systemic adverse events, four (monovalent OPV2), three (novel OPV2-c1), and two (novel OPV2-c2) of which were considered severe. In IPV-vaccinated participants, solicited adverse events occurred in 16 (94%) of 17 who received novel OPV2-c1 (including one severe) and 13 (81%) of 16 who received novel OPV2-c2 (including one severe), compared with 15 (88%) of 17 placebo recipients (including two severe). In previously OPV-vaccinated participants, 286 (97%) of 296 were seropositive at baseline; after one dose, 100% of novel OPV2 vaccinees and 97 (97%) of monovalent OPV2 vaccinees were seropositive.

Interpretation

Novel OPV2 candidates were as safe, well tolerated, and immunogenic as monovalent OPV2 in previously OPV-vaccinated and IPV-vaccinated adults. These data supported the further assessment of the vaccine candidates in children and infants.

5.2 Introduction

Global eradication of wild-type 2 and 3 polioviruses has been declared, (188) with wild-type 1 now only endemic in Afghanistan and Pakistan. (189) However, intestinal reversion to neurovirulence of attenuated Sabin oral poliovirus vaccine (OPV) viruses can occur and, when shed in stools and transmitted through populations with low OPV coverage, it can cause cases of paralysis. (190) Reported numbers of such circulating vaccine-derived poliovirus cases have increased every year since 2016, mainly due to type 2. (191) The Global Polio Eradication Initiative has developed a response strategy, which includes the development of new vaccines. (192)

A consortium has been working since 2011 on research and development of novel poliovirus strains engineered to be more genetically stable with less likelihood of reversion to neurovirulence while retaining the benefits of Sabin OPV. Because more than 94% of circulating vaccine-derived poliovirus cases were due to type 2, initial focus was on novel type 2 OPVs (OPV2s), (193) and has produced two candidates, OPV2-c1 and OPV2-c2. (79), (194) Both candidates are attenuated serotype 2 polioviruses derived from a modified Sabin 2 infectious clone with different combinations of five distinct modifications of the Sabin 2 genome, propagated in Vero cells. Novel OPV2-c1 includes a genetically stabilized domain V (the primary attenuation site for Sabin 2), relocation of the cis-acting replication element, and modifications to the polymerase to enhance fidelity and reduce recombination. (79) Novel OPV2-c2 includes the same genetically stabilized domain V and codon deoptimization in the capsid-coding region. (194) These modifications aimed to stabilize the genetic sequence against reversion in the 5′ untranslated region with additional attenuation provided by introducing about 87 additional silent mutations in the capsid region.

After reporting the first phase 1 study of both candidates in healthy adults, (130) we now report a larger phase 2 assessment of the safety, tolerability, immunogenicity, and genetic stability of both candidates in adults vaccinated with OPV or inactivated polio vaccine (IPV) to support further clinical development in children and infants. (195) This investigation is unique because global withdrawal of type 2-containing OPVs during the development of the novel OPV2 vaccines before clinical trial lots were available made it impossible to concurrently compare monovalent OPV2 and novel OPV2. Therefore, we did a prospectively designed phase 4 study with monovalent OPV2 vaccine to provide historical control data against which to assess each novel OPV2 candidate. Both studies are reported here.

5.3 Methods

Study design and participants

We did two partially masked studies at two centers: a phase 4 study of monovalent OPV2 (historical control study) at the Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp (Antwerp, Belgium); and a phase 2 study of the two novel OPV2 candidates at the same center and at the CEVAC, Center for Vaccinology, Ghent University Hospital (Ghent, Belgium). Study protocols were approved by each center's institutional review board and the Belgian national authority. The studies were conducted according to the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. All participants provided written informed consent.

Eligible participants were healthy adults aged 18–50 years with documented history of at least three polio vaccinations, including OPV in the phase 4 study and either OPV or IPV in the novel OPV2 phase 2 study, with no dose within 12 months of study start. Other inclusion criteria were being a resident in Belgium and being available for the study duration, and being in good mental and physical health at enrolment on the basis of medical history and examination. Females of childbearing potential had to have a negative pregnancy test at enrolment and agree to use an approved contraceptive method during and for 3 months after the study. Main exclusion criteria were any medical condition likely to affect the participant's wellbeing or immune response, including a low baseline total serum IgA level, any travel intended or within the previous 6 months to polio-endemic countries, breastfeeding, any professional food handling duties, any professional or household contact with immunosuppressed or incompletely polio-vaccinated people (e.g., young infants), or participation in another clinical trial within 28 days of this one.

Randomization and masking

Historical control study participants were enrolled and randomly assigned 1:1 to receive one or two doses of monovalent OPV2. In the novel OPV2 study, the novel OPV2-c2 candidate was prioritized so the first 100 OPV-vaccinated participants were randomly assigned 1:1 to groups 3 (one dose) and 4 (two doses) to receive novel OPV2-c2. The second 100 OPV-vaccinated participants were randomly assigned 1:1 to groups 1 (one dose) and 2 (two doses) to receive novel OPV2-c1. IPV-vaccinated adults were enrolled in parallel and randomly assigned 2:1 to group 6 (two doses of novel OPV2-c2) or group 7 (two doses of placebo), until

group 6 enrolment was complete, when 2:1 randomization was continued for group 5 (two doses of novel OPV2-c1) and group 7 (two doses of placebo). Block randomization was used throughout to ensure balanced randomization across time using a preprepared computer-generated randomization schedule (Assign Data Management and Biostatistics, Innsbruck, Austria). The study nurses (administration team) who gave the vaccine or placebo were unmasked according the randomization schedule, but each participant and the medical staff who assessed adverse events and drew blood samples for immunogenicity assessments were masked as to vaccine to placebo assignment.

Procedures

The monovalent OPV2 vaccine was Polio Sabin Mono Two (oral), manufactured by GlaxoSmithKline Biologicals, Belgium; lot number mOPV2-007, batch number DOP2A004AZ. The vaccine is a licensed, monovalent, live-attenuated poliomyelitis virus vaccine of the Sabin strain type 2 (P 712, Ch, 2ab), propagated in MRC5 human diploid cells. Each two-drop dose (0·1 mL) nominally contained $10^{5·7}$ 50% cell culture infective dose ($CCID_{50}$) units of type 2 poliovirus at release.

Both novel OPV2 candidates, novel OPV2-c1 (lot number 2060416C) and novel OPV2-c2 (lot number 2060316C), were manufactured by Bio Farma (Jawa Barat, Indonesia). High doses of novel OPV2 containing about 1 000 000 $CCID_{50}$ to ensure robust safety assessments, were administered orally as six drops (0·3 mL) delivered from a supplied dropper. Placebo was six orally administered drops of sugar syrup, propylene glycol (batch number 18B06/V89669; Conforma, Destelbergen, Belgium). One-dose groups received their only dose on day 0; two-dose groups received one dose on day 0 and the second on day 28.

Participants were monitored for 30 min after vaccination for immediate reactions, then asked to complete 7-day diary cards soliciting systemic adverse events and daily oral temperature, which were graded for severity as follows: mild (easily tolerated with minimal discomfort, 37·5–38·0°C), moderate (sufficiently discomforting to interfere with normal everyday activities, 38·1–39·0°C) or severe (prevents normal everyday activities, >39·0°C). Unsolicited adverse events were recorded for 28 days after each vaccination and assessed for causality and severity by the study investigator. Terms used to identify adverse events were coded according to the Medical Dictionary for Regulatory Activities (version 22.0). A standard panel of clinical laboratory assessments in the historical study was augmented with measurements of creatine phosphokinase, γ-glutamyl transferase, and albumin in the novel OPV2 study after observation of increased

levels of creatine phosphokinase and some liver enzymes in some participants in the phase 1 study of both novel OPV2 candidates. (130)

Sera obtained on days 0, 28, and 56 (after two doses) were stored and shipped at a maximum temperature of –20°C to the Centers for Disease Control and Prevention (CDC) laboratories (Atlanta, GA, USA) for measurement of poliovirus type 2-specific antibodies concurrently for both studies using the WHO standard microneutralization assay (WHO EPI GEN 93.9), adapted as previously described. (130), (175)) The lower limit of quantitation (LLOQ) was 2·5 $\log_2$ titer and the upper limit of quantitation (ULOQ) was 10·5 $\log_2$ titer. At each timepoint we calculated seroprotection rates (group proportions with a neutralizing antibody titer ≥1:8), group geometric mean titers using a logarithmic (base 2) scale, and seroconversion rates (total proportions of each group who changed from seronegative to seropositive or, for those who were initially seropositive, who displayed an at least four-fold rise in antibody titers after vaccination). Seroconversion was only calculated in individuals whose baseline antibody titer was low enough to allow observation of a four-fold increase without breaching the ULOQ.

Daily stool samples collected at days 0–10, 14, 21, 28, and 42 in one-dose groups, and additionally at days 29–38, 42, 49, 56 and 70 in two-dose groups, were stored and shipped to the CDC laboratory as were the serum samples. Nucleic acid was extracted from stool samples to detect poliovirus using RT-PCR and, in positive samples, the viral load was measured as $CCID_{50}$. (185) Deep-sequencing was done in exploratory endpoint stool samples, representing each participant's last polio type 2-positive stool samples containing more than 4·00 $\log_{10}$ $CCID_{50}$ per g of stool, using cDNA synthesis and full-length poliovirus genome amplification as described previously. (130)

Outcomes

Coprimary objectives were to assess and compare safety of novel OPV2 versus monovalent OPV2 in OPV-vaccinated groups, or novel OPV2 versus placebo in IPV-vaccinated groups, in terms of serious and severe adverse events up to day 28 after the first dose of vaccine, and immunogenicity as seroprotection rate 28 days after one dose in OPV-vaccinated groups. Secondary objectives were assessments of systemic reactogenicity, assessed as solicited adverse events for 7 days after each vaccination and as unsolicited adverse events for 28 days after each vaccination; and immunogenicity. Immunogenicity parameters included geometric mean titers of poliovirus neutralizing antibodies at all measured

timepoints, seroprotection rates at timepoints other than day 28 (primary objective), and seroconversion rates. Exploratory objectives were measurements of viral shedding and the genetic stability of any shed virus in stool viral samples. Ultimately, samples of shed virus will be assessed for neurovirulence, but this is beyond the scope of this report.

Statistical analysis

Sample size for OPV-vaccinated groups for each study was selected considering a non-inferiority comparison of seroprotection rates between each candidate and the control after one vaccination, assuming a 95% seroprotection rate, one-sided α=0·025, margin 10%, and 80% power, and augmented to ensure at least 50 participants were allocated to each dose group to achieve a 90% probability of observing an adverse event of interest when the true rate was 5%, allowing for a 5% dropout. Sample sizes for IPV-vaccinated groups were selected to detect a four-times increase in the risk of specific increased laboratory values assuming a background rate of 6%, using one-sided α=0·05 and 80% power, and allowing for 5% dropout.

All adverse events, including serious adverse events, severe adverse events, and solicited and unsolicited adverse events were summarized by type, seriousness, severity, and causality and by group and overall, and primary safety endpoints were compared between corresponding monovalent OPV2 (groups 1 and 2) and novel OPV2 (groups 1–4) and between novel OPV2 and placebo for exclusively IPV-vaccinated participants (groups 5 and 6 *vs* group 7) using the two-sided Fisher's exact test after each dose individually, and across all doses. The primary immunogenicity endpoint, the seroprotection rate after one dose of either vaccine candidate in the OPV-vaccinated groups (novel OPV2 study combined groups 1 and 2, and combined groups 3 and 4), was compared with the corresponding endpoint from the historical monovalent OPV2 control study (combined groups 1 and 2) via a non-inferiority test of the difference of each of the novel candidates to the monovalent OPV2 control, each using one-sided α=0·025 and a non-inferiority margin of 10%, computed using two-sided α=0·05 Miettinen and Nurminen score-based CIs for inference. The method used was described previously. (196) The independent variables are the vaccine group indicator and the baseline titer; the dependent variable is the post-baseline titer, which is considered to be observed, right censored (if result is ≥ULOQ), or left censored (if result is ≤LLOQ), to avoid bias in estimation due to the expected high frequency of responses exceeding ULOQ because of previous vaccinations received.

Secondary endpoints for OPV-vaccinated participants involved similar comparisons between corresponding groups across studies (monovalent OPV2 study groups 1 and 2 compared with novel OPV2 study groups 1 and 2, and groups 3 and 4) using two-sided 95% CIs for the rate difference (seroconversion rate), the difference in medians (log_2 neutralizing titers, using bootstrap methods), or the neutralizing antibody geometric mean titer ratio, using survival regression analysis on the log_2 titers with normal errors, incorporating the baseline log_2 titers as a covariate, and using maximum likelihood estimation to accommodate censoring at ULOQ and LLOQ, with reverse transformation of the model-estimated difference in means and corresponding CI. Immunogenicity data from IPV-vaccinated participants (monovalent OPV2 study groups 5–7) were summarized with the seroprotection rates, seroconversion rates, and geometric mean titers, but not compared between groups.

For each timepoint, viral shedding positivity and concentration were summarized. A viral shedding index estimate calculated for each participant as the average of log_{10}-transformed values of $CCID_{50}$ per g in stool samples established using quantitative PCR (viral identity) and $CCID_{50}$ (titers) from select stool samples taken 7, 14, 21, and 28 days after each vaccination was summarized by group and dose. Assay LLOQ (2·75 log_{10} $CCID_{50}$ per g) and ULOQ (8·25 log_{10} $CCID_{50}$ per g) were used as observed values where necessary.

Primary immunogenicity analyses were done in the per-protocol population. Safety was assessed in the total vaccinated population—i.e., all participants who received at least one dose of their assigned vaccine.

An independent data and safety monitoring board monitored the novel OPV2 development program, including the previous phase 1 study, (130) the present novel OPV2 study, and another in children and infants. (149) The phase 4 control study is registered with EudraCT (2015-003325-33) and the phase 2 novel OPV2 study is registered with EudraCT (2018-001684-22) and ClinicalTrials.gov (NCT04544787).

5.4 Results

In the historical control study, between Jan 25 and March 18, 2016, 112 volunteers were screened and 100 were enrolled and assigned to receive one or two doses of monovalent OPV2. All 100 participants received the assigned number of doses and remained in the study to the end of follow-up (day 42 for those in the one-dose group and day 70 for those in the two-dose group; figure A). In the novel OPV2 study, between Oct 15, 2018, and Feb 27, 2019, 277 volunteers were screened and 250 were enrolled (200 OPV vaccinated and 50 IPV vaccinated). Enrolment of IPV-vaccinated participants was truncated, per protocol, because of low enrolment rates, with data and safety monitoring board concurrence on the accumulation of sufficient safety data in these groups. Of the OPV-vaccinated participants, 50 were assigned to each of the four groups and of the 50 IPV-vaccinated participants, 17 were assigned to novel OPV2-c1, 16 to novel OPV2-c2, and 17 to placebo (figure B).

All participants received at least one vaccination and were included in the total vaccinated population for analysis of safety. Eight participants were excluded from the per-protocol population for immunogenicity analyses, either because they had low IgA, did not receive their assigned second vaccinations, or received concomitant medication not permitted by the protocol.

Demographics were generally similar across studies and groups in terms of age, race, and body-mass index, except for the male to female ratio (table 1). In OPV-vaccinated groups across both studies, 133 (44%) of 300 were men and 167 (56%) were women, and in the IPV-vaccinated groups, 12 (24%) of 50 were men and 38 (76%) were women. Most OPV-vaccinated participants had received three or four vaccinations and most IPV-vaccinated participants had received four to six vaccinations.

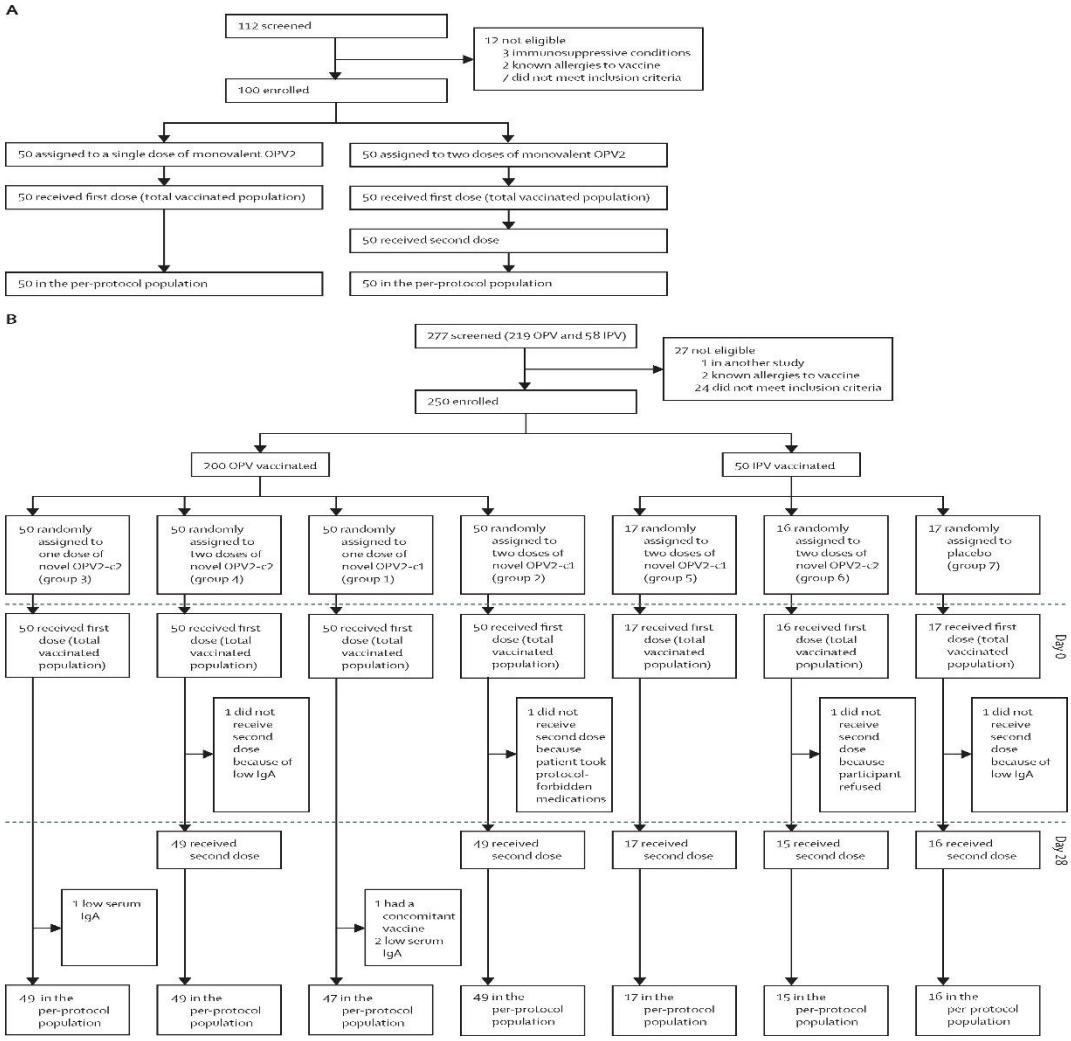


Fig.1: Trial Profiles for the historical study with mOPV2 (A) and the novel OPV2 candidates (B)

Table 1. Demographics of the populations in the two studies (TV Set)

		Historic control study		nOPV2 study - OPV vaccinated				nOPV2 study - IPV vaccinated		
		mOPV2		nOPV2-c1		nOPV2-c2		nOPV2-c1	nOPV2-c2	Placebo
		Group 1	*Group 2*	*Group 1*	*Group 2*	*Group 3*	*Group 4*	*Group 5*	*Group 6*	*Group 7*
	N	50	50	50	50	50	50	17	16	17
Age (years)	Mean ± SD	26 ± 8	28 ± 9	31 ± 10	31 ± 10	32 ± 10	34 ± 10	23 ± 10	31 ± 9	24 ± 8
Male	n (%)	19 (38)	25 (50)	28 (56)	21 (42)	22 (44)	18 (36)	6 (35)	3 (19)	3 (18)
BMI (kg/m²)	Mean ± SD	22.8 ± 3.3	24.7 ± 4.5	24.2 ± 4.2	23.8 ± 3.3	25.0 ± 4.4	25.3 ± 3.6	23.1 ± 4.8	23.9 ± 4.7	24.7 ± 4.2
Race	n (%)									
White		48 (96)	49 (98)	49 (98)	49 (98)	49 (98)	49 (98)	16 (94)	16 (100)	15 (88)
Asian		2 (4)	0 (0)	0 (0)	) (0)	1 (2)	) (0)	0 (0)	0 (0)	ɔ (0)
Black or African American		0 (0)	1 (2)	0 (0)	) (0)	0 (0)	) (0)	0 (0)	0 (0)	1 (6)
Other		0 (0)	0 (0)	1 (2)	I (2)	0 (0)	I (2)	1 (6)	0 (0)	1 (6)
Polio vaccination history										
OPV doses	n (%) =									
3		6 (12)	6 (12)	49 (98)	ɔ0 (100)	41 (82)	I0 (80)			
4		44 (88)	44 (88)	1 (2)	) (0)	9 (18)	) (18)	0 (0)	0 (0)	ɔ (0)
5		0 (0)	0 (0)	0 (0)	) (0)	0 (0)	I (2)			
IPV doses	n (%) =									
0		46 (92)	47 (94)	46 (92)	I9 (98)	48 (96)	I7 (94)	0 (0)	0 (0)	ɔ (0)
1		4 (8)	3 (6)	4 (8)	I (2)	2 (4)	ɜ (6)	0 (0)	0 (0)	ɔ (0)
4		-	-	-		-		6 (35)	4 (25)	10 (59)
5		-	-	-		-		10 (59)	5 (31)	5 (29)
6 or more		-	-	-		-		1 (6)	7 (44)	2 (12)

Safety

No deaths, life-threatening conditions, or definitely related serious adverse events were reported, no participant withdrew from either study because of adverse events, and no differences in proportions of patients with primary safety endpoint events were observed, except for a higher rate of any severe unsolicited events after first dose (one [6%] of 16 with novel OPV2-c2 *vs* seven [41%] of 17 with placebo) in IPV-vaccinated participants (table 2). Of the four serious adverse events, all in the novel OPV2 study, one was possibly related to vaccination; an IPV-vaccinated (group 6) participant had an influenza-like illness with onset 12 days after a second dose of novel OPV2-c2 that lasted for 6 days before resolving. Three other serious adverse events were considered unrelated to vaccination; a new onset ileitis terminalis (group 1, novel OPV2-c1) diagnosed 56 days after vaccination, and cases of severe vomiting (group 2, novel OPV2-c1) due to medication for shoulder surgery and anaphylaxis (group 4, novel OPV2-c2) due to medication for cystitis.

There were no meaningful differences in reactogenicity between the monovalent OPV2 and novel OPV2 groups. Most OPV-vaccinated participants reported solicited adverse events within 7 days of their first vaccination, 62 (62%) of 100 after monovalent OPV2, 71 (71%) of 100 after novel OPV2-c1, and 74 (74%) of 100 after novel OPV2-c2 (table 2). Most frequent adverse events were headache, fatigue, abdominal pain, diarrhea, and myalgia, with no difference in frequency or severity across groups (Appendix 1). Most were mild to moderate, but across both studies, nine OPV-vaccinated participants reported severe adverse events, including cases of headache (six participants), myalgia (two participants), and fatigue and paresthesia (one participant each), all of which resolved. Rates of solicited adverse events in OPV-vaccinated groups were lower after the second dose than after the first dose, reported by 18 (36%) of 50 after monovalent OPV2, 26 (53%) of 49 after novel OPV2-c1, and 21 (43%) of 49 participants after novel OPV2-c2 (Appendix 2). One case of abdominal pain and one of fatigue were described as severe.

In the IPV-vaccinated groups, solicited adverse event rates were higher, with 16 (94%) of 17 in the novel OPV2-c1 group, 13 (81%) of 16 in the novel OPV2-c2 group, and 15 (88%) of 17 in the placebo group (table 2). Four participants reported solicited severe adverse events (Appendix 1-2): three after first doses of either placebo (one with headache and fatigue and one with fatigue) or novel

OPV-c1 (one with severe headache) and one after the second dose of novel OPV-c2 (severe fatigue).

Most participants reported an unsolicited adverse event during the study (table 2), with severe unsolicited adverse events reported by 17 (17%) of 100 monovalent OPV2 recipients in the historical study, compared with 23 (23%) of 100 after novel OPV2-c1 and 11 (11%) of 100 after novel OPV2-c2 in the OPV-vaccinated groups. In IPV-vaccinated participants, four (24%) of 17 after novel OPV2-c1 and five (31%) of 16 after novel OPV2-c2 reported severe unsolicited adverse events, compared with nine (53%) of 17 placebo recipients. Relationship to vaccination was considered to be possible or probable for four severe unsolicited adverse events after monovalent OPV2, and for two after novel OPV2-c1 and four after novel OPV2-c2 in OPV-vaccinated and IPV-vaccinated groups, and for three participants who received placebo. These severe adverse events mainly consisted of gastrointestinal disorders—diarrhea, nausea, and abdominal pain occurring after the 7-day solicited adverse event reporting period.

There were no consistent abnormalities in clinical laboratory assessments related to receipt of either novel OPV2-c1 or novel OPV2-c2 in OPV-vaccinated or IPV-vaccinated participants (Appendix 3). Four clinically relevant grade 4 laboratory abnormalities were observed; three increases of creatine kinase—two in OPV-vaccinated participants at day 28 after the first dose of novel OPV2-c2 (which were linked to practicing sport) and one in an IPV-vaccinated participant 7 days after placebo—and a grade 4 potassium level increase observed at day 56 after two doses on monovalent OPV2 in the historical study, possibly due to hemolysis. Overall, frequencies of grade 3 or 4 outcomes were no greater after vaccination than at baseline (day 0). Furthermore, no grade 3 or 4 changes in alanine aminotransferase or aspartate aminotransferase, or the related parameters γ-glutamyl transferase, bilirubin, or albumin, were observed.

	Historical control study	Novel OPV2 study—OPV vaccinated		Novel OPV2 study—IPV vaccinated		
	Monovalent OPV2, groups 1 and 2	Novel OPV2-c1, groups 1 and 2	Novel OPV2-c2, groups 3 and 4	Novel OPV2-c1, group 5	Novel OPV2-c2, group 6	Placebo, group 7
Solicited systemic adverse events after dose 1						
N	100	100	100	17	16	17
Any	62 (62%)	71 (71%)	74 (74%)	16 (94%)	13 (81%)	15 (88%)
Mild	47 (47%)	45 (45%)	60 (60%)	15 (88%)	12 (75%)	14 (82%)
Moderate	11 (11%)	23 (23%)	12 (12%)	4 (24%)	7 (44%)	5 (29%)
Severe	4 (4%)	3 (3%)	2 (2%)	1 (6%)	0	2 (12%)
Solicited systemic adverse events after dose 2						
N*	50	49	49	17	15	16
Any	18 (36%)	26 (53%)	21 (43%)	11 (65%)	9 (60%)	12 (75%)
Mild	10 (20%)	18 (37%)	14 (29%)	8 (47%)	3 (20%)	7 (44%)
Moderate	7 (14%)	8 (16%)	6 (12%)	3 (18%)	5 (33%)	5 (31%)
Severe	1 (2%)	0	1 (2%)	0	1 (7%)	0
Unsolicited adverse events after dose 1						
N	100	100	100	17	16	17
Any	65 (65%)	68 (68%)	69 (69%)	13 (76%)	12 (75%)	16 (94%)
Probably or possibly related	28 (28%)	19 (19%)	19 (19%)	1 (6%)	2 (13%)	1 (6%)
Severe	13 (13%)	18 (18%)	7 (7%)	3 (18%)	1 (6%)	7 (41%)
Unsolicited adverse events after dose 2						
N*	50	49	49	17	15	16
Any	26 (52%)	33 (67%)	33 (67%)	11 (65%)	12 (80%)	14 (88%)
Probably or possibly related	7 (7%)	5 (10%)	7 (14%)	1 (6%)	1 (7%)	0
Severe	5 (10%)	6 (12%)	5 (10%)	1 (6%)	4 (27%)	5 (31%)

Table 2: Participants reporting solicited adverse events within 7 days of vaccination, and unsolicited adverse events within 28 days, of each vaccination in all vaccinated participants Data are n (%) unless otherwise stated *Only includes two-dose groups

Humoral Immunogenicity

At baseline, 286 (97%) of 296 OPV-vaccinated participants across both studies—(97 [97%] of 100 in the monovalent OPV2 study, 189 [96%] of 196 in the novel OPV2 study)—were already seropositive for poliovirus type 2 (table 3), precluding any meaningful comparisons between vaccine groups. Overall, immune responses to the novel OPV2 candidates as seroprotection rate, median titers, or geometric mean titers appeared to be similar to or greater than those observed after monovalent OPV2 in the historical control study. (Appendix 4) Monovalent OPV2 in 100 OPV-vaccinated participants increased the median log_2 titer from 7·83 (95% CI 7·34 to 8·50) to 9·67 (8·34 to 10·17) after one dose, and to 10·17 (8·50 to ≥10·50) after a second dose (table 3). The seroprotection rate was 97% (95% CI 92 to 99) both before and 28 days after one monovalent OPV2 dose, and 98% (89 to 100) after two doses. Seroconversion was observed in 18 (29%) of 62 evaluable participants after one dose of monovalent OPV2, and 11 (38%) of 29 after the second dose.

97 (99%) of 98 in the novel OPV2-c1 groups 1 and 2 and 92 (94%) of 98 in the novel OPV2-c2 groups 3 and 4 were seroprotected before vaccination, and the seroprotection rate was 100% at days 28 (after first dose) and 56 (after second dose) of either novel OPV2 candidate. Median log_2 titers increased in both groups after one dose, to the ULOQ (10·50) with novel OPV2-c1 and to 10·17 (95% CI 9·67 to ≥10·50) with novel OPV2-c2. A further increase to the ULOQ (10·50) was observed after a second novel OPV2-c2 dose. Measurable seroconversion was observed in 41 (75%) of 55 participants after one dose and 20 (74%) of 27 after two doses of novel OPV-c1. In novel OPV2-c2 vaccinees, seroconversion occurred in 24 (51%) of 47 participants after first dose and 15 (58%) of 26 after the second dose.

At baseline, 42 (89%) of 47 IPV-vaccinated participants were seropositive, increasing to 100% in both novel OPV2 groups after one dose and with median titers greater than the ULOQ. Seroconversion rates were 100% for novel OPV2-c1 and 92% for novel OPV2-c2 (table 3). Although no changes of seroprotection rate or median titer were observed in most placebo recipients, one initially seropositive placebo recipient seroconverted after the second injection.

	Historical control study	Novel OPV2 study—OPV vaccinated		Novel OPV2 study—IPV vaccinated		
	Monovalent OPV2, groups 1 and 2	Novel OPV2-c1, groups 1 and 2	Novel OPV2-c2, groups 3 and 4	Novel OPV2-c1, group 5	Novel OPV2-c2, group 6	Placebo, group 7
Poliovirus neutralising antibody titres						
Day 0, baseline						
N	100	98	98	15	16	16
Median (95% CI), log_2	7·83 (7·34–8·50)	8·34 (7·83–8·83)	8·83 (8·00–9·50)	7·83 (6·50–9·17)	7·17 (4·83–8·50)	6·50 (3·83–8·00)
Day 28, after dose 1						
N	100	96	98	17	16	16
Median (95% CI), log_2	9·67 (8·34–10·17)	10·50 (10·50–10·50)	10·17 (9·67–10·5)	10·50 (10·50–10·50)	10·50 (10·17–10·50)	5·67 (3·50–7·83)
Day 56, after dose 2*						
N	50	49	49	17	15	16
Median (95% CI), log_2	10·17 (8·50–10·50)	10·50 (10·50–10·50)	10·50 (9·50–10·50)	10·50 (10·50–10·50)	10·50 (9·17–10·5)	7·00 (4·50–8·50)
Seroprotection rates						
Day 0, baseline						
N	100	98	98	15	16	16
n (%; 95% CI)	97 (97%; 92–99)	97 (99%; 94–100)	92 (94%; 87–98)	14 (93%; 68–100)	15 (94%; 70–100)	13 (81%; 54–96)
Day 28, after dose 1						
N	100	96	98	17	16	16
n (%; 95% CI)	97 (97%; 92–99)	96 (100%; 96–100)	98 (100%; 96–100)	17 (100%; 81–100)	16 (100%; 79–100)	12 (75%; 48–93)
Day 56, after dose 2*						
N	50	49	49	17	15	16
n (%; 95% CI)	49 (98%; 89–100)	49 (100%; 93–100)	49 (100%; 93–100)	17 (100%; 81–100)	15 (100%; 78–100)	13 (81%; 54–96)
Seroconversion rates†						
Day 28, after dose 1						
N	62	55	47	10	12	12
n (%; 95% CI)	18 (29%; 18–42)	41 (75%; 61–83)	24 (51%; 36–66)	10 (100%; 69–100)	11 (92%; 62–100)	0 (0%; 0–26)
Day 56, after dose 2*						
N	29	27	26	10	11	12
n (%; 95% CI)	11 (38%; 21–58)	20 (74%; 54–89)	15 (58%; 37–77)	10 (100%; 69–100)	9 (82%; 48–98)	1 (8%; 0–38)

Log_2 titer values shown as 2·5 should be interpreted as 2·50 or less and the use of 10·50 should be interpreted as 10·50 or greater.
*Only includes two-dose groups. †Seroconversion was only measured in those whose initial antibody titer allowed observation of a four-fold increase.
Table 3: median poliovirus neutralizing antibody titers and seroprotection and seroconversion rates in the per protocol population

Shedding

Viral shedding rates after monovalent OPV2 or novel OPV2 candidates were lower in OPV-vaccinated than in IPV-vaccinated participants, illustrating the induction of intestinal immunity by OPV (table 4). PCR-positive stools were obtained from 15 (15%) of 100 monovalent OPV2 recipients after the first dose. In OPV-vaccinated recipients, 31 (31%) of 100 after the first dose of novel OPV-c1 and 20 (20%) of 100 after novel OPV-c2 had PCR-positive stools. Peak rates of shedding were observed at day 8 after monovalent OPV2, day 7 after novel OPV2-c1, and day 8 after novel OPV2-c2. All assessed participants had stopped shedding poliovirus by day 28 after receiving monovalent OPV2 (n=66) or novel OPV-c1 (n=64), and only one of 66 novel OPV-c2 recipients was still shedding at this timepoint.

In IPV-vaccinated participants, shedding was observed in 15 (88%) of 17 novel OPV2-c1 recipients and 14 (88%) of 16 novel OPV2-c2 recipients. Shedding was effectively finished by day 28 in IPV-vaccinated participants, when one of ten novel OPV-c1 recipients still had a PCR-positive stool, and none of 12 tested in the novel OPV-c2 group were positive. One placebo recipient was found to shed a very low titer of poliovirus in one stool sample collected on day 8. This participant did not display any serological indication of exposure and, although we have no explanation for this observation, it was potentially a case of contamination of the stool sample at the vaccination center or the laboratory.

After the second dose, numbers of vaccine recipients shedding and the magnitude of viral excretion ($CCID_{50}$) were lower than after the first dose, and similar across groups, including IPV-vaccinated groups (table 4), showing that one dose of either novel OPV2 candidate had induced intestinal immunity.

Genetic stability

In OPV-vaccinated participants, genetic stability was assessed in two exploratory endpoint stool samples obtained 5 days after monovalent OPV2, nine samples from days 4–10 after novel OPV2-c1, and five obtained 5–7 days after novel OPV2-c2 (Appendix 5). No variants were observed at the main sites for loss of attenuation, nucleotide 481 or VP1-aa143, or in any other regions of the genome in the monovalent OPV2 samples. In novel OPV2-c1 samples we did not observe any mutations in the relocated cis-acting replication element, including at nucleotides 123 and 179 or at domain IV nt.398 (nucleotide 459 in novel OPV2-c1). No variants consistent with reversion in domain V (nucleotides 468–535), the

main determinant for restoration of virulence after monovalent OPV2 administration in humans, or in the Rec1 or Hifi modification locations of the 3D polymerase were observed.

Reversion of an unprotected secondary attenuation site, VP1-aa143, was observed in one sample from day 7 but not in samples from days 8, 9, and 10. In novel OPV2-c2 samples, no mutations were observed in domain IV (U398C, equivalent to U459C in novel OPV2 candidate 1) or in domain V, nor any reversions of VP1- aa143 or the modified CpG sites in the P1 region. Of the eight evaluable samples from IPV-vaccinated novel OPV2-c1 recipients, no reverting variants were detected in domain V whereas two day-21 samples showed partial reversion at VP1–143 (Appendix 6). Variants were observed in cis-acting replication element 5 at positions 123/179. In five evaluable samples from IPV-vaccinated novel OPV2-c2 recipients, no reverting variants were observed in domain V but two samples (day 8 and day 9) showed partial reversion at VP1–143.

	Monovalent OPV2 control study	Novel OPV2 study—OPV vaccinated		Novel OPV2 study—IPV vaccinated		
	Monovalent OPV2, groups 1 and 2	Novel OPV2-c1, groups 1 and 2	Novel OPV2-c2, groups 3 and 4	Novel OPV2-c1, group 5	Novel OPV2-c2, group 6	Placebo, group 7
After dose 1						
N	100	100	100	17	16	17
PCR positive, n (%; 95% CI)	15 (15%; 9–24)	31 (31%; 22–41)	20 (20%; 13–29)	15 (88%; 64–99)	14 (88%; 62–98)	1 (6%; 0–29)
Participants with SIE, n (%)	58 (58%)	94 (94%)	89 (89%)	15 (88%)	15 (94%)	14 (82%)
Median SIE (95% CI)	0 (0–0)	0 (0–0)	0 (0–0)	1·10 (0·35–2·56)	0·92 (0–1·04)	0 (0–0)
Shedders*, n	11	21	12	12	9	0
Median SIE in shedders (95% CI)	0·94 (0·92–1·21)	1·07 (0·94–1·41)	0·98 (0·75–1·36)	1·27 (0·86–2·79)	1·03 (0·92–3·44)	NC
After dose 2						
N†	50	49	49	17	15	16
PCR positive, n (%; 95% CI)	3 (6%; 1–17)	9 (18%; 9–32)	2 (4%; 1–14)	6 (35%; 14–62)	1 (7%; 0–32)	0 (0%; 0–21)
Participants with SIE, n (%)	27 (54%)	42 (88%)	47 (96%)	13 (77%)	14 (93%)	12 (75%)
Median SIE (95% CI)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Shedders*, n	1	6	1	1	0	0
Median SIE in shedders (95% CI)	0·95 (NC)	1·09 (0·92–1·76)	0·92 (NC)	1·05 (NC)	NC	NC

CIs obtained via the percentile bootstrap method. All titers in stool samples with negative shedding results are set to zero; lower limit of quantitation (2·75 log10) is used as observed, where necessary. SIE was calculated as the arithmetic mean of $\log_{10}CCID_{50}$ per g from days 7, 14, 21, or 28 after dose 1, and days 35, 42, 49, and 56 after dose 2. Medians among shedders were calculated by excluding participants who were PCR negative for shedding at all the respective timepoints. NC=not calculated. SIE=shedding index estimate. *Shedders are participants with non-missing endpoint and with at least one positive result at one of the timepoints used for the respective endpoint. †Only includes two-dose groups.

Table 4: Poliovirus shedding in all vaccinated participants after dose 1 and in the per-protocol population after dose

5.5 Discussion

These two interlinked studies done 2 years apart— a prospective, historical control study using Sabin monovalent OPV2 and the later study with two novel OPV2 candidate vaccines—were designed to compare the novel candidates with monovalent OPV2 in terms of safety and immunogenicity, with exploratory assessments of viral shedding and enhanced genetic stability. We observed that all vaccines were safe and well tolerated, with no serious adverse events or withdrawals definitely related to vaccination. Most solicited systemic adverse events were reported as mild or moderate and transient, with similar reactogenicity profiles for all groups who received monovalent OPV2 or the two novel OPV2 candidates.

Observations of increased creatine phosphokinase and liver enzymes in some participants in the phase 1 study of these novel OPV2 candidates (130) led to inclusion of additional parameters in the protocol of the novel OPV2 study that had not been included in the monovalent OPV2 study. However, the original suspicion that this was due to excessive exercise by the affected participants living in containment appears to be confirmed, as grade 3 or 4 increases were rare and no consistent changes were observed in this larger novel OPV2 study.

Within the constraints of high baseline immunity, neither novel OPV2 candidate appeared to be inferior immunologically to the monovalent OPV2 vaccine. Although fewer previous vaccinations were registered for the novel OPV2 vaccination study, coverage with four vaccinations is high in Belgium and documented numbers were influenced by availability of vaccination cards. Both novel OPV2 candidates were also immunogenic in IPV-immunized adults, with 100% seroprotection rates after one dose, as previously shown in the phase 1 study. (130)

Both novel OPV2 candidates and monovalent OPV2 were shed in stools at a similar rate in OPV-vaccinated participants. Shedding was higher in IPV-vaccinated participants, which is expected because, unlike OPV, IPV induces little to no primary intestinal immunity. (197) Peak rates of shedding were observed within 10 days of vaccination and virtually all participants had stopped shedding within the 28-day follow-up period. For the novel OPV2 candidates, the sequencing results remain promising and consistent with the phase 1 study (130) with no reverting modifications of the genetically stabilized domain V detected in any samples from any cohorts, while Sabin-2 reversion in domain V is common at day 7 after vaccination and beyond. (185), (186) More detailed analysis of the genetic

variations together with ongoing analyses of the neurovirulence of the shed virus will be reported subsequently.

With increasing numbers of circulating vaccine-derived poliovirus outbreaks globally, the WHO–Global Polio Eradication Initiative strategy to interrupt transmission relies on the development of new vaccines with more genetically stable poliovirus strains like those described here. (192) With reports of outbreaks due to types 1 and 3, development of similar novel OPV candidates for types 1 and 3 has already been initiated and a phase 1 study with these new candidate vaccines is scheduled to start in early 2021 (NCT04529538).

The main limitation of this investigation was the necessity to do two separate studies. Global withdrawal of Sabin OPV2 in 2016 before novel OPV2 lots became available made direct contemporaneous comparison of monovalent OPV2 and novel OPV2 candidates impossible, necessitating the historical study for monovalent OPV2 baseline data. To enable comparisons between studies, both protocols were designed to be as similar as possible using volunteers from the same population in Belgium. Although essentially open label for safety because monovalent OPV2 was studied first, immunogenicity analyses were done simultaneously in a masked manner in the same laboratory to minimize potential bias. We assessed novel OPV2 shedding in participants with different background polio vaccination histories because exclusively IPV-vaccinated participants have low or no intestinal immunity, unlike OPV vaccinees. As well as circulating reverted viruses, other rare consequences of OPV use are cases of vaccine-associated paralytic poliomyelitis, occurring in vaccinees or their contacts at a rate of about four cases per million births. (103) Clinical studies, including this one, are too small to detect such a phenomenon so it is speculative whether the improved genetic stability of novel OPV2 will have an effect on rates of vaccine-associated paralytic poliomyelitis. Another limitation is that this was done in fully vaccinated adults, whereas the most likely recipients of novel OPV2 will be children and infants, who might be unvaccinated or incompletely immunized. For that reason, following initial safety assessments by the data and safety monitoring board of the present adult novel OPV2 study, a study of both novel OPV2 candidates (with a historical monovalent OPV2 control study) was done in Panama in children and bivalent OPV-immunized or IPV-immunized infants to simulate the situation with minimal intestinal immunity against type 2 virus in the post-OPV2 withdrawal era. (195)

In our studies, both novel OPV2 candidates appeared to be as safe, well tolerated, and immunogenic as monovalent OPV2, with similar profiles of viral shedding.

Further study is underway to confirm the objective of mitigating reacquisition of neurovirulence by these novel OPV2 vaccines, but the data thus far suggests that the goal of developing more genetically stable, attenuated OPV2s with no effect on the immunogenicity has been achieved.

Acknowledgments

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Supplementary appendices

Appendix 1. Summary of Solicited Adverse Events (AEs) after the first dose – TVP.

		Historical control study	nOPV2 study OPV-vaccinated		nOPV2 study IPV-vaccinated		
Category	**Severity***	**Groups 1 & 2**	**Groups 1 & 2**	**Groups 3 & 4**	**Group 5**	**Group 6**	**Group 7**
	Vaccine	mOPV2	nOPV2-c1	nOPV2-c2	nOPV2-c1	nOPV2-c2	Placebo
				n subjects (%)			
Solicited AEs		N = 100	N = 100	N = 100	N = 17	N = 16	N = 17
	Any	62 (62)	71 (71)	74 (74)	16 (94)	13 (81)	15 (88)
	Severe	*4 (4)*	*3 (3)*	*2 (2)*	*1 (6)*	*0*	*2 (12)*
Abdominal pain	Any	19 (19)	21 (21)	20 (20)	8 (47)	3 (19)	8 (47)
	Severe	*0*	*0 (0)*	*0*	*0*	*0*	*0*
Anaesthesia	Any	1 (1)	2 (2)	0	-	-	-
	Severe	*0*	*0*	*0*			
Arthralgia	Any	4 (4)	9 (9)	4 (4)	2 (12)	0	0
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Diarrhoea	Any	20 (20)	22 (22)	24 (24)	9 (53)	3 (19)	5 (29)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Fatigue	Any	35 (35)	37 (37)	35 (35)	10 (59)	9 (56)	9 (53)
	Severe	*0*	*0*	*1 (0)*	*0*	*0*	*2 (12)*
Fever	Any	4 (4)	3 (3)	2 (2)	1 (6)	3 (19)	1 (6)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Headache	Any	28 (28)	39 (39)	48 (48)	12 (71)	9 (56)	9 (53)
	Severe	*3 (3)*	*2 (2)*	*1 (0)*	*1 (6)*	*0*	*1 (6)*
Myalgia	Any	12 (12)	16 (16)	12 (12)	3 (18)	3 (19)	2 (12)
	Severe	*1 (1)*	*1 (1)*	*0*	*0*	*0*	*0*
Nausea	Any	8 (8)	18 (18)	9 (9)	4 (24)	3 (19)	5 (29)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Paresthesia	Any	6 (6)	5 (5)	3 (3)	3 (18)	3 (19)	1 (6)
	Severe	*1 (1)*	*0*	*0*	*0*	*0*	*0*
Vomiting	Any	2 (2)	0	2 (2)	0	2 (13)	0
	Severe	*0*	*0*	*0*	*0*	*0*	*0*

Appendix 2. Summary of Solicited Adverse Events (AEs) after the second dose – TVP.

		Historical control study	nOPV2 study OPV-vaccinated		nOPV2 study IPV-vaccinated		
Category	**Severity***	**Group 2**	**Group 2**	**Group 4**	**Group 6**	**Group 5**	**Group 7**
	Vaccine	mOPV2	nOPV2-c1	nOPV2-c2	nOPV2-c2	nOPV2-c1	Placebo
				n subjects (%)			
Solicited AEs		N = 50	N = 49	N = 49	N = 17	N = 15	N = 16
	Any	18 (38)	26 (53)	21 (43)	11 (65)	9 (60)	12 (75)
	Severe	*1 (2)*	*0*	*1 (2)*	*0*	*1 (7)*	*0*
Abdominal pain	Any	3 (6)	8 (16)	2 (4)	5 (29)	4 (27)	4 (25)
	Severe	*0*	*0 (0)*	*1 (1)*	*0*	*0*	*0*
Anaesthesia	Any	1 (2)	0	0	-	-	-
	Severe	*0*	*0*	*0*			
Arthralgia	Any	1 (2)	3 (6)	2 (4)	0	1 (7)	0
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Diarrhoea	Any	6 (12)	9 (18)	4 (8)	7 (41)	2 (13)	3 (19)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Fatigue	Any	9 (18)	14 (29)	9 (18)	6 (35)	7 (47)	10 (63)
	Severe	*1 (2)*	*0*	*0*	*0*	*1(7)*	*0*
Fever	Any	0	2 (4)	1 (2)	0	1 (7)	0
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Headache	Any	8 (16)	15 (31)	13 (27)	7 (41)	5 (33)	6 (38)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Myalgia	Any	4 (8)	2 (4)	1 (2)	0	4 (27)	4 (25)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Nausea	Any	2 (4)	4 (8)	1 (2)	1 (6)	1 (7)	4 (25)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Paresthesia	Any	2 (4)	0	0	1 (6)	1 (7)	1 (6)
	Severe	*0*	*0*	*0*	*0*	*0*	*0*
Vomiting	Any	1 (2)	0	0	0	0	0
	Severe	*0*	*0*	*0*	*0*	*0*	*0*

Appendix 3. Clinically relevant (Grade 3 or 4) laboratory abnormalities up to 28 days after vaccination in TVP of the two studies.

		Historical control study		nOPV2 study						
				OPV-vaccinated				IPV-vaccinated		
	Group	Group 1	Group 2	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
	Vaccine	mOPV2	mOPV2	nOPV2-c1		nOPV2-c2		nOPV2-c1	nOPV2-c2	Placebo
Laboratory assessment	N =	100	50	100	50	100	50	17	16	17
Any clinically relevant change		21 (21)	18 (36)	28 (28)	18 (36)	30 (30)	15 (30)	4 (24)	6 (38)	9 (53)
Any Grade 3 or 4 anomaly										
Albumin		nd	nd	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
APTT		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ALT		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AST		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CPK		nd	nd	0 (0)	0 (0)	2 (2)	0 (0)	2 (12)	0 (0)	3 (18)
Creatinine		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fibrinogen		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GGT		nd	nd	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Glucose	n (%)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Haemoglobin		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lymphocytes		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Neutrophils		0 (0)	1 (2)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Phosphate		0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	3 (6)	0 (0)	0 (0)	0 (0)
Platelets		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Potassium		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (6)	1 (6)	1 (6)
Total bilirubin		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
White blood cell count		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

nd = not determined. APTT = activated partial thromboplastin time; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phosphokinase; GGT = gamma-glutamyl transferase.

Appendix 4 Primary immunogenicity objective: seroprotection rate 28 days after one dose of mOPV2 or nOPV2 candidates in the PPP

		Historical control study	nOPV2 study - OPV vaccinated		ΔSPR, % (95% CI)	
		mOPV2	**nOPV2-c1**	**nOPV2-c2**	**nOPV2-c1 – mOPV2**	**nOPV2-c2– mOPV2**
		Groups 1 and 2	*Groups 1 and 2*	*Groups 3 and 4*		
Seroprotection rates[a]	**(n/N)** % (95% CI)					
Day 0		**97 / 100** 97 (92–99)	**97 / 98** 99 (94–100)	**92 / 98** 94 (87–98)	2·0 (-2·9–7·6)	-3·1 (-10·1–3·2)
Day 28		**98 / 100** 98 (93–100)	**96 / 96** 100 (96–100)	**98 / 98** 100 (96–100)	2·0 (-1·9–7·0)	2·0 (-1·8–7·0)
Median neutralising titres[b]	(Q1–Q3)					
Day 0		**228** (144–362)	**324** (228–455)	**455** (256–724)		
Day 28		**815** (324–1152)	**≥ 1448** (≥1448– ≥1448)	**1152** (815– ≥1448)		

a: Seroprotection rate based on type 2 neutralising titres, calculated as n/N x 100.

b: Interquartile statistics calculated on log_2-transformed type 2 neutralising titres back transformed for this table.

Appendix 5. Frequency of genetic variants at known attenuation sites and modified regions of the candidates.

Genome unit (nucleotides)		cre5 (121-181)	Dom IV 459	S15 Dom V (529-596)	VP1-143 (2969-2971)	2C cre KO (4508-4560)
		nOPV2-c1 in OPV-vaccinated adults				
Variant observed[2]				C547U	A2969G (I143V)	U4540C
EES Day[1]	D4 (SI)	n.d.	n.d.	n.d.	n.d.	n.d.
	D6 (SSI)	n.d.	n.d.	n.d.	n.d.	0, 0, 0.01
	D6 (SSI)	n.d.	n.d.	n.d.	n.d.	n.d.
	D7 (SSI)	n.d.	n.d.	n.d.	0·56, 0·52, 0·97	n.d.
	D7(SSI)	n.d.	n.d.	n.d.	n.d.	0·02, 0·03, 0
	D8 (SSI)	n.d.	n.d.	n.d.	n.d.	n.d.
	D9 (SS)	n.d.	n.d.	n.d.	n.d.	n.d.
	D10 (S)	n.d.	n.d.	n.d.	n.d.	n.d.
	D10 (SS)	n.d.	n.d.	0·21, 0	n.d.	n.d.
		nOPV2-c2 in OPV-vaccinated adults				
		No variations or mutations were observed in EES from Day 5 to Day 7				

Appendix 6.	Frequency of genetic variants at known attenuation sites and modified regions of the candidates in IPV-vaccinated adults						
	nOPV2-c1 in IPV-vaccinated adults						
Variant observed[2]	123C/G19A	U459C		VP1 A2969G (I143V)	VP1 U2970C (I143T)	VP1 U2970G (I143S)	C4519U
EES Day[1] D3 (SSI)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0, 0.03, 0.02
D6 (SI)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D8 (I)	n.d.	n.d.	n.d.	0.04	n.d.	n.d.	n.d.
D8 (SSI)	1·00, 0·97, 1·00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D9 (SI)	0, 0·03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D9 (SSI)	0·31, 0·05, 0·53	0·05, 0, 0·10	n.d.	n.d.	n.d.	n.d.	n.d.
D21 (SSI)	0·96, 0·96, 0·55	0, 0·01, 0	n.d.	0·53, 0·52, 0·56	0·03, 0·04, 0·07	0·09, 0·12, 0·03	n.d.
D21 (SI)	0·07, 0·38	0·93, 0·62	n.d.	n.d.	0·10, 0·38	n.d.	n.d.
	nOPV2-c2 in IPV-vaccinated adults						
Variant observed[2]				J2909A I143N)	U2909C (I143T)		
EES Day[1] D4 (SSI)		n.d.	n.d.	n.d.	n.d.		
D8 (SI)		n.d.	n.d.	0·08, 0·01	0, 0·15		
D9 (SS)		n.d.	n.d.	n.d.	n.d.		
D9 (SSI)		n.d.	n.d.	0·16, 0·25, 0·02	0, 0·02, 0		
D10 (SSI)		n.d.	n.d.	n.d.	n.d.		

Chapter 6:
Safety, tolerability, and immunogenicity of inactivated poliovirus vaccine with or without E.coli double mutant heat-labile toxin (dmLT) adjuvant in healthy adults; a phase 1 randomized study

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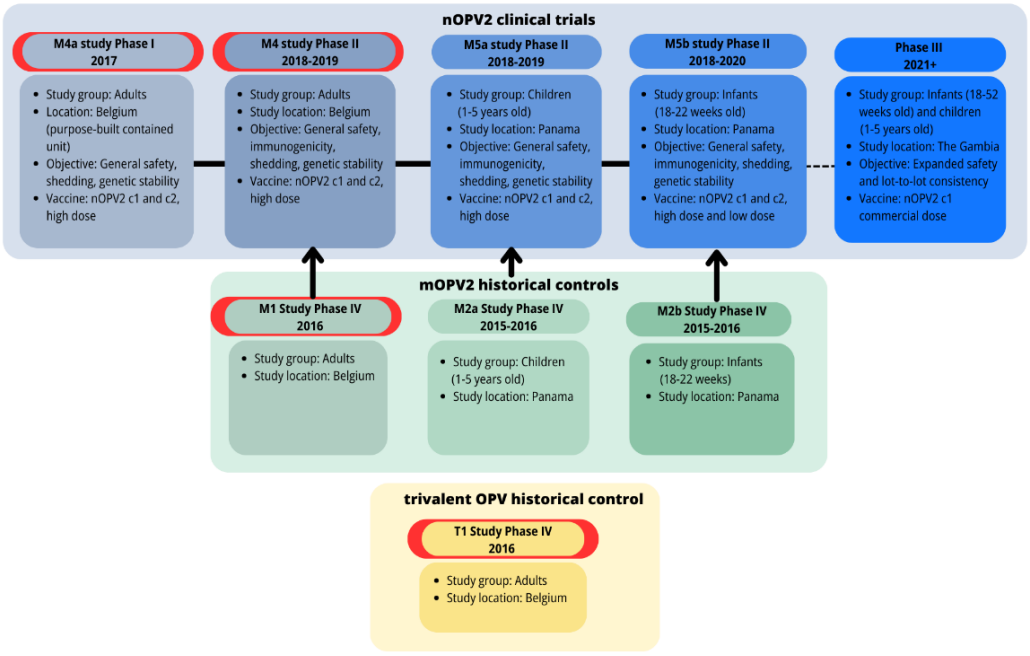
nOPV2 clinical trials
M4a study Phase I
2017
Study group: Adults
Location: Belgium (purpose-built contained unit)
Objective: General safety, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M4 study Phase II
2018-2019
Study group: Adults
Study location: Belgium
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5a study Phase II
2018-2019
Study group: Children (1-5 years old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose
M5b study Phase II
2018-2020
Study group: Infants (18-22 weeks old)
Study location: Panama
Objective: General safety, immunogenicity, shedding, genetic stability
Vaccine: nOPV2 c1 and c2, high dose and low dose
Phase III
2021+
Study group: Infants (18-52 weeks old) and children (1-5 years old)
Study location: The Gambia
Objective: Expanded safety and lot-to-lot consistency
Vaccine: nOPV2 c1 commercial dose
mOPV2 historical controls
M1 Study Phase IV
2016
Study group: Adults
Study location: Belgium
M2a Study Phase IV
2015-2016
Study group: Children (1-5 years old)
Study location: Panama
M2b Study Phase IV
2015-2016
Study group: Infants (18-22 weeks)
Study location: Panama
trivalent OPV historical control
T1 Study Phase IV
2016
Study group: Adults
Study location: Belgium
IPV+dmLT
CVIA065 Study Phase I
2020
Study group: Adults
Study location: Belgium
Study design: IPV+dmLT vs. IPV, challenge bOPV

6.1 Abstract

Background

Inactivated trivalent poliovirus vaccine (IPV) induces humoral immunity, which protects against paralytic poliomyelitis but does not induce sufficient mucosal immunity to block intestinal infection. We assessed the intestinal immunity in healthy adults in Belgium conferred by a co-formulation of IPV with the mucosal adjuvant double mutant Labile Toxin (dmLT) derived from Escherichia coli.

Methods

Healthy fully IPV-vaccinated 18–45-year-olds were randomly allocated to three groups: on Day 1 two groups received one full dose of IPV (n = 30) or IPV + dmLT (n = 30) in a blinded manner, and the third received an open-label dose of bivalent live oral polio vaccine (bOPV types 1 and 3, n = 20). All groups received a challenge dose of bOPV on Day 29. Participants reported solicited and unsolicited adverse events (AE) using study diaries. Mucosal immune responses were measured by fecal neutralization and IgA on Days 29 and 43, with fecal shedding of challenge viruses measured for 28 days. Humoral responses were measured by serum neutralizing antibody (nAb).

Results

Solicited and unsolicited AEs were mainly mild-to-moderate and transient in all groups, with no meaningful differences in rates between groups. Fecal shedding of challenge viruses in both IPV groups exceeded that of the bOPV group but was not different between IPV and IPV + dmLT groups. High serum nAb responses were observed in both IPV groups, alongside modest levels of fecal neutralization and IgA.

Conclusions

Addition of dmLT to IPV administered intramuscularly neither affected humoral nor intestinal immunity nor decreased fecal virus shedding following bOPV challenge. The tolerability of the dose of dmLT used in this study may allow higher doses to be investigated for impact on mucosal immunity.

Registered on ClinicalTrials.gov - NCT04232943.

6.2 Introduction

Following the eradication of smallpox, the extensive use of vaccines has nearly achieved the global eradication of a second human infectious disease, paralytic poliomyelitis. Wild polioviruses (WPVs) type 2 and 3 have been officially declared eradicated globally (198), with WPV type 1 endemic only to Afghanistan and Pakistan (199). Most cases of paralytic poliomyelitis are now caused by rare cases of vaccine-associated paralytic poliomyelitis (VAPP) or, more frequently, due to circulating vaccine-derived polioviruses (cVDPV) reacquiring neurovirulence during passage through the intestines of vaccinees and their contacts in under-immunized communities (3). As most cVDPV cases are due to Sabin type 2 virus circulating in environments conducive to transmission, the Global Polio Eradication Initiative (GPEI) coordinated a global effort to cease routine use of live Sabin type 2 vaccine following global eradication of WPV2. This step in the eradication strategy involved replacement of Sabin trivalent live oral poliovirus vaccines (tOPV) with a combination immunization schedule of bivalent live vaccine (bOPV; types 1 and 3) and at least one dose of inactivated poliovirus vaccine (IPV). The removal of Sabin type 2 from routine use was completed globally in May 2016 (200). However, following the tOPV to bOPV switch, population-level immunity to type 2 decreased, leaving communities susceptible to new cVDPV2 outbreaks, resulting from ongoing pre-cessation chains of transmission and outbreak response immunizations with monovalent OPV2 (122). cVDPV2 outbreaks remain a major challenge to eradication with 1,081 and 682 cases of cVDPV2 confirmed from 24 and 22 countries in the recent 2020 and 2021 peaks, respectively. Several strategies are being used to address cVDPV2, including the recent introduction of novel OPV2 (nOPV2) under a WHO Emergency Use Listing for outbreak response. Additionally, administration of IPV in routine immunization is critical for the successful replacement of tOPV with bOPV. Although IPV protects the recipient from symptomatic disease through humoral immunity, it does not stimulate the robust mucosal immunity necessary at intestinal sites to arrest shedding (76). In the period since OPV2 cessation, however, a global IPV shortage has limited and delayed supplies in low- and middle-income countries (201). One strategy to address supply shortages and limited intestinal immunity induced by IPV is the development of adjuvanted inactivated vaccines enabling use of fractional antigen quantities (dose sparing) while improving intestinal immunity (44). Fractional dosing has been investigated in clinical studies using an established vaccine adjuvant, aluminum hydroxide ((202), (75)), but no mucosal activity was observed. More recently, double mutant Labile Toxin (dmLT), a protein toxoid derived from wild-type Enterotoxigenic Escherichia coli (ETEC) labile toxin (LT), has

been shown to have mucosal adjuvant effects in preclinical (203) - (205)) and early phase clinical studies ((76), (206), (207)). This phase 1 clinical trial investigated the safety of dmLT-adjuvanted IPV (IPV + dmLT) in healthy adults, as well as the humoral and intestinal immune responses to a full dose of IPV with or without dmLT relative to bOPV vaccination, including the impact on fecal viral shedding following a bOPV challenge.

6.3 Methods

This was a single-center phase 1 randomized study to compare the safety, tolerability, and immunogenicity of a single dose of IPV with or without dmLT in healthy adults. The study was conducted at the Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Belgium following approval of the Antwerp University Hospital Ethics Committee. It was performed in accordance with the Declaration of Helsinki and the ICH GCP and guidelines of the Federal Agency for Medicines and Health Products (FAMHP), Belgium. The primary objectives were to evaluate and compare the safety of IPV + dmLT versus IPV alone and to compare the rate of fecal viral shedding throughout 28 days after a bOPV challenge dose at Day 29 post vaccination. The key secondary objectives were the evaluation and comparison of intestinal immune responses and the extent of fecal viral shedding following bOPV challenge. Eligible participants were healthy 18–45-year-old males or females with a history of complete IPV vaccination (at least three doses of IPV) who were available for the duration of the study. Main exclusion criteria were receipt of OPV at any time or IPV vaccination within the previous 5 years, having routine contact with children incompletely vaccinated against polio, i.e., those under 6 months of age, or any known conditions that might interfere with immune responses. IPV or IPV + dmLT were administered intramuscularly on Day 1 to groups of 30 participants each, in a blinded manner. A positive control group (unblinded) was included, composed of 20 adults who received bOPV. A challenge dose of bOPV was given to all participants on Day 29. Eight participants per day (the maximum capacity of the study site) were randomized in a 3:3:2 ratio to one of the three treatment groups, IPV, IPV + dmLT, and bOPV, using a permuted-block design generated and maintained by the Statistical Data Coordinating Center (SDCC) at The Emmes Company, LLC (Emmes). Subsets of 10 participants per group, one per group per day, were randomly selected for assessment of antibody secreting cells (ASC) $\alpha_4\beta_7$ integrin gut homing marker.

Vaccines

The licensed trivalent Salk IPV used was IMOVAX®Polio (Sanofi Pasteur, France); each 0.5 mL dose contains 40 D-antigen units of type 1 (Mahoney strain), 8 DU type 2 (MEF-1 strain) and 32 DU type 3 (Saukett strain) polioviruses produced in VERO cells. The bOPV vaccine was Bivalent Polio Sabin™ One and Three produced by GSK (Rixensart, Belgium); each 0.1 mL oral dose contained not less than $10^{6.0}$ $CCID_{50}$ of type 1 and $10^{5.8}$ $CCID_{50}$ of type 3 polioviruses. The adjuvant dmLT (lot 001-08-16), also known as LT (R192G/L211A), was manufactured by IDT Biologika (Dessau- Rosslau, Germany). The IPV + dmLT formulation was prepared under aseptic conditions by an unblinded qualified research pharmacist at the clinical site. On the day of administration, a single vial of lyophilized dmLT was rehydrated with 0.5 mL of Sterile Water for Injection to produce a 1 mg/mL stock solution. Serial dilutions of dmLT were performed with pooled IMOVAX® Polio vaccine, by combining the contents of single-dose syringes (0.5 mL) in a sealed, sterile glass vial. Diluted dmLT was mixed with pooled IMOVAX_ Polio vaccine in a quantity sufficient to vaccinate all scheduled participants on the day of preparation. The final IPV + dmLT formulation contained 0.5 µg of dmLT per 0.5 mL dose.

Endpoints

The primary safety endpoints were the frequencies and incidences of serious adverse events (SAEs) throughout the study, unsolicited adverse events (AE), especially those graded as severe during the 28 days following study vaccination and solicited reactogenicity (local and systemic reactions) during the 7 days following vaccination and challenge. The primary efficacy endpoint, the proportion of participants without detectable fecal shedding of bOPV vaccine viruses in the IPV + dmLT and IPV arms, 7 days after challenge, was chosen as a direct measure of the intestinal immunity conferred by vaccination.

Secondary endpoints included the proportions of participants with type-specific poliovirus fecal IgA and neutralizing responses 28 days after vaccination and 14 days after challenge; the serum neutralizing antibody (nAb) seroconversion rate and NAb levels 28 days after vaccination with IPV + dmLT or IPV; the area under the curve (AUC) of fecal shedding measured by $CCID_{50}$ per gram of stool in the 28 days following challenge; and the proportions of participants developing type-specific poliovirus antibody secreting cell (ASC) responses at any time point following both vaccination and challenge.

Safety

SAEs evaluated throughout the study were any events resulting in death or were life-threatening, required hospitalization, and/or resulted in a persistent incapacity that disrupted normal life. General health and clinical laboratory assessments—complete blood counts (CBC) with differential for white blood cell (WBC), hemoglobin, absolute neutrophil count (ANC), platelets, creatinine, albumin, total bilirubin, alanine transaminase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), and antibodies against HBsAg, HIV and HCV were performed during screening before vaccination, and on Day 8 post-vaccination for serum chemistry and hematology. Solicited local injection site reactions were pain, erythema/redness, swelling, induration and hyperpigmentation for the two IPV arms, and solicited systemic adverse events were chills, fatigue, headache, muscle aches/myalgia, joint ache/ arthralgia, rash, nausea, vomiting, diarrhea, and fever defined as an oral temperature > 38.0 °C for all participants. Unsolicited adverse events were reported from Day 1 to Day 57. Solicited and unsolicited AEs were graded for severity on a scale of 0 (normal), 1 (mild), 2 (moderate), and 3 (severe).

Biological samples

Blood and stool samples were temporarily stored at the Centre for the Evaluation of Vaccination (CEV) or in a central biorepository at the Laboratory of Experimental Hematology (LEH), Vaccine and Infectious Disease Institute, University of Antwerp after processing until they were shipped to the appropriate laboratories for analyses. Fresh whole blood was shipped to the Institute for Medical Immunology, Université Libre de Bruxelles (ULB, Brussels, Belgium) for determination of polio type-specific IgA/IgG ASC and ASC positive for the $\alpha 4\beta_7$ integrin gut homing marker in a subset of samples (208). Serum samples for poliovirus nAb and stool samples to assess for presence and quantity of shed virus were processed and temporarily stored frozen at the CEV for transportation on dry ice to the laboratory at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA (130), (175). Stool samples for fecal IgA and fecal nAb were processed and temporarily stored frozen at the CEV for transportation on dry ice to Dartmouth College (Geisel School of Medicine, Lebanon, NH, USA).

Viral shedding

Stool samples were obtained on Day 29 post-vaccination, before bOPV challenge, and then on Days 33, 36, 39, 43, 46, 50 and 57 (equivalent to Days 4, 7, 10, 14, 17,

21 and 28 post-bOPV challenge). Type-specific fecal viral shedding was assessed using reverse transcription polymerase chain reaction (RT-PCR) to detect viral RNA, and then total infectious virus, measured as 50% cell culture infective dose ($CCID_{50}$), was titered in those positive for viral RNA by standardized methods at the CDC as previously described (185).

Immunogenicity endpoints

Titers of type-specific nAb on Days 1 and 29 post-vaccination (serum samples collected prior to vaccination and challenge, respectively) measured by standard micro-neutralization assay methods (130), (175) were expressed as the reciprocal of the highest serum dilution with no cytopathic effect (CPE). The seroconversion rate is defined as the proportion of participants demonstrating a minimum four-fold increase in type-specific poliovirus serum nAb titers between Days 1 and 29, or a Day 29 reciprocal neutralizing titer 2:8 if seronegative at baseline. Also calculated were geometric mean titers (GMT), geometric mean-fold rises (GMFR) between Days 1 and 29, and seropositivity (seroprotection) rates (proportions of each group with a titer ≥ 8) on Days 1 and 29.

Intestinal immunogenicity was measured as poliovirus fecal neutralization and fecal IgA in samples obtained at screening before vaccination, and then on Days 8, 29 (prior to challenge), 36, 43, 50, and 57 using standardized methods. Fecal neutralizing activity was measured by limiting dilution inhibition of luciferase-expressing wild-type-derived polio pseudoviruses and expressed as the titer needed to achieve 60% neutralization (titers >2 were considered detectable) (209). Total and polio-type specific concentrations of fecal IgA were measured in a Luminex assay using monovalent IPV covalently conjugated to fluorescent coated beads (210). The assay was developed using the Salk poliovirus strains from IPV vaccine, but for this study the assay was also run using the Sabin strains from IPV. Results are expressed as group proportions of participants who developed type-specific poliovirus fecal neutralization responses (minimum 4-fold increase from baseline) or fecal IgA and as GMTs and GMFR between baseline (Day 1, pre-vaccination) and post-baseline measurements on 29 days (pre-challenge) and 43 days (14 days after bOPV challenge).

Responses of type-specific poliovirus antibody-secreting cells (ASC) measured by a standard method (208) were defined as achieving ≥ 8 ASC/10^6 PBMC at any time point following both study vaccination and bOPV challenge. Type-specific circulating IgG- and IgA-secreting $\alpha_4ß_7$ ASC homing markers were measured ex vivo

by ELISPOT in randomly selected subsets of 10 samples per group. Briefly, after PBMC isolation, B cells were enriched by using the EasySepTM Human B Cell Enrichment Kit from Stemcell. After antibody staining and gating, a pattern of three populations of cells were sorted by flow cytometry and analyzed by ELISPOT: $\alpha_4\beta_7$-, $\alpha_4\beta_7$ dim and $\alpha_4\beta_7$ bright, with the two latter populations considered positive. The GMT and frequency of type-specific poliovirus ASCs were calculated before and after study vaccination, as well as the GMFR between baseline and post-baseline measurements.

Statistics

With 30 participants per IPV group, this study had an 80% probability of detecting at least one AE that occurs at a rate of 5.3% or higher. With 27 evaluable participants per IPV arm, this study was designed to provide at least 96% power to detect 2:60% reduction in shedding rate 8 days post-challenge in the IPV + dmLT group assuming the shedding rate in the IPV alone group was at least 80%. All adverse events were summarized for the total vaccinated population, according to treatment received. All participant-level percentages were supplemented with two-sided 95% confidence intervals (CIs) computed via the Clopper-Pearson method.

The primary viral shedding endpoint was assessed in the per protocol population. The proportion of participants with stool positive for poliovirus was summarized by time point and group including corresponding 95% CIs. Proportions shedding in IPV groups were compared for each serotype and overall via one minus the relative risk and accompanied by a 95% CI computed using the Farrington and Manning method (211). The type-specific time to cessation of shedding was analyzed by Kaplan-Meier methods, including right-censoring where appropriate. Quartiles of time to cessation of shedding and the shedding cessation rate at each post-challenge day were estimated along with corresponding 95% CIs, using the Greenwood method (212). Cessation of viral shedding was defined as the day of the first PCR-negative stool for challenge virus after which the next two consecutive stool samples were also PCR-negative. Additionally, viral shedding ($\log_{10}$ $CCID_{50}$/g, not type-specific) was summarized descriptively as a continuous variable with LLOQ (2.75 $\log_{10}$) and ULOQ (8.25 $\log_{10}$) used as the observed value whenever these limits were met and 0 for PCR- negative samples. A viral shedding index estimate was calculated using the arithmetic mean of the $\log_{10}$ $CCID_{50}$/g samples collected on Days 36, 43, 50, and 57 and supplemented with the

difference in medians (IPV + dmLT minus IPV alone) with corresponding two- sided 95% CIs computed using the percentile bootstrap method. The ratio of the shedding index was also calculated as the difference on the log scale, with accompanying 90% CI computed using the same bootstrap method, then back-transformed using the antilog. Here, the 90% CI is used to enable a one-sided level 0.05 non-inferiority test.

Immunogenicity assessments conducted in the per protocol population were summarized descriptively as GMTs, GMFRs, and seroresponse or seroconversion rates and compared between groups using baseline-adjusted GMT ratios. Geometric means were analyzed on the log scale, adjusted for baseline measures, and using survival regression analysis to accommodate censoring at LLOQ or ULOQ with antilog transformations of model-based estimates and corresponding 95% CIs.

6.4 Results

This study was initiated on January 22, 2020, but enrolment was halted on March 16, 2020 due to the COVID-19 pandemic and specific COVID-19 prevention measures instituted in Belgium at that time; the study resumed on July 27, 2020, with completion on February 1, 2021. A total of 152 volunteers were enrolled, of whom 87 were randomized to one of three groups to receive one dose of either IPV alone (n = 32), IPV + dmLT (n = 33) or bOPV (n = 22). As shown in Fig. 1, 80 participants received a study vaccine; 60 received IPV with or without dmLT and 20 received bOPV. The numbers of participants eligible for the per protocol immunogenicity and shedding analyses were 77 (96%) and 76 (95%), respectively. Two participants voluntarily withdrew from the study for reasons unassociated with the study, with three excluded after dose verification indicated a reduced dose of dmLT had been administered (Fig. 1). The demographics of participants who received study vaccines were comparable across the three groups (Table 1) .

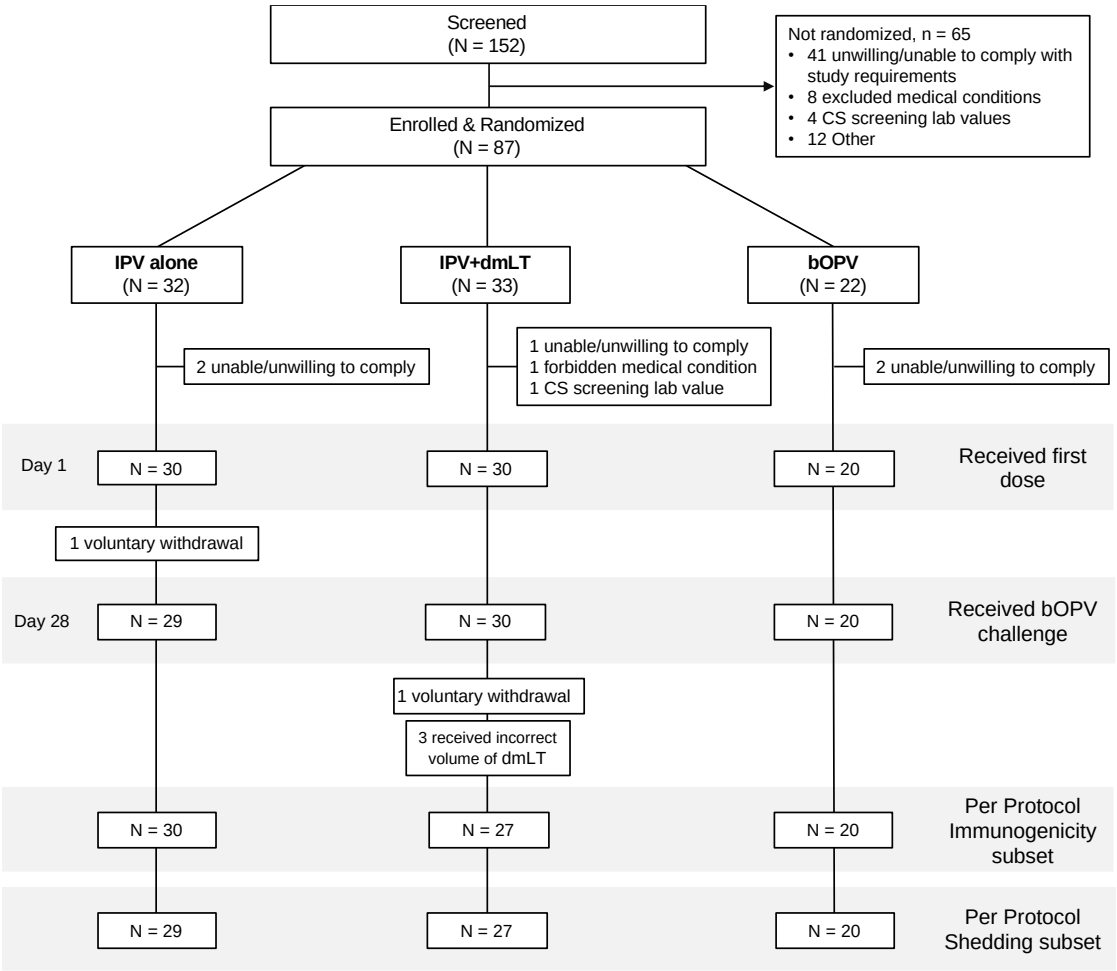

Fig.1 Study Flow chart

Table 1: Demographics of the total vaccinated study population.

	IPV (N = 30)	**IPV+dmLT (N = 30)**	**bOPV (N = 20)**
Sex, n (%)			
Male	18 (60.0)	17 (56.7)	13 (65.0)
Female	12 (40.0)	13 (43.3)	7 (35.0)
Age, years			
Mean (SD)	18.9 (1.61)	18.8 (1.35)	20.1 (4.18)
Minimum, maximum	18–27	18–25	18–33
Ethnicity, n (%)			
Hispanic or Latino	-	-	1 (5.0)
Not Hispanic or Latino	30 (100)	29 (96.7)	19 (95.0)
Unknown	-	1 (3.3)	-
Race n, (%)			
Black or African American	2 (6.7)	-	1 (5.0)
Native Hawaiian or Other Pacific Islander	-	-	1 (5.0)
White	28 (93.3)	30 (100)	18 (90.0)
BMI, (kg/m^2)			
Mean (SD)	22.93 (3.71)	22.33 (3.10)	22.57 (2.97)
Minimum, maximum	17.6–34.0	17.0–27.6	18.5–27.6

Safety and reactogenicity

Overall, study vaccinations were well tolerated with acceptable reactogenicity; there were no deaths, serious AEs or study withdrawals due to adverse events. Two reported immediate reactions within 30 min of vaccination were mild cases of headache in the IPV group and nausea in the IPV + dmLT group. On the day of vaccination, 19 (63%) of 30 IPV recipients and 24 (80%) of 30 IPV + dmLT recipients reported a local reaction, all graded as mild or moderate in severity. The majority of these reactions consisted of mild pain at the injection site with only two cases of induration (one in each group), a single case of swelling (IPV group) and single cases of erythema and hyperpigmentation (both in the IPV + dmLT group). Reports of local reactions declined at similar rates in both groups over the subsequent three days (Fig. 2).

Frequencies of solicited systemic AEs were comparable in all three groups, reported by 40%, 43% and 30% of IPV, IPV + dmLT and bOPV groups on Day 1, respectively (Fig. 2). Systemic AEs graded as moderate or severe were significantly more frequent on Day 1 ($p = 0.029$) in the IPV + dmLT group (6 events in 30 participants, 20%) than IPV (1 event in 30 participants, 3.3%) or bOPV (0 events). The most frequent systemic AEs were fatigue and headache, both reported by 11 (37%) of 30 IPV, 13 (43%) of 30 IPV + dmLT and 9 (45%) of 20 bOPV recipients. Rates of systemic AEs declined more gradually than local reactions and participants in all three groups continued to report them through Day 7 with no significant differences between study groups (Fig. 2), but all had resolved spontaneously by Day 15.

Unsolicited AEs up to Day 28 were reported by 20 (67%) of the 30 IPV recipients, compared with 18 (60%) of the 30 IPV + dmLT recipients and 11 (55%) of 20 who received bOPV. Unsolicited AEs were mainly mild or moderate in severity; although there were 3 and 4 events described as severe after IPV and IPV + dmLT, respectively; only one of these was considered to be related to vaccination – a case of severe transient elevated aspartate aminotransferase (AST) in an IPV + dmLT vaccinee which spontaneously resolved 10 days after first being observed. There were no other clinically significant changes from baseline or differences between treatment groups in laboratory values, vital signs, or physical examinations (see Table 2).

Table 2: Unsolicited adverse events in the total vaccinated study population up to Day 29.

	IPV (N = 30)	**IPV+dmLT (N = 30)**	**bOPV (N = 20)**
All adverse events, n (%) *e* *			
Any AE	20 (67) *31*	18 (60) *46*	11 (55) *30*
Any severe AE	3 (10) *3*	4 (13) *4*	0
Any serious AE	0	0	0
Any AE leading to withdrawal	0	0	0
All adverse events within 28 days of vaccination, n (%) *			
Any	14 (47) *19*	15 (50) *24*	10 (50) *20*
Severe	2 (7) *2*	2 (7) *2*	0
Serious AE	0	0	0
All related adverse events within 28 days of vaccination, n (%) *			
Any	5 (17) *5*	4 (13) *6*	5 (25) *6*
Severe	0	1 (3) *1*	0
Serious AE	0	0	0

***** n = number of participants reporting an AE; e = number of events

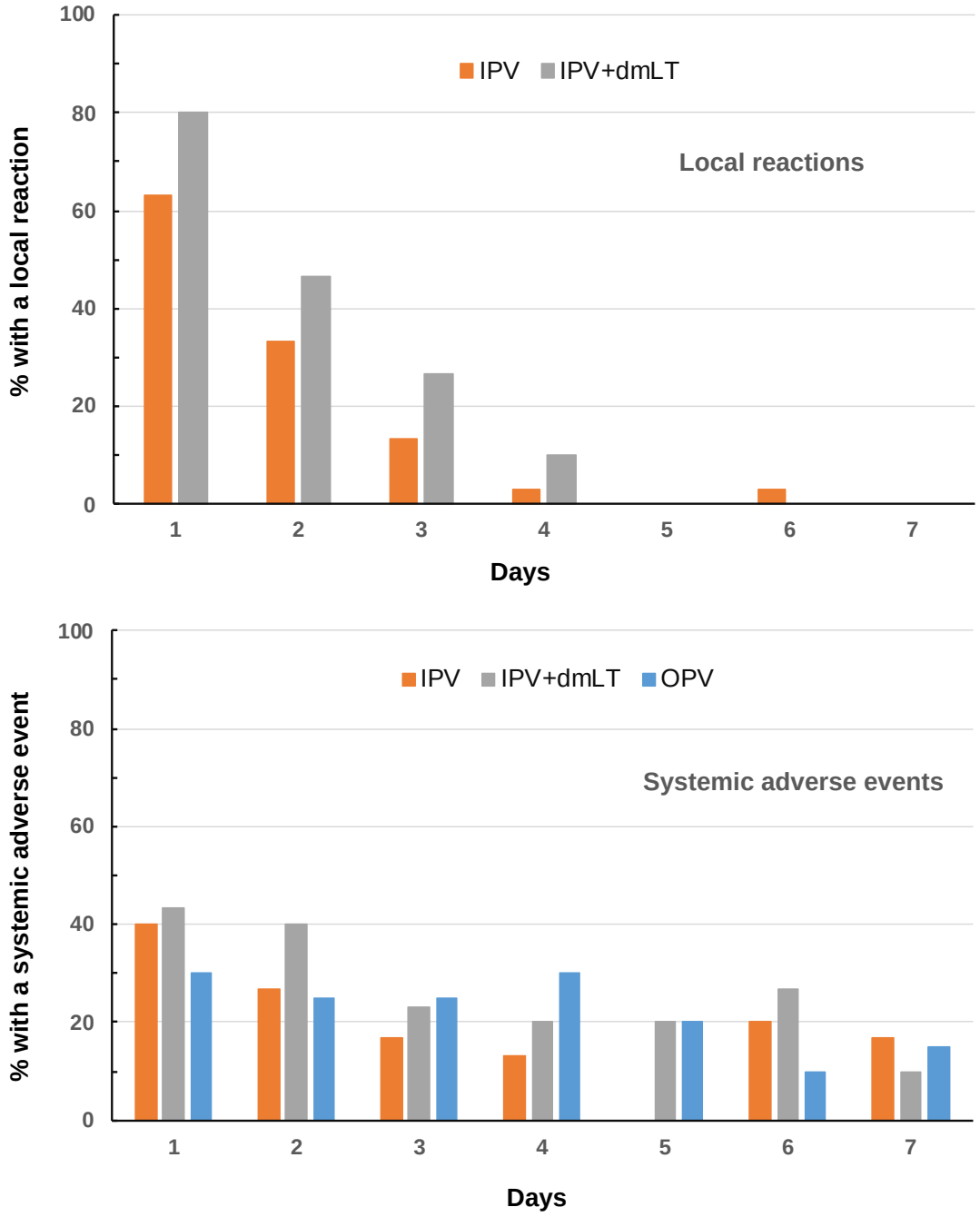

Fig.2. Frequencies of solicited local reactions and systemic adverse events in the study groups for 7 days after vaccination on Day 1.

Stool viral shedding

Viral stool shedding peaked four days after bOPV challenge in all study groups regardless of virus type. For both types 1 and 3 the proportions of both IPV-treated groups who were shedding were similar through 28 days after bOPV challenge (Fig. 3). There was an observable trend to lower rates of shedding in the bOPV group, which was clearest for type 3, in which there was a lower rate in the bOPV group than the similar rates in IPV and IPV + dmLT groups. Shedding was indistinguishable and rare across all three groups by 28 days post-challenge. Median time to cessation of type 1 shedding was 6 days (95% CI: 5–9) for IPV, 7 days (95% CI: 5–14) for IPV + dmLT and 5 days (95% CI: 4–9) for bOPV groups. For type 3 the respective times were 9 days (95% CI: 4–18) for IPV, 19 days (95% CI: 10–27) for IPV + dmLT and 5 days (95% CI: 4–11) for bOPV.

At the predefined time-point of Day 36, 7 days after challenge, the relative risk (RR) for type-specific viral shedding (IPV + dmLT/IPV) was 1.17 (CI: 0.56–2.46) for type 1 and 0.97 (CI: 0.56–2.46) for type 3. Percentage reductions were -0.17 (CI:-1.464–0.440) and 0.03 (CI: -0.510–0.394) for poliovirus type 1 and 3, respectively. Confidence intervals for the RR contained 1.0 for treatment with IPV + dmLT compared with IPV alone and estimated risk reduction in shedding of any virus type was modest (<20%), suggesting no significant difference in viral shedding for either poliovirus type with the addition of dmLT.

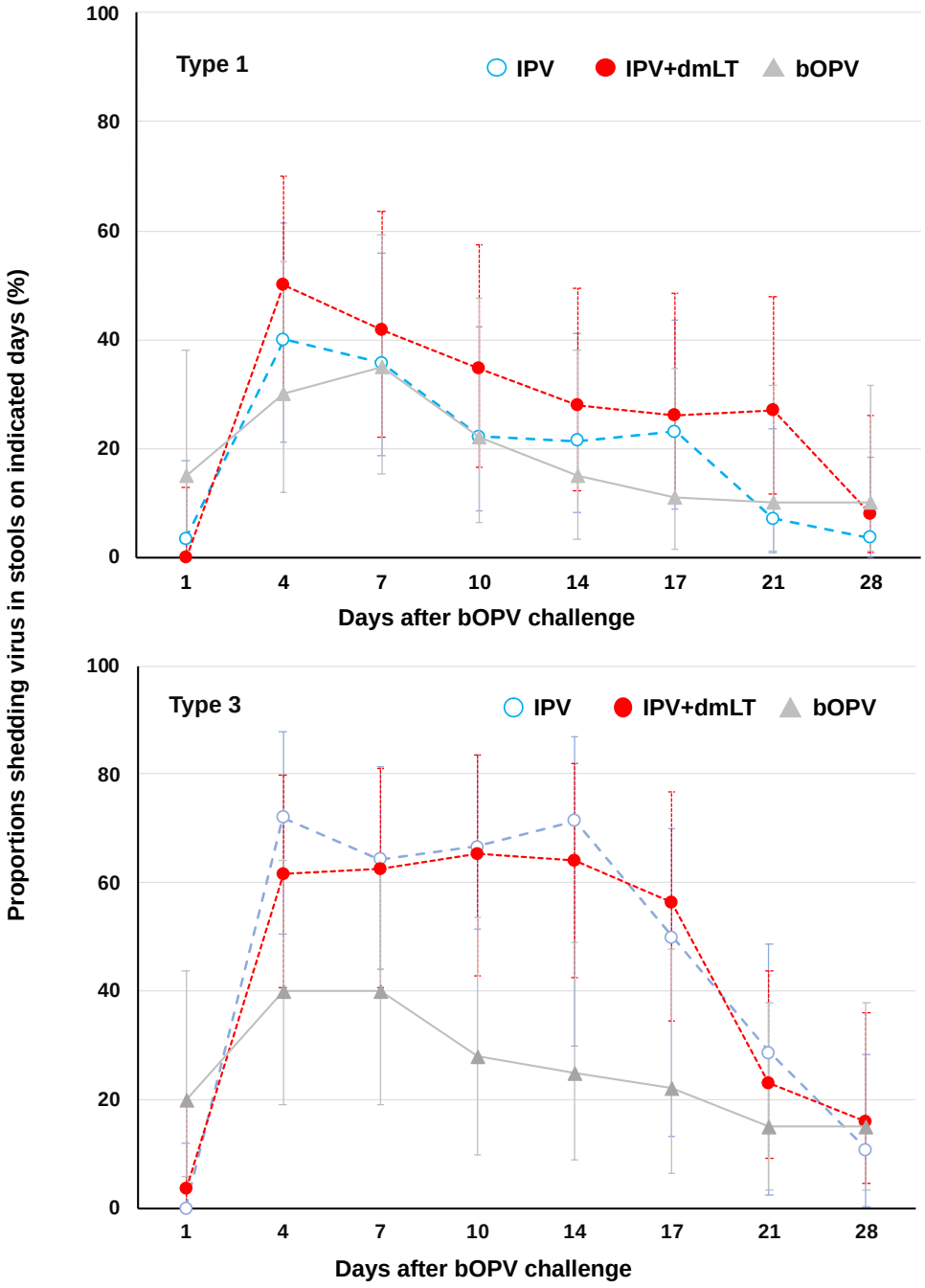

Fig.3. Shedding of poliovirus types 1 and 3 over the 28 days after challenge with bOPV in the three study groups. Shown as percentages of each group shedding with 95% CI bars.

Intestinal Immunity

All positive fecal neutralization responses occurred after bOPV challenge except for one response in the IPV group before bOPV challenge (Fig. 4). Positive fecal neutralization responses to type 1 were detected in no more than two participants at any timepoint in IPV or IPV + dmLT groups, and there were no positive type 1 responses in any bOPV participant at any time up to Day 57. Type 3 responses were observed in no more than three participants at each timepoint in the IPV group, in no >2 participants in the IPV + dmLT group, and in only 1 participant in the bOPV group.

As with the fecal neutralizing responses, only a small proportion of participants demonstrated any measurable changes in fecal IgA over time. Generally, higher levels of fecal IgA were observed when measured using the Sabin strains which also resulted in more variable results than the Salk strains. However, fecal IgA levels using the Salk strains were higher in the IPV group compared with the IPV + dmLT group, particularly for serotypes 1 and 2, and on Day 29.

No meaningful differences were observed in IgG (Table 3) or IgA (Table 4) ASC cells between treatment groups. Large proportions of the IPV groups demonstrated IgG ASC against type 1 at Day 8 after vaccination, 90.0% and 74.1% in IPV and IPV + dmLT groups, respectively, with lower proportions against type 2 (56.7% and 51.9%) and type 3 (40.0% and 40.7%). Proportions with ASC for all three types were lower in the bOPV group (Table 3). IgG ASC were undetectable at Day 29 in all groups, and increases were much lower in all groups after bOPV challenge. The same profile of responses was observed for IgA ASC but with much lower proportions with detectable responses at Days 8 and 29, with the highest responses being observed for type 1 and within the bOPV group (Tab Few $\alpha_4ß_7$ integrin gut homing ASCs were observed. Positive IgA ASC responses were more frequently observed to poliovirus type 1 (Table 5) than to types 2 or 3 (data not shown), and particularly in cells with high expression of the $\alpha_4ß_7$ marker; post-bOPV challenge 44.4% of participants in the IPV group, 42.9% in IPV + dmLT group, and 50% in the bOPV group had $\alpha_4ß_7^{high}$ IgA ASCs to poliovirus type 1, but there were no participants with high levels of IgA homing ASCs to poliovirus type 2 or type 3.

Positive homing IgG ASC responses was more widespread, with observed responses to all three poliovirus types, the proportions of participants with $\alpha_4ß_7^{high}$ IgG ASCs to poliovirus type 1 post-bOPV challenge were 50%, 62.5%, and 20% in IPV, IPV + dmLT and bOPV groups, respectively. Similarly, IgG homing ASC

response rates to poliovirus type 2 post-bOPV challenge were 50%, 0%, and 100%, respectively while no participants with high levels of IgG had homing ASCs to poliovirus type 3.

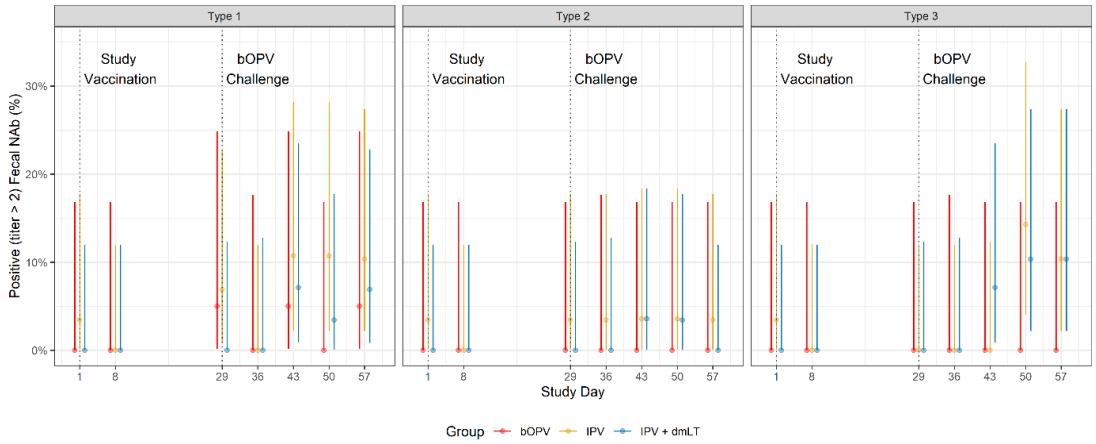

Fig.4. Presence of fecal type-specific poliovirus neutralization activity in the three study groups. Shown as percentages of each group with detected activity with 95% CI bars.

Table 3: Proportions with type-specific circulating IgG antibody-secreting cells (ASC) by timepoint in each per protocol study group

	IPV group		IPV+dmLT group		bOPV group	
Time	n/N **%**	(95% CI)[a]	n/N **%**	(95% CI)[a]	n/N **%**	(95% CI)[a]
Poliovirus type 1						
Baseline	0/30 **0**	(0.0–11.6)	0/27 **0**	(0.0–12.8)	0/20 **0**	(0.0–16.8)
Day 8	27/30 **90.0**	(73.5–97.9)	20/27 **74.1**	(53.7–88.9)	10/20 **50.0**	(27.2–72.8)
Day 29	0/29 **0**	(0.0–11.9)	0/26 **0**	(0.0–13.2)	0/20 **0**	(0.0–16.8)
Day 36	8/29 **27.6**	(12.7–47.2)	6/26 **23.1**	(9.0–43.7)	2/20 **10.0**	(1.2–31.7)
Poliovirus type 2						
Baseline	0/30 **0**	(0.0–11.6)	0/27 **0**	(0.0–12.8)	0/20 **0**	(0.0–16.8)
Day 8	17/30 **56.7**	(37.4–74.5)	14/27 **51.9**	(32.0–71.3)	2/20 **10.0**	(1.2–31.7)
Day 29	0/29 **0**	(0.0–11.9)	0/26 **0**	(0.0–13.2)	0/20 **0**	(0.0–16.8)
Day 36	1/29 **3.4**	(0.1–17.8)	1/26 **3.8**	(0.1–19.6)	0/20 **0**	(0.0–16.8)
Poliovirus type 3						
Baseline	0/30 **0**	(0.0–11.6)	0/27 **0**	(0.0–12.8)	0/20 **0**	(0.0–16.8)
Day 8	12/30 **40.0**	(22.7–59.4)	11/27 **40.7**	(22.4–61.2)	4/20 **20.0**	(5.7–43.7)
Day 29	0/29 **0**	(0.0–11.9)	0/26 **0**	(0.0–13.2)	0/20 **0**	(0.0–16.8)
Day 36	4/29 **13.8**	(3.9–31.7)	1/26 **3.87**	(0.1–19.6)	1/20 **5.0**	(0.1–24.9)

a. 95% CI = Confidence interval computed via Clopper-Pearson method.
Positivity defined as a background-subtracted ASC count ≥ 8 cells per 10^6 PBMC.

Table 4: Proportions with type-specific IgA antibody-secreting cells (ASC) by timepoint in each per protocol study group

	IPV group		IPV+dmLT group		bOPV group	
Time	n/N **%**	(95% CI)[a]	n/N **%**	(95% CI)[a]	n/N **%**	(95% CI)[a]
Poliovirus type 1						
Baseline	0/30 **0**	(0.0–11.6)	0/27 **0**	(0.0–12.8)	0/20 **0**	(0.0–16.8)
Day 8	3/30 **10.0**	(2.1–26.5)	2/27 **7.4**	(0.9–24.3)	5/20 **25.0**	(8.7–49.1)
Day 29	0/29 **0**	(0.0–11.9)	0/26 **0**	(0.0–13.2)	0/20 **0**	(0.0–16.8)
Day 36	2/29 **6.9**	(0.9–22.8)	4/26 **15.4**	(4.4–34.6)	0/20 **0**	(0.0–16.8)
Poliovirus type 2						
Baseline	0/30 **0**	(0.0–11.6)	0/27 **0**	(0.0–12.8)	0/20 **0**	(0.0–16.8)
Day 8	2/30 **6.7**	(0.8–22.1)	1/27 **3.7**	(0.1–19.0)	2/20 **10.0**	(1.2–31.7)
Day 29	0/29 **0**	(0.0–11.9)	0/26 **0**	(0.0–13.2)	0/20 **0**	(0.0–16.8)
Day 36	1/29 **3.4**	(0.1–17.8)	2/26 **7.7**	(1.0–25.1)	0/20 **0**	(0.0–16.8)
Poliovirus type 3						
Baseline	0/30 **0**	(0.0–11.6)	0/27 **0**	(0.0–12.8)	0/20 **0**	(0.0–16.8)
Day 8	1/30 **3.3**	(0.1–17.2)	1/27 **3.7**	(0.1–19.0)	3/20 **15.0**	(3.2–37.9)
Day 29	0/29 **0**	(0.0–11.9)	0/26 **0**	(0.0–13.2)	0/20 **0**	(0.0–16.8)
Day 36	1/29 **3.4**	(0.1–17.8)	2/26 **7.7**	(1.0–25.1)	0/20 **0**	(0.0–16.8)

a. 95% CI = Confidence interval computed via Clopper-Pearson method.
Positivity defined as a background-subtracted ASC count ≥ 8 cells per 10^6 PBMC.

Table 5: Proportions with type 1-specific circulating IgA and IgG-secreting α4β7 ASC Homing Marker 7 days after vaccination (Day 8) or bOPV-challenge (Day 29) in each per protocol study group striated according to expression (neg, dim and high).

		IPV group		IPV+dmLT group		bOPV group	
	Time	n/N %	(95% CI)[a]	n/N %	(95% CI)[a]	n/N %	(95% CI)[a]
Homing IgG ASC							
Neg	**Day 8**	7/10 **70%**	(35–93)	6/9 **67%**	(30–93)	0/10 **0%**	(0–31)
	Day 29	1/10 **10%**	(0–45)	0/9 **0%**	(0–34)	2/10 **20%**	(3–56)
Dim	**Day 8**	6/10 **60%**	(26–88)	7/9 **78%**	(40–97)	3/10 **30%**	(7–65)
	Day 29	2/10 **20%**	(3–56)	3/9 **33%**	(7–70)	2/10 **20%**	(3–56)
High	**Day 8**	5/10 **50%**	(19–81)	6/9 **67%**	(30–93)	4/9 **44%**	(14–79)
	Day 29	5/10 **50%**	(19–81)	5/8 **63%**	(24–91)	2/10 **20%**	(3–56)
Homing IgA ASC							
Neg	**Day 8**	1/9 **11%**	(0–48)	1/8 **13%**	(0–53)	0/10 **0%**	(0–31)
	Day 29	0/10 **0%**	(0–31)	1/9 **11%**	(0–48)	0/10 **0%**	(0–31)
Dim	**Day 8**	2/7 **29%**	(4–71)	2/7 **29%**	(4–71)	1/8 **13%**	(0–53)
	Day 29	1/10 **20%**	(0–45)	0/9 **0%**	(0–34)	0/10 **0%**	(0–31)
High	**Day 8**	2/5 **40%**	(5–85)	0/3 **0%**	(0–71)	1/5 **20%**	(1–72)
	Day 29	4/9 **44%**	(14–79)	3/7 **43%**	(10–82)	2/4 **50%**	(7–93)

a. 95% CI = Confidence interval computed via Clopper-Pearson method.
Positivity defined as samples expressing gut-homing marker (ASC count > 0).

Humoral immunogenicity

As a full polio immunization history with IPV was required for participation, the seropositivity status in the 77 per protocol participants at baseline was high; seropositivity rates were 97.4%, 93.5% and 97.4% for polio types 1, 2, and 3, respectively, and >90% in individual study groups (Table 6). Four weeks post-vaccination all participants were seropositive for all three types after IPV or IPV + dmLT vaccination. All but one participant in the bOPV group were seropositive for all three types, the exception being one person who remained seronegative for type 2. Three participants in the bOPV arm seroconverted for type 2 after vaccination, resulting in a 15% seroconversion rate, despite the absence of type 2 in bOPV. This is consistent with previously observed induction heterotypic immunity (213). Type 1 and 2 seroconversion rates were lower for IPV + dmLT (84.0% and 92.0%) than IPV (93.1% and 100%) and type 3 seroconversion was higher after IPV + dmLT (96.0%) than IPV alone (86.2%). Geometric mean-fold increases for all three types were more than twice as high with IPV than IPV + dmLT and lower after bOPV (Table 6).

Table 6: Type-specific humoral neutralizing antibodies (per protocol population)

	Day 1 pre-vaccination		Day 29 post- vaccination			
Group	**Geometric mean titer (95% CI)**	**Seropositive (%)**	**Geometric mean titer (95% CI)**	**Seropositive (%)**	**Geometric mean-fold rise (95% CI)** [a]	**Seroconversion (%)** [b]
Poliovirus type 1						
	n = 30	28/30	n = 29	29/29	n = 29	27/29
IPV	**191**	**93.3**	**18731**	**100**	**134**	**93.1**
	(110–329)	(77.9–99.2)	(10502–33405)	(88.1–100)	(53.9–334)	(77.2–99.2)
	n = 27	27/27	n = 25	25/25	n = 25	21/25
IPV+dmLT	**423**	**100**	**25048**	**100**	**54.2**	**84.0**
	(239–748)	(87.2–100)	(13152–47705)	(86.3–100)	(20.3–145)	(63.9–95.5)
	n = 20	20/20	n = 20	20/20	n = 20	16/20
bOPV	**249**	**100**	**15657**	**100**	**40.8**	**80**
	(129–480)	(83.2–100)	(7770–31549)	(83.2–100)	(13.69–122)	(56.3–94.3)
Poliovirus type 2						
	n = 30	28/30	n = 29	29/29	n = 29	29/29
IPV	**188**	**93.3**	**45241**	**100**	**347**	**100**
	(93.4–378)	(77.9–99.2)	(24760–82665)	(88.1–100)	(122–985)	(88.1–100)
	n = 27	26/27	n = 25	25/25	n = 25	23/25
IPV+dmLT	**205**	**96.3**	**43251**	**100**	**152**	**92.0**
	(100–418)	(81.0–99.9)	(23053–81147)	(86.3–100)	(49.5–466)	(74.0–99.0)
	n = 20	18/20	n = 20	19/20	n = 20	3/20
bOPV	**221**	**90.0**	**241**	**95.0**	**0.8**	**15**
	(96.3–508)	(68.3–98.8)	(120–486)	(75.1–99.9)	(0.22–2.7)	(3.2–37.9)
Poliovirus type 3						
	n = 30	30/30	n = 29	29/29	n = 29	25/29
IPV	**1022**	**93.3**	**91419**	**100**	**104**	**86.2**
	(555–1882)	(88.4–100)	(55229–151324)	(88.1–100)	(40.7–265)	(68.3–96.1)
	n = 27	27/27	n = 25	25/25	n = 25	24/25
IPV+dmLT	**1266**	**100**	**52165**	**100**	**41.5**	**96.0**
	(666–2406)	(87.2–100)	(30175–90179)	(86.3–100)	(15.1–114)	(79.73–99.9)
	n = 20	18/20	n = 20	20/20	n = 20	10/20
bOPV	**2041**	**90**	**7875**	**100**	**8.6**	**50.0**
	(957–4355)	(68.3–98.8)	(4332–14315)	(83.2–100)	(2.8–26.5)	(27.2–72.8)

a. Geometric mean-fold rise and confidence interval computed via the maximum likelihood method on the difference in $\log_2$ titers then back-transformed.

b. ≥4-fold increase in serum neutralizing activity from baseline or post-vaccination reciprocal titer ≥1:8 if seronegative at baseline

6.5 Discussion

Intramuscular addition of dmLT mucosal adjuvant did not have any meaningful impact on the safety or tolerability of IPV vaccine. There were no SAEs, deaths, or withdrawals due to an AE reported and only one related adverse event was considered to be severe – a participant in the IPV + dmLT group displayed a transient elevation of aspartate aminotransferase (AST) level which resolved spontaneously within 10 days. There were no other clinically significant changes from baseline in laboratory values, vital signs, or physical examinations. Local and systemic reactogenicity was transient and generally mild to moderate and typical of IPV vaccine in the study population of Belgian adults (214) and was only slightly increased by dmLT; the duration of local reactions was not affected by dmLT.

In adult subjects primed with IPV, four weeks after vaccination, both IPV and bOPV induced high levels of humoral neutralizing antibodies and seroconversion for all three poliovirus types (with the obvious exception of type 2 for bOPV). Neither humoral nor intestinal immunogenicity were increased by dmLT; indeed, the magnitude of humoral responses measured as geometric mean-fold rises were generally lower after IPV + dmLT than IPV alone.

The addition of dmLT did not affect fecal viral shedding following bOPV challenge in comparison with IPV alone. Fecal viral shedding was generally higher in IPV-treated participants for poliovirus type 3 compared with type 1. Previous studies have hypothesized that $\alpha_4ß_7$ integrin gut homing ASCs could serve as a surrogate marker of polio vaccine-induced mucosal immune protection (215). Further studies were recommended on subjects with and without polio vaccination exposure to generate additional data to solidify any conclusions on the relevance of these cells as such a surrogate marker, so assessment of $\alpha_4ß_7$ integrin gut homing ASCs were included in this study. There were modest levels of IgA and IgG ASC expressing the $\alpha_4ß_7$ gut homing integrin induced in response to poliovirus type 1 and few homing cells induced in response to poliovirus types 2 and 3. No differences were observed between the two IPV-treated groups in levels of fecal neutralization or fecal IgA responses, consistent with previously published findings among adults but different from infants ((54), (216)).

Although dmLT has been shown to have potent adjuvant capacity in preclinical animal models when administered via intramuscular or intradermal routes ((203) – (205)) and in early clinical studies ((76), (206), (207)) we failed to observe any

impact of dmLT on the intestinal or humoral immunogenicity of co-administered IPV. Some limitations however, should be taken into account. In this study we only selected and evaluated one dose of dmLT which was generally well tolerated by the vaccinees. Further, the different age and routes of administration – adults from a high-income country given intramuscular dmLT in the present study rather than 6–59- month-old children from a LMIC who received dmLT with oral ETEC vaccine (207), as well as the high baseline seropositivity of participants in this study may have limited any measurable adjuvanting effect of the dmLT. Although the current data do not support IPV and dmLT as a solution to improving the intestinal immune response to IPV, studies of dmLT formulation in preclinical models and the tolerability of the dmLT dose used in this study may suggest the utility of another clinical assessment with higher doses of dmLT. Future evaluations should be conducted in a younger study population who are more likely to demonstrate measurable fecal neutralization and IgA responses (49). Additionally, the interpretation of post-challenge shedding comparisons between the IPV arms and the bOPV arm were limited in this study due to ongoing vaccine shedding among 15% of bOPV recipients at the time of challenge. Future clinical assessments among OPV-naïve individuals should extend the time between vaccination and challenge to avoid this coinfection.

Alternative routes of IPV administration, e.g., intradermal injection with fractional doses of IPV (f-IPV) alone provides a viable option for dose-sparing [28], although the WHO SAGE currently recommends the use of two doses of f-IPV for routine immunization together with bOPV (165). Individuals whose primary immunization includes IPV in conjunction with bOPV will not have the intestinal immunity required to prevent transmission of type 2 poliovirus should they be exposed (217). In the absence of alternatives, there remains an unmet need for induction of type 2 intestinal immunity that may be addressed with development of an improved IPV or inclusion of nOPV2 which can confer intestinal immunity. Despite promising data from preclinical studies, dmLT at the dose used in the present study does not appear to address this need, and further investigation is necessary.

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Chapter 7
General discussion and conclusion

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Following global OPV2 cessation in May 2016, (also referred to as 'the switch') monovalent OPV2 only remained stockpiled for outbreak response. As IPV induces only limited mucosal immunity mOPV2 was the only appropriate vaccine for stopping cVDPV2 outbreaks. However, if an insufficient number of children are reached new circulating strains will be seeded, leading eventually to new outbreaks. In addition, if sufficient upscaling of IPV would not be achieved timely to meet the global need the intestinal mucosal immunity to PV2 on population level would decrease in regions lacking this IPV supply, increasing the risk of cVDPVs and new paralytic polio cases. In order to meet these risks researchers developed new IPV and OPV vaccine candidates to address current and future needs. In 2011 a scientific consortium was established by the BMGF with the objective to create more genetically stable, novel OPVs to support the GPEI. The initial focus was on poliovirus type 2, to develop a safer vaccine to be used in outbreak control, with later on extension to all 3 serotypes. In 2015, 2 lead polio type 2 vaccine candidates were identified for clinical development but were not yet ready to be tested before the planned global withdrawal of OPV2 in April-May 2016. To prepare the evaluation of these vaccine candidates as well as future novel tOPV, two historical control studies had, however, timely been designed and performed which we summarize here to put the main findings in perspective.

The UAT1 study (EudraCT 2015-003324-32) has been conducted in Belgium at CEV, Antwerp and at The Queen Astrid Military hospital, Neder-Over Heembeek between December 2015 and June 2016. In this study 128 healthy OPV-primed adults were enrolled and randomized to receive 1 or 3 doses of tOPV with 28 days in between. As expected, the vaccine was well tolerated in both groups, with no safety signals observed during the study.
Baseline median $\log_2$ titers of serotype specific neutralizing antibodies and seroprotection rates were already high at baseline and increased by Day 28 after first vaccination for all 3 serotypes in both groups combined but low seroconversion rates were seen. The second and third dose induced slight increases in seroprotection rates, seroconversion rates and median $\log_2$ titers.

Analysis of viral shedding and assessment of genetic sequence of stool samples obtained in this study will be done as soon as a comparative study with novel tOPV has been conducted in order to process and evaluate samples of both studies in a blinded way.
In conclusion, this study showed that tOPV is safe and immunogenic in OPV-vaccinated subjects and the results can be used as comparison data in future studies with novel polio vaccines.
These results are not published and will not be further discussed as no comparison study with trivalent nOPV has been conducted yet.

The UAM1 study (EudraCT 2015-003325-33) has been conducted at CEV, Antwerp in 2016 before global withdrawal of OPV2. One hundred OPV- primed healthy adults have been enrolled to receive 1 or 2 doses of mOPV2. Safety, immunogenicity, shedding and genetic sequencing results will be discussed below in comparison with the results of the nOPV2 phase2 study.

The aim of this thesis is to evaluate nOPV2-c1 and nOPV2-c2 candidates in adults for safety, immunogenicity, viral shedding and genetic stability in order that, in case of positive results, studies in children and infants could be moved forward in Panama.

In addition, a novel adjuvanted IPV vaccine has been evaluated for safety, humoral immunogenicity and its ability to generate mucosal responses in comparison with IPV.

7.1 Main Findings of this PhD thesis

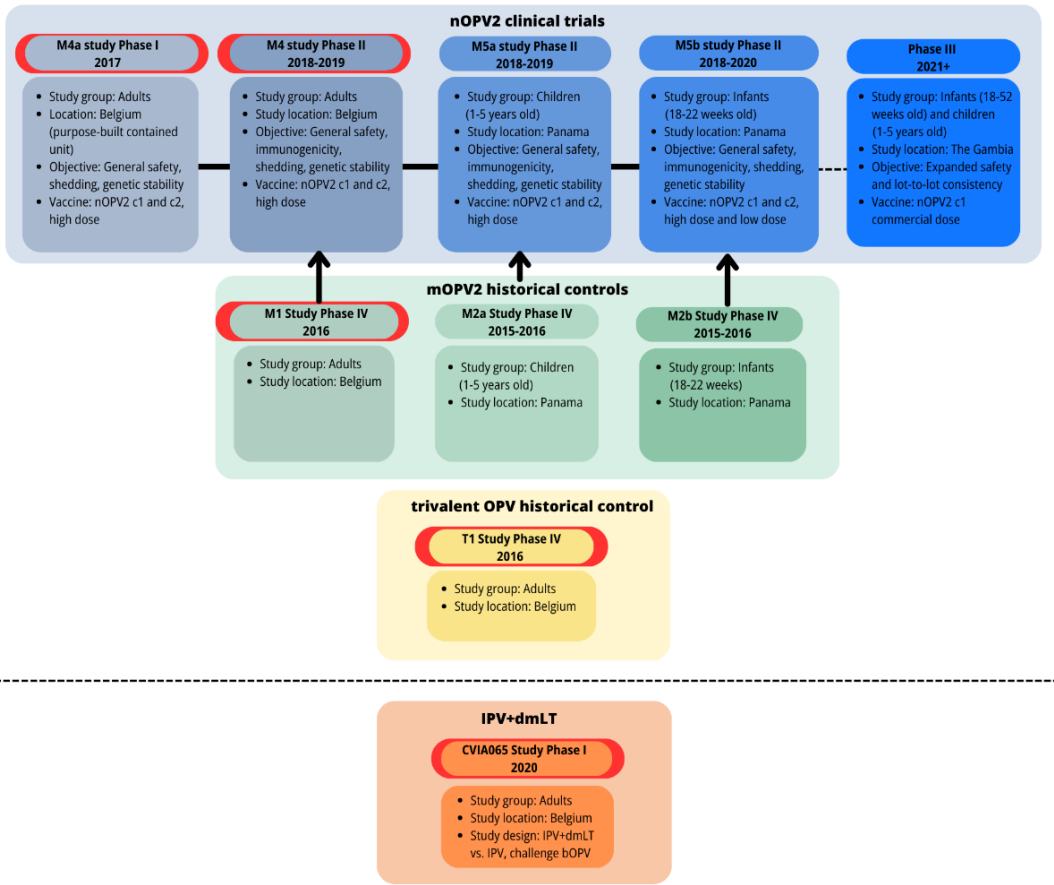


Fig. 1. Overview of studies discussed in this thesis. Titles in red are the studies that are subject of this PhD. Inspired by (131)

7.1.1 nOPV2 vaccine candidates

7.1.1.1 Safety of nOPV2-c1 and nOPV2-c2

We evaluated safety of the nOPV2 vaccine candidates for the first time in the **phase 1 study UAM4a** (also referred to as M4a)(EudraCT 2017-000908-21), conducted in 2017 in contained conditions at CEV. The aim of the study was to provide a preliminary evaluation of the clinical safety, viral shedding and genetic stability of the vaccine candidates, and to evaluate whether they could pose an environmental threat to the broader population if used in future larger studies outside containment. For this initial study no comparisons of safety and immunogenicity were made with licensed vaccine because of the small number of participants and their IPV vaccine history. The 2 nOPV2 vaccine candidates were well tolerated by the subjects. The only remarkable and unexpected observation was a relative high number of transient elevations of liver enzymes, of which some were severe or even reached the level of life-threatening according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 which was used in the study for toxicity grading, although subjects did not report any symptoms. These high levels were mainly observed in aspartate transaminase (AST) and creatine kinase (CK) tests with no changes in bilirubin or gamma-glutamyl transferase results. Similar rates of lab abnormalities were observed after vaccination of either vaccine candidate. At that time a causal relationship could not be totally excluded because no placebo control group was included in the study. However, based on lack of symptoms (even with CK levels approximately 30-times higher than considered as normal), fast recovery and thorough anamnesis of the subjects the most likely explanation is that these abnormal lab results were caused by unaccustomed and excessive use of exercise equipment and daily supervised fitness training that was available in the facility. Intensive muscular exercise has been described in literature as a cause of asymptomatic elevations of liver function tests, mentioning in particular elevations of AST, ALT and CK. (218)Therefore, we can conclude that this phase 1 study showed preliminary evidence that the nOPV2 candidates were safe and well-tolerated with the reservation that the lab abnormalities should be further investigated in next studies. In agreement with the DSMB the study design of the next Phase 2 study was modified to further assess the lab parameters and by adding a cohort of IPV subjects to be randomized to one of the nOPV2 candidates or to placebo. The addition of IPV-primed subjects was necessary to exclude

vaccination history as contributing factor to the lab abnormalities and to be able to compare with a placebo group.

In the larger **Phase 2 UAM4 (M4) study** (EudraCT 2018-001684-22) in 2018 safety of both vaccine candidates has been further investigated and confirmed. The study has been conducted at 2 centers in Belgium: CEV, Antwerp and CEVAC, Ghent. It was of interest to evaluate the candidate vaccines in cohorts of former OPV recipients and former IPV recipients prior to initiating studies in younger age groups. The OPV cohort aligned with the mOPV2 cohort in the historical UAM1 study, and the IPV cohort allowed an additional evaluation of specific safety parameters for the laboratory abnormalities observed in the UAM4a study. At the symptoms level, assessments of severe and serious AEs showed no meaningful differences of nOPV2-c1 and nOPV-c2 compared to mOPV2 in OPV-primed subjects and compared to placebo in IPV primed subjects. No related SAEs were observed in OPV primed subjects of UAM4 and UAM1 study and only 1 SAE (influenza like illness) was considered possibly related to vaccination with nOPV-c2 in the IPV primed group. The number of severe unsolicited adverse events was comparable between all groups and only few in each group were considered related and regarded gastro-intestinal complaints. Systemic reactogenicity was slightly higher for both nOPV2 candidates than for mOPV2 in the OPV-primed subjects but no differences were seen in the IPV-primed groups.

For the laboratory assessments in OPV and IPV primed subjects, no consistent signals of potential harms related to dosing with nOPV2-c1 or nOPV2-c2 were identified. In the OPV-primed groups 2 subjects showed grade 4 CK elevations at day28 after receiving nOPV2-c2, no grade 3 or 4 CK elevations were seen after nOPV2-c1 and no grade 3 or 4 lab abnormalities were detected up to day 28 post-dose mOPV2. In the IPV-primed groups 2 subjects in the nOPV2-c1 group showed a grade 3 CK elevation at day 42 and day 56, respectively whereas no grade 3 or 4 lab events occurred after receiving nOPV-c2. In the placebo group 3 subjects showed CK elevations after dosing: 1 grade 4 at day 7, 2 grade 3 at days 3 and 35, respectively. By anamnesis all events could be linked to sport activities shortly before blood draw. Moreover, the overall frequencies of severe or life-threatening laboratory outcomes were no greater than the baseline (Day 0) frequencies and no severe or life-threatening clinically-relevant outcome was reported for investigated liver enzymes (ALT, AST,GGT, bilirubin or albumin). This suggested that the low frequencies of severe or life-threatening laboratory outcomes observed in the UAM4a study, represent events unrelated to the receipt of nOPV2 vaccine candidates.

Risk on VAPP in our studies was not expected as polio vaccination in Belgium is mandatory since 1966 and all participants provided evidence of at least 3 priming doses of IPV or OPV.

In conclusion, based on the safety results observed in the phase I and phase II studies we show that both nOPV1-c1 and nOPV-c2 are well tolerated and have an acceptable safety profile similar to that of mOPV2.

7.1.1.2 Humoral Immunogenicity

Protection against paralytic polio is defined by type specific neutralizing antibodies, which is reflected in the established immune correlate of protection, commonly used to evaluate vaccine efficacy. A type-specific serum neutralizing antibody titer ≥1:8 is considered protective against disease. Therefore, the efficacy of a specific polio vaccine is assessed by the seroprotection rate (SPR) it generates in the population. Because the nOPV2 vaccine is intended to be used as a tool for outbreak response, likely in a heterogeneous population including vaccinated individuals, the SPR was considered the most relevant primary endpoint. In addition, absolute and relative (to mOPV2) levels of neutralizing antibodies have been evaluated, as well as the seroconversion rate (SCR), defined as the seroprotection rate among initially seronegative individuals, or a 4-fold rise among baseline seroprotected individuals.

The immunogenicity results of the 30 IPV primed subjects in the **phase 1 study (UAM4a)** indicated already that both nOPV2 candidates were immunogenic. Most participants received a number of 5 or more vaccinations in the past and all but one participant was seroprotected at baseline. Nevertheless, vaccination induced high elevations of type-2 neutralizing antibodies (nAB) and most participants (with baseline titer low enough to allow detection of a 4-fold rise) showed seroconversion one month after vaccination. In addition, seroprotection levels were 100% for all participants after vaccination.

We confirmed these results in the **phase 2 study** with OPV as well as IPV primed subjects. Also in this study both OPV- and IPV vaccinated cohorts showed a high baseline immunity regarding nAB titers and SPR. Nevertheless, by comparison with the mOPV2 data of the historical UAM1 study after vaccination we could show non-inferior immunogenicity of both nOPV2 candidates to mOPV2. Seroprotection rates reached 100 % for either nOPV2 candidate after first dose. Moreover, PV2-specific geometric mean titers (GMT) and SCR at Day 28 in OPV

primed groups may indicate that the immunogenicity of nOPV2-c1 and nOPV2-c2 at the 10^6 $CCID_{50}$ level may even be superior to a standard dose of mOPV2 (10^5CCID_{50}).

The IPV cohorts were relatively small in this study and no comparator control data were available but the results showed a similar trend as in the OPV cohorts with high levels of immunity before vaccination and seroconversion reaching 100% and 92% for nOPV2-c1 and nOPV2-c2 respectively.

Overall, we can conclude that both nOPV2 vaccine candidates met the humoral immunogenicity criterium of non-inferiority in comparison with mOPV2 with even the suggestion of being superior to mOPV2.

7.1.1.3 Viral shedding

The main risks associated with the use of mOPV2 are the rare incidence of VAPP in vaccine recipients or their close contacts and reversion of attenuation with generation of circulating vaccine derived viruses in the community. Reducing these risks was the primary objective of the nOPV2 development. Estimates of these risks can be provided by comparison of the speed and extent to which nOPV2 candidates and mOPV2 reacquire neurovirulence and by assessing the likelihood of transmission. To address the latter, we evaluated the rate, magnitude and duration of viral shedding in UAM1, UAM4a and UAM4 study.

Because nOPV2 candidates were only ready to be tested after the global withdrawal of OPV2 GAP III conditions applied. Therefore, **FIH study M4a** was conducted in contained conditions and designed specifically to evaluate all shedding characteristics of both nOPV2 candidates before proceeding to ambulatory trials. IPV only-vaccinated subjects were selected for this study to ensure sufficient shedding could be observed as IPV induces only limited mucosal immunity. In Belgium polio vaccination is mandatory since 1966 and OPV has been used for several decades before switching to IPV in 2001. As a consequence, at the time of the study in 2017 no Belgian IPV only-vaccinated adults were available (only adolescents). Therefore, we reached out to neighboring countries who used IPV already for a longer time and eventually we enrolled 30 adults of Dutch origin with IPV-only vaccination history in the study.

The eradication of wild-type polio viruses in several northern European countries (France, The Netherlands, Sweden, Finland, Iceland) established by IPV-use only is

most likely due to the ability of IPV to reduce nasopharyngeal shedding. Oral-oral transmission route is presumably more predominant in industrialized countries. Yet, only few studies investigated the impact of IPV or OPV on shedding of PV in the nasopharynx and inconclusive results are provided in literature. (148) Therefore, we investigated nasopharyngeal shedding in the IPV-primed subjects of the M4a study at several timepoints during their stay in the unit. No shedding could be evidenced in any participant after challenge with nOPV2 candidates.

By contrast, viral shedding in stool was detected in most of the participants of the phase 1 study and differences in shedding characteristics were seen between both nOPV2 candidates. Subjects were asked to collect samples daily and until 3 consecutive samples were PCR negative. Shedding started for most participants within a few days after vaccination and the duration and the magnitude (median viral shedding index) were higher with nOPV2-c1 than with nOPV2-c2. More subjects (7/15) after nOPV2-c1 were still shedding at D28 with last subject ceasing shedding on day 89 versus 4/15 subjects after nOPV2-c2 were still shedding at D28 with longest duration ending on day 48. The risk analysis threshold for reduced risk of transmission for Sabin OPV strains is determined as 4.0 $\log_{10}$ $CCID_{50}$. In our study we observed only in a few individual samples a median viral index score of more than 5.0 $\log_{10}$ shortly (within 7 days) after nOPV2-c1 vaccination and only in 1 sample after nOPV2-c2 vaccination. This concentration didn't last longer than 2 days in any sample. In comparison, approximately 25% of IPV primed infants in a Chilean study showed similarly high viral concentration in stools and in a study in Lithuania 73% of IPV primed children (1-5 years old) reached a titer of ≥8.25 $\log_{10}$ $CCID_{50}$ in ≥1 sample after one challenge dose with mOPV2. (179), (197)This indicates that shedding rates after nOPV2 candidates may be reduced.

Interestingly, we observed resumption of shedding in some candidates after 3 consecutive PCR negative samples. As common use for determination of poliovirus shedding cessation the time to cessation of shedding was defined as the time interval between administration of vaccine and the last day of shedding positivity prior to cessation (three consecutive PCR-negative samples). However, 3 subjects of either group resumed shedding after reaching this threshold. A possible explanation could be re-infection as toilets had to be shared and were appointed per 3 subjects (only applicable to male subjects due to the small number of female volunteers) but the subject with the longest shedding duration resumed shedding after 3 negative days at day 73, being at home already for 45 days.

Two subjects of the nOPV2-c1group exceeded the expected shedding duration time much longer than other participants of that group. No explanation has been found as both subjects were healthy with no remarkable medical history. One of them lived in Belgium and could return to his home at Day 28 and continued daily stool collection until shedding cessation at Day 89. Viral shedding titers were most of the time below 4.0 $\log_{10}$ $CCID_{50}$ but this titer was reached at D46 and D56 (4.16 and 4.06, respectively), lasting in both cases only for 1 day. The other long-term shedder was domiciled in the Netherlands and had to stay longer in Belgium due to the shedding. His viral shedding course was different as his shedding titers remained constantly low fluctuating around culture negativity (2.75 $\log_{10}$ $CCID_{50}$) until Day 83. As it remained unclear why both subjects showed prolonged shedding both subjects were proposed to receive 1 dose of IPV. Studies have shown that while IPV alone has little impact on mucosal immunity in some cases IPV has shown to be able to boost immunity after OPV mucosal priming. (219) One of the subjects agreed and received 1 dose of IPV at D57, however this didn't seem to shorten further shedding duration.

In **the Phase 2 study** stool samples were collected daily for the first 10 days and at day 14,21,28 and 42 after each vaccination. If shedding was still ongoing at last visit subjects were asked to collect 3 consecutive stool samples each 3 weeks. As expected, due to mucosal priming in the past stool viral shedding rate was low in OPV primed subjects after mOPV2 as well as after nOPV2 vaccination. Overall shedding rate and extent after vaccination with either of the nOPV2 candidates were similar and not increased compared to mOPV2. In UAM1 shedding didn't last longer than 14 days after first vaccination and this was also the case in the UAM4 study for nOPV2-c1. For nOPV-c2 only 1 subject was still shedding at D27. After the second vaccination only a few subjects showed shedding for a short time with a similar magnitude of shedding for the 3 vaccines.

Shedding in IPV primed subjects of the UAM4 study was more frequent and lasted longer in comparison with the OPV primed subjects in that study but showed a lower rate and shorter duration in comparison with the IPV primed subjects of the M4a study. This can be due to individual variability and small sample size but it is possible that differences in sample storage and duration before sample processing between both studies also has played a role. All IPV primed participants received a second dose of nOPV2 at D28 and from the samples provided at that timepoint only 1 was PCR positive for nOPV2-c1 (1/10) and none for nOPV2-c2 (0/12). Decrease in shedding rate after second dose compared to the first dose was

clearly shown in these IPV primed subjects, indicating some indirect evidence of mucosal immunity generated by the first dose of nOPV2.

In conclusion, viral shedding characteristics were quite similar to those observed after mOPV2 for both candidates.

7.1.1.4 Genetic stability and neurovirulence

Enhanced genetic stability was the main objective for development of novel oral polio vaccines. In Sabin OPV2 loss of attenuation occurs fast (usually within 7 days) in 3 specific regions of the genome. Nucleotide changes at the primary attenuation site G481A in domain V of the 5'untranslated region are critical for reacquisition of neurovirulence and this site functions as gatekeeper for other reversion processes. In addition, U398C nucleotide changes in domain IV and polymorphisms in the codon for amino acid 143 of the VP1 capsid protein (VP1-143) can cause early loss of attenuation. (220) Both candidate vaccines have been adapted specifically to make reversion less likely. In nOPV2-c1 stabilization of domain V (called S15domV) is combined with changes of the essential cis-acting replication element (cre) to reduce risk of loss of this stabilization through recombination with other enteroviruses. In addition, mutation rate and recombination frequency are decreased by further modifications to the genome (HiFi3 and Rec1). The same stabilization of domain V is used in nOPV2-c2 and is further supported by changes in the capsid region (CpG40) to reduce replicative fitness and as such, transmission risk.

Genetic stability of the viruses shed by the participants in our studies (M1-M4a-M4) has been assessed by next generation sequencing (NGS) for evaluation of retention of the key modifications. Additionally, potential neurovirulence of viruses in the stool samples was investigated by a modified transgenic mouse neurovirulence test (mTmNVT). Both tests were qualified and executed at Viroclinics Biosciense B.V. and have been applied to a subset of stool samples. The Exploratory Endpoint Specimen (EES) is the latest sample of a subject containing at least 4 log $CCID_{50}$/g, generally used as a risk analysis threshold for reduced risk of transmission for Sabin OPV strains and is the minimum virus titer needed for both tests. (221) Only samples after the first dose have been evaluated because of limited shedding after second dose of nOPV2 in M1 and M4 studies.

Evidence in the literature demonstrates high and rapid reversion for Sabin OPV at the primary site of attenuation. As shown by Stern, all type 2 viruses shed 7 days

after administration contained significant (33 to 96%) reversion at the primary site of attenuation (nucleotide 481). (222) Another study showed consistent results with almost 100% reversion within 14 days after trivalent OPV (tOPV) vaccination. (185)

In the M4a study 15 EES were provided for nOPV2-c1, ranging from D2 to D56. Overall mouse paralysis rate for shed nOPV2-c1 viruses was 1.6% (range 0-10) compared to 0% for nOPV2-c1 vaccine bulk. No reversion was observed at the 481 nucleotide of domain V. Reversion at VP1-143 (unprotected site) was seen in samples of D7 or later, similarly as expected for Sabin OPV2. Some samples showed variants in cre5 and domain IV (position 459, known to be related to virus fitness/adaptation) but even a sample with fixed mutations in cre5, domain IV and VP1-143 only showed 10% paralysis in the mouse test. In comparison, 90% paralysis of mice was caused by a Day 7 sample with 88% A481G reversion after mOPV2 vaccination in a previous trial (177), which was used as informal comparator. For nOPV2-c2 6 EES were identified (range Day 2-28). No variants were seen in domain V and IV but some variants at the VP1-143 were observed in Day 9 and Day 28. All CpG-modifications were retained. Only 2 EES (Day 2-3) could be successfully amplified in cell culture to be used for the neurovirulence testing. Paralysis rate of 14% was shown for 1 sample resulting in an overall paralysis rate of 6.9% (range 0-14) across both samples.

In the **M1 study** only 2 EES (both Day 5 samples) were available for mOPV2 while in the **M4 study** for nOPV2-c1 9 EES (range Days 4-10) and 8 EES (range Days 3-21) were identified for OPV- and IPV vaccinated subjects respectively. In OPV vaccinated subjects, no key reverting variants (cre5, domain IV, domain V) were seen in EES of OPV-vaccinated subjects for mOPV2 as well as for nOPV2-c1 and no transgenic mice became paralyzed in the test. The absence of reversion in the mOPV2 samples is in line with expectations as these were early day 5 samples. Also in IPV-vaccinated subjects no domain V reverting variants were shown in nOPV2-c1 shed virus. Key variants seen caused 0% paralysis in transgenic mice for the D8 sample and 10% for both D21 samples. Most EES that were lacking key site variants didn't cause paralysis in the mouse test except for 1 sample (11.1%).

Regarding nOPV2-c2 in previously OPV vaccinated subjects 5 EES were identified (range Day 5-7). No reversion in domain V and IV was seen and also the modified CpG sites in the P1 region were retained. In addition, no EES showed any reversion of VP1-143. Overall paralysis rate in mice was 2.02% (associated with variants carrying amino acid changes in other regions of the candidate) In IPV-vaccinated

subjects 5 EES samples were available (range Day 4-10). No reversion was observed in domain V and IV. Only 2 EES of Day 8 and day 9 showed reversion of VP1-143 resulting in 10% mice paralysis by the EES Day 8 shed viruses.

In conclusion: overall paralysis rates (all mice paralyzed/ all valid mice) of EES were 0.0% for candidate 1 and 2.02% (range 0.0% to 5.3%) for candidate 2 in OPV-vaccinated cohorts indicating the low virulence anticipated for these candidates. The overall paralysis rates observed for the IPV vaccinated cohorts were 3.1% (range 0 to 11.1%) for candidate 1 and 1.7% (range 0 to 10%) for candidate 2, and confirm results observed previously with cell culture passaged nOPV2 candidate 1 and candidate 2 research materials (preclinical studies), as well as the phase 1 M4a study . As no IPV vaccinated adults were included in mOPV2 control studies no comparisons can be made between the M1 and M4 nOPV2 cohorts. However, a day 7 shed Sabin-2 virus used as a control in the mouse test shows high paralysis rate (66.7% in these tests), while the nOPV2 candidates show very low rates of paralysis (e.g. both day 21 nOPV2 candidate 1 EES show 10% paralysis).

In our studies for shed nOPV2-c1 and nOPV2-c2 we observed no or limited increases in neurovirulence in the modified transgenic mouse model compared to the bulk vaccine, in contrast to a marked loss of attenuation that would be expected from corresponding samples from Sabin OPV2 vaccinees after 7 days. No significant changes to the primary attenuation site domain V were seen for either candidate vaccine, regardless of prior vaccination history of the subject.

7.1.2 Conduct of a study in containment and GAPIII

In line with the Polio Eradication & Endgame Strategic Plan 2013-2018 the first step towards global cessation of OPV was taken by withdrawal of OPV type 2 in a synchronized global switch by May 2016. Since then, tOPV was replaced by bOPV (types 1 and 3) with at least 1 dose of IPV and mOPV2 stockpile only remained available for outbreak response. To minimize the risks of a facility-associated reintroduction of viruses the WHO developed the GAP III, a global action plan for implementation of poliovirus safe-handling and containment measures after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use. (152) Consequently, these guidelines applied also to the nOPV2 vaccine candidates, especially for the FIH study in which the shedding and genetic stability of both candidates would be intensively investigated. In addition, a relative long stay (28 days) of the volunteers was foreseen in the unit, based on literature data showing a range of 63-100% shedding during the first 10 days after

OPV challenge in IPV vaccinated children (223), (156), (157)and a mean shedding duration of 13 days in adults after OPV1 challenge. (224)

As the CEV in 2017 only consisted of an ambulatory unit and no suitable facilities compliant with the GAPIII requirements were found in the neighborhood, we built a temporary self-contained unit, constructed of 66 containers, equipped for a sequential stay of 2 cohorts of 16 individuals (15 volunteers and 1 supervisor) during 4 weeks. To make the facility resembling as much as possible to a common phase 1 unit several risks had to be taken into account and for which stringent standard operating procedures had to be developed. In the third week after the study start an inspection by the Belgian regulatory authorities (FAMPH) took place. The aim of the inspection was to verify that the Poliopolis phase 1 unit met all legal and regulatory obligations of good clinical practice in clinical trials (relating to the Belgian Law of 7 May 2004 related to experiments on humans, and the applicable royal decrees, European guidelines in Eudralex vol.10 and ICH guideline E6). No critical or major deficiencies were identified and adjustments regarding minor comments were applied in due time.

In this thesis we describe all procedures and measurements taken in the unit to minimize risk on contamination of the environment with the candidate viruses. To avoid contamination of the first evaluated vaccine candidate to the second cohort the whole facility had to be decontaminated with Chlorine dioxide gas and more than sufficient time (3 weeks) was respected before start of the second cohort.

In addition, special precautions had to be taken to take care of the mental wellbeing of the volunteers during their long stay. In addition to medical screening participants were also evaluated beforehand by a team of psychologists to evaluate their coping ability with longtime containment and how they would fit in the group of 15 volunteers. Medical personnel performed daily visits 7/7 not only to check physical safety but also their mental welfare. To reduce risk of boredom relaxation and fitness rooms were foreseen, however nothing will guarantee that subjects will not quit before shedding cessation. It is a fundamental right of the volunteer to end participation whenever he or she wants. On the other hand, minimizing of risk on contamination of environment and protection of non-participating, potentially vulnerable individuals had to be ensured. The participation of Dutch volunteers entailed an additional risk in case of premature withdrawal. A shedding Dutch participant returning to his home in the Netherlands would potentially pose a risk to a specific population subset known for refusing vaccination because of religious regions and living scattered

throughout the country (referred to as the 'bible belt'). Therefore, a code of conduct described the measures that were asked of the subjects in case of consent withdrawal while shedding was still ongoing. Subjects were asked to stay in Belgium and continue daily stool sampling with containing of all stools in portable toilets to be brought to the center for further decontamination and waste management. All volunteers were well informed and screened on their understanding of the reason for this request. Unfortunately, for both cohorts at Day 28 no confirmation of shedding cessation was reached for about half of the participants and while cessation for most of the shedding participants after nOPV-c2 ceased shortly thereafter 1 subject continued shedding until Day 48. For nOPV2-c1 several subjects with ongoing shedding at Day 28 continued shedding for an extra 10 days or even longer as was seen in 2 participants reaching shedding cessation at Day 83 and Day 89. As it was not realistic for subjects domiciled in the Netherlands to remain in Belgium for such a longtime Dutch authorities were consulted and subjects were allowed to return to the Netherlands provided that they continued all precautions and measures as well as daily stool sampling to be investigated both by CDC and RIVM.

Another complication was the event of re-shedding which occurred in some participants after 3 consecutive negative stools which defined end of shedding in the study. All stool samples in the study were sent to CDC for analysis resulting in a turn-around time of minimum 5 days for results to be available. Consequently, subjects were informed about their shedding status on Day 27, a day before discharge, based on the last results at that time available. Unfortunately, some subjects had ceased shedding according to the last results and returned to the Netherlands while new results of later timepoints revealed they had resumed shedding in the meantime. As soon as this was apparent regulatory and health authorities were informed and it was decided to inform all volunteers and ask them to collect another 3 stool samples regardless of the timepoint of their last positive stool sample. In this way we could ensure all subjects definitively ceased shedding. All subjects responded to this request and all of these samples were negative.

In conclusion, we were able to design and built a unique temporary infrastructure suitable for a long stay of volunteers taking into account all precautions of GAPIII to minimize risk of contamination of the environment. As possible for all research unexpected findings did occur and have been dealt with. Minimal risks to the environment were ensured and at the same time ethical considerations for the volunteers have been taken into account, showing the importance of good

communication and information of the volunteers beforehand and during the study as well as with Ethics committee and Regulatory and Health Authorities.

7.1.3 IPV+dmLT: an adjuvanted IPV vaccine

IPV is known to generate a strong humoral response, protective to symptomatic polio disease but induces only limited mucosal response, irrespective of the number of doses, antigen amount, or route of administration. (221) As such, it has shown little impact on transmission in areas with predominant faeco-oral route. Since 2016 tOPV is replaced by bOPV with at least one dose of IPV to ensure a minimal polio type 2 immunity (partial humoral and minimal mucosal) as a first step in the global transition process to IPV-only vaccination. (225) However, since then population type 2 mucosal immunity has been decreasing which Is reflected in the rising number of cVDPV2 outbreaks and paralytic cases after the switch, therefore SAGE recommended in September 2020 to add a second IPV dose to the bOPV schedule to enhance seroprotection. (226) Furthermore, the necessary upscaling of IPV production after the switch encountered difficulties resulting in global IPV shortages affecting many low-and middle-income countries. Adding an adjuvant to IPV with the potential of enhancing intestinal immunity and reducing the amount of antigen to fractional doses would solve both problems.

Pre-clinical and clinical studies with vaccines adjuvanted with double mutant [LT(R192G/L211A)] heat labile toxin (dmLT), derived from enterotoxigenic *Escherichia coli* labile toxin, demonstrated its potential to improve mucosal immunity. ((203), (227)) A recent study with fractional IPV adjuvanted with dmLT administrated in healthy IPV-only vaccinated adults via intradermal route showed boosting of serum nAB to the 3 polio serotypes with significant increase to serotype 1. However, only very low or absent levels of fecal neutralization antibodies and serotype-specific IgA were observed. (225)

In our study CVIA065 (EudraCT 2019-002415-25) IPV-only vaccinated subjects were randomized to receive 1 intramuscular dose of IPV + dmLT, IPV or bOPV at Day 1, followed by a challenge dose of bOPV at Day 29 to assess safety and immunogenicity (humoral and mucosal) of IPV+dmLT in comparison with IPV.

7.1.3.1 Safety

Local reactions after IPV and IPV + dmLT were well tolerated and of short duration. Frequency of solicited and unsolicited systemic reactions were comparable between the 3 groups except a significant higher frequency of moderate or severe solicited systemic AEs at Day 1 for IPV + dmLT. For all 3 groups the duration of these solicited systemic events (mostly headache and fatigue) was longer than 7 days but all resolved by Day 15. Unsolicited adverse events were comparable in frequency between IPV and IPV + dmLT and similar to bOPV. All treatment-related AEs were mild except for 1 moderate and 1 severe AE, which occurred in the same participant who was dosed with IPV+ dmLT. The severe related AE consisted of severe transient elevated AST level which resolved spontaneously within 10 days. No serious adverse events or withdrawals due to adverse events occurred.

Overall, we can conclude that the administration of IPV+ dmLT was safe and well tolerated.

7.1.3.2 Humoral immunogenicity

As all subjects were fully IPV primed baseline titers were high for all 3 serotypes with seroprotection >95% for all 3 groups. Boosting response with high levels of humoral neutralizing antibodies and seroconversion for all 3 serotypes of >80% was seen in both IPV groups and similarly for type 1 and 3 in the bOPV group. Addition of dmLT to IPV did not improve seroprotection or seroconversion over IPV alone. Moreover, levels with IPV alone were generally higher than IPV+ dmLT.

7.1.3.3 Stool viral shedding

After bOPV challenge, viral stool shedding was generally highest at Day 4 for all groups. While lower rates of shedding were seen for the bOPV group especially for serotype 3, no differences in shedding rate were seen between IPV and IPV+ dmLT groups through Day 57. At Day 8 the relative risk was at or near 1 comparing IPV and IPV+ dmLT, suggesting absence of differences in viral shedding for poliovirus types 1 and 3 by adding dmLT to IPV. Also, time to cessation was similar between both IPV groups for serotype 1 as well as serotype 3.

7.1.3.4 Intestinal immunity

Only few fecal neutralization responses were seen across the 3 groups and also fecal IgA changes were observed in a small number of participants but with no meaningful difference in type specific responses for both IPV groups. These results are consistent with other studies of IPV in adults and of intradermal fractional IPV + dmLT. ((228), (225))

IgG antibody secreting cells (ASC)[2] show clear, type-specific responses to vaccination and challenge. Overall, these were higher for both IPV groups: at Day 8 shown in 90% and 74.1 % of IPV and IPV + dmLT groups respectively for serotype 1, 56.7% and 51.9% for serotype 2, and 40.0% and 40.7% for serotype3. In the bOPV group percentages were lower at 50, 10 and 20 for serotype 1, 2 and 3, respectively. As $\alpha_4\beta_7$integrin gut homing ASCs currently is investigated to serve as surrogate marker for polio vaccine-induced mucosal immune protection these cells were also assessed in this study. Modest levels of IgG and IgA ASCs expressing $\alpha_4\beta_7$ integrin were seen against type 1 and a few to serotypes 2 and 3 but not sufficiently for further analysis.

In conclusion, we demonstrated in our study that intramuscular administration of 1 dose of IPV + dmLT was safe and well tolerated in adults, but no beneficial effect of dmLT addition to IPV could be demonstrated for humoral or intestinal immunity.

7.2 Strengths and limitations

A **strength** of this thesis is that we were able to evaluate 2 cohorts of 15 subjects for 28 days in contained conditions. Although the number of participants was low, this allowed us to follow up these subjects closely after vaccination, not only for safety but also for viral shedding. Subjects were daily checked during medical visits and as such also constantly reminded on the purpose of the study, hence the large number of daily stool samples that were provided. Being able to process them very fast after collection has definitively contributed to better detection of viral load, especially in case of low viral titer. In addition, also shedders beyond D28

[2] ASC are differentiated B-cells (plasma blasts) which later on evolve into tissue plasma cells to produce antibodies. In response to antigen exposure some ASC will migrate from the primary lymph nodes to effector lymphoid tissues and are transiently present in the blood. Assessment of circulating antigen-specific ASC expressing mucosal homing receptors, such as $\alpha_4\beta_7$ gives an indication of recent exposure to antigens in mucosal tissues. (266)

were asked to provide daily 1 stool sample which allowed us to investigate shedding characteristics of both nOPV2 candidates intensely.

Furthermore, the strong commitment of the personnel has largely contributed to the almost absence of drop-out in this study: only 1 subject declined to spend the night of Day 27 in the unit but returned the next morning to complete all assessments as defined by the protocol. Subjects had daily medical visits throughout the 4 weeks of containment, not only to check their physical health but also for mental support. In addition, weekly meetings with the whole cohort were organized by the investigators to give the participants feedback of study status and background information about global cVDPV2 outbreaks. In this way, subjects were reminded of the objectives of the study and felt more involved in the project.

This experience of conducting a trial in contained conditions has served as basis for the current Vaccinopolis facility which includes a unit for controlled human infection model (CHIM) trials.

Another strength is the exhaustive collaboration between many stakeholders and their joined efforts to push the program forward, which has determined the speed with which this entire project has been carried out.

Also, all serological blood samples and stool samples collected across all studies were analyzed at the same laboratory in the US (CDC) and similarly all neurovirulence tests and genetic sequencing were carried out at Viroclinics using the same methods for all studies.

A strength regarding safety assessment is the fact that elevation of lab parameters (CK and liver enzymes) was detected as a safety signal in the phase 1 UAM4a study. Furthermore, because of this finding we adapted the design of the phase 2 study UAM4 and were able to justify our conclusion of bias by extensive physical exercises.

In addition, several inspections by the Belgian regulatory authorities (FAMPH) were conducted. The Poliopolis phase 1 unit was inspected in June 2017 regarding its fulfilment of legal and regulatory obligations of good clinical practice. During the UAM4 study an inspection took place in November 2018 to verify whether the study was conducted in accordance with the WHO GAPIII and in April 2019 a GCP inspection at the Poliopolis site verified that all study documents met legal and regulatory obligations of good clinical practice (Belgian Law of 7 May 2004 related to experiments on humans, and the applicable royal decrees, European guidelines

in Eudralex vol.10 and ICH guideline E6). These inspections contributed to improving both sites internal procedures and our oversight as a sponsor and ensured high quality of the conducted studies. No major findings were observed at the Poliopolis site.

A **limitation** of the infrastructure was the absence of individual toilets which made that bathrooms had to be shared by 3 to 4 men each (only applicable to the male participants as the number of female volunteers was sufficiently small in both cohorts they didn't need to share). As in both cohorts a substantial number of participants was still shedding at Day 28 and some re-started shedding after 3 consecutive negative days, it is possibly a consequence of re-infection.. However, this risk was assumed to be very low as toilets were disinfected after each use, all participants within the same cohort had received the same oral vaccine (no placebo groups) and induction of mucosal immunity is inherently linked to cessation of shedding. This recent established intestinal immunity was assumed to protect the subjects from re-infection with the same strain in the timeframe so shortly after vaccination. However, in the phase 2 study a second dose of nOPV2 at D28 showed indeed a decrease in numbers of shedding participants and magnitude of the shedding, indicating intestinal immunity was induced but the shedding number was not zero. (229)

Because of the global OPV2 withdrawal in 2016 no direct comparison could be made between nOPV2 and mOPV2 recipients and this is a limitation of the study methodology. To accommodate this as much as possible the UAM1 study was specifically designed to collect sufficient data to compare with as soon as the nOPV2 candidates would be ready to be tested, so both studies have an almost identical design. The same volunteer population in Belgium was used, be it with 1 additional center (CEVAC, Ghent) participating in the M4 study, which was reflected in similar demographics for the OPV-primed subjects of both studies.

A limitation for safety assessment in these comparative studies was the inevitable open label design for the OPV-primed subjects. As they knew the very good safety profile of the Sabine vaccine and the global use of it for many decades may have unintentionally influenced the determination of adverse events to mOPV2 to a lower rate than if participants could have been randomized at the same time to nOPV2. With regards to the IPV group, the numbers of volunteers in the different groups were rather low with the consequence that only large differences in safety parameters compared to the placebo group would be detected. However, numbers were sufficient to ensure the absence of safety signals for lab

parameters. In contrast, immunogenicity samples were not analyzed until both studies had been completed in order to investigate them at the same time and in a blinded way by the same lab.

While vaccine associated paralytic polio is the main adverse event associated with mOPV use the rarity of this event (4/million births) and the fact that all participants were fully vaccinated against polio makes it impossible to detect this in a clinical trial. The improved genetic stability we observed in the samples collected in these studies are encouraging but will have to be further evaluated in children and infants.

A limitation for the immunogenicity results observed in UAM4a as well as in UAM4 study is the higher dose level (10^6 $CCID_{50}$) evaluated for nOPV2 candidates relative to the dose level of mOPV2 ($\geq 10^5$ $CCID_{50}$). This might give an optimistic view of the immunogenicity comparison between nOPV2 and mOPV2 and may represent a higher dose than eventually might be released. The higher dose was chosen to be evaluated in adults for safety before proceeding to dosing in children and infants, who would be randomized to receive one of both dose levels 10^6 $CCID_{50}$ or 10^5 $CCID_{50}$.

A limitation regarding neurovirulence is the low number of samples that could be tested in the modified transgenic mouse test, especially for nOPV2-c2 because reduced shedding resulted in fewer EES. However, in none of the genetically analyzed samples domain V was reversed. In addition, strains have been molecular cloned in the lab to incorporate combinations of mutations seen individually in samples of shed nOPV2 candidates and also in these combinations of variants neurovirulence remains much less than Sabin 2 vaccine with only the S481G reversion of domain V. (220)

A limitation regarding the study with adjuvanted IPV was the fact that it has been performed in adults while studies investigating mucosal immunity determinants are suggesting that the ability of generating mucosal immunity might wane with age and therefore adults might not be sufficiently representative and further tests in children will be needed to gain more conclusive information.

Another limitation of the CVIA065 study is that only 1 dose level has been tested in individuals that were already highly seropositive at baseline. As the vaccination was very well tolerated exploration of higher dose levels can be considered for further evaluation.

7.3 What happened next

Based on the promising results of the adult studies the evaluation of both nOPV2 candidates could be continued at lower ages. Phase 2 studies with low ($10^5 \log_{10}$ $CCID_{50}$) and high ($10^6 \log_{10}$ $CCID_{50}$) doses have been conducted in 100 children (1-4 years old) and 574 infants (18-22 weeks old) at several sites in Panama from Sep 2018 to Sep 2019 (ClinicalTrials.gov, NCT03554798). (230) Similar to the adult studies a historical control study with mOPV2 (50 children and 110 infants) was performed in 2015-2016 before tOPV withdrawal (ClinicalTrials.gov, NCT02521974). (231) Because of the nOPV2 withdrawal in 2016 the immunization history of the children was different between the 2 studies. In the historical study with mOPV2 most children had received tOPV and a minority IPV-only, while in the study with nOPV2 the majority of children had a bOPV/IPV immunization history. All infants were primed with 3 doses of bOPV + 1 IPV. These studies confirmed the safety and tolerability of both vaccine candidates and no elevated liver enzymes or creatine phosphokinase were observed, which confirmed the results of the phase 2 adults studies. In children, seroprotection rates were high already at baseline (100%, 100% and 94% for mOPV2, nOPV2-c1 and nOPV2-c2 respectively) and were 100% for all groups at Day 28 after 1 dose. In infants, seroprotection rates at D28 after 1 dose were 94% for mOPV2, 93% and 94% for low and high dose of nOPV2-c1 respectively, whereas 91% and 95% seroprotection was reported for low and high dose of nOPV-c2. As such, the predefined non-inferiority criterion for seroprotection at D28 in comparison with mOPV2 was met for both low-dose and high dose of nOPV2-c1. However, regarding nOPV2-c2 this was only the case for the high dose as the seroprotection level for low dose exceeded (although minimal) the allowed deviation of 10%(-10.6%). (195) Due to differences in polio vaccination history shedding in children after mOPV2 was less and not comparable with nOPV2 data. ((195), (232)) Viral shedding rates in infants (PCR positive) were similar between mOPV2 and nOPV2 candidates in the first week after first dose but proportions with infectious virus ($\log_{10} CCID_{50} \geq 2.75$) were lower for both nOPV2 candidates low and high doses and nOPV2 showed lower shedding rates towards D28, which was promising for potential reduced transmissibility. (221) Genetic sequencing and neurovirulence test on the samples of the children showed the stability of the novel domain V. Genetic changes in other regions were not unexpected and may increase virus fitness and virulence after several weeks of intestinal replication but differed greatly from the fast reversion of Sabin-2 with high virulence of viruses seen in samples after Day7. Also, paralysis of mice was accordingly much lower with shed nOPV2 than after mOPV2 vaccination. (232) Ultimately, these data were

confirmed in the shedding samples of the infants in which the most virulent samples after nOPV2 were found at Day28 and showed a much lower neurovirulence than partially reverted Sabin samples at Day7. Although data were limited to this relative short duration after vaccination the difference in genetic stability for domain V and neurovirulence with mOPV2 indicated a reduced risk for VAPP (usually occurring within 21 days post-vaccination) and lower likelihood to generate cVDPVs. (233)

While these studies were going on cVDPV outbreaks increased tremendously. While in 2016 only 2 countries where known with cVDPV2 transmission this number increased each year and by the end of 2020 cVDPV2 was circulating in 30 countries. Genetic sequencing demonstrated that the first few outbreaks shortly after the switch were linked to supplementary vaccination campaigns with tOPV in high risk populations. These outbreaks were expected because low routine immunization and immunity were recognized in these areas. However, subsequent emergences were increasingly linked to mOPV2 used as outbreak response after the global OPV2 withdrawal and concerningly, transmission spread to neighboring countries that had not reported cVDPV2 circulation before. (234) cVDPV2s that share 4 or more nucleotide mutations in VP1 divergent from mOPV2 are classified in the same genetic emergence group. During this period 2016-2020 68 genetic cVDPVs emergences were identified, with the highest increase (40 new emergences) in 2019, in total responsible for almost 1600 AFP cases. Several factors played a role in this evolution, such as poor routine immunization, global IPV shortage up to 2018 and the rapid waning of population polio type 2 mucosal immunity after cessation of tOPV. From 2020 onwards, the situation became worse because of the covid pandemic, which interrupted significantly routine and supplementary immunization activities. (235) It became clear that globally the situation was deteriorating and that use of mOPV2 was rather paradoxical: the only way to interrupt outbreaks was through the use of mOPV2 but with the inherent risk of seeding new cVDPV2s. (234)

Proactively, in 2018 already discussions between the nOPV2 consortium and WHO started to define which data would be required to allow emergency use authorization by the relevant National Regulatory Authority and Emergency Use listing (EUL) by the WHO of the selected nOPV2 vaccine. EUL is time limited risk-benefit assessment for emergency use of vaccines (or other medicinal products) when a Public health Emergency of International Concern (PHEIC) is in place and no sufficient data are yet available for WHO prequalification. Although the EUL is only granted after a comprehensive scientific risk/benefit assessment involving

quality, safety, and immunogenicity data it is not the same as a prequalification and each country can choose to deploy nOPV2 under EUL or not. (236)

The application process is based on a rolling submission with agreed timelines between WHO and the manufacturer and nOPV2 assessment would start once sufficient clinical phase 2 data of children and infants would be available. To choose between the 2 nOPV2 candidates selection criteria for safety, genetic stability and immunogenicity were predefined but also manufacturing data were very important. To be able to produce sufficient quantity of vaccines by the end of 2020, when EUL was expected, the choice of vaccine candidate had to be made in 2019 while the phase 2 studies were still running. The EUL timelines could only be met with production of low dose (10^5 $CCID_{50}$) of either candidate. Because preliminary data indicated higher potency of nOPV2-c1 at that dose decision was taken to start at-risk, at-scale production of this candidate vaccine. As soon as immunogenicity data of infants became available which showed inferiority of nOPV2-c2 for seroprotection at low dose compared to mOPV2 the selection of nOPV2-c1 became definitive. (131)The EUL assessment started Feb 2020 and after thorough review of all clinical data and rigorous inspections and follow up of the manufacturing and production processes eventually, on 13 Nov 2020 nOPV2 became the first vaccine for which EUL was issued by the WHO. (236) By that time 200 million doses were manufactured by Biofarma (Indonesia), ready to be distributed in the first countries that agreed to nOPV2 use for outbreak response and had met the verification requirements established by the GPEI and endorsed by SAGE. In order to qualify countries should demonstrate VDPV2 detection and have an efficient system in place to acquire and distribute vaccine in case of outbreak. They should have the capacity of adequate environmental and clinical surveillance, with specific attention to AFP and AESI surveillance and the ability to respond appropriately to an unanticipated finding. Use of nOPV2 should be spread minimally 12 weeks from Sabin OPV campaigns in the same area and preferably at least 6 weeks from bOPV campaigns in the same area. ((131), (237)) At the same time broad communication actions should be undertaken to ensure population acceptance of the new vaccine.

In March 2021 the initial phase of nOPV2 roll-out started with Nigeria and Liberia as the first countries, followed by Benin and Congo. By October 2021 more than 65 million doses were administrated in these 4 countries in response to outbreaks and an independent safety review of these data didn't reveal any major safety concern. Based on this positive review the most stringent initial requirements could be lifted and on 11 Oct 2021 nOPV2 became the vaccine of choice to

respond to cVDPV2 outbreaks. More countries could now become eligible to use the vaccine and by Sep 2022 more than 500 million doses in more than 20 countries had been used. Post-deployment monitoring requirements as outlined by EUL were still maintained. In the meantime safety, immunogenicity and shedding data of a larger phase 2 trial (N = 330 infants) in polio-vaccine naïve infants in Bangladesh conducted between Sep 2020 and Aug 2021 became available and confirmed the promising data of the previous studies. Two doses of nOPV2 in naïve infants induced seroprotective titers of neutralizing antibodies by week 8 with low amounts of shedding and no safety signals were reported. (238)

In April 2022 real world safety data of the first 100 million doses were reported by SAGE and confirmed again the results of the clinical studies. Only 6 reported AESIs were considered related to nOPV2, of which 3 recovered completely (anaphylaxis, allergic reaction and meningoencephalitis). The other cases were VAPP incidents after initial vaccination of 44 million children, corresponding to a rate of 0.007 cases per 100 000 nOPV2 vaccinees which is much less than the expected 0.025-0.4 cases per 100 000 after Sabin OPV vaccination. (237) Genetic sequencing of environmental isolates demonstrated the improved genetic stability of the vaccine with no reversion in domain V and low recombination rates. (239) Breakthrough transmission of cVDPV after two outbreak response campaigns occurred only in a minority of the countries and the number of new genetic emergences of cVDPV2 seems to decline. (237)

However, the battle is not won yet. In February 2021 the first detection of cVDPV2 in WHO European region occurred when an outbreak emerged in Tajikistan with 36 paralytic polio cases. Viruses don't respect country borders, this was also apparent in the covid pandemic, and continuous migration of people can cause importation and spread of the vaccine-derived polioviruses in populations with insufficient coverage and background immunity. Due to global IPV supply shortages between 2016 and 2018 a substantial number of children in Tajikistan lacked the essential protection against PV2 and outbreak could only be stopped by implementing an initial round of IPV vaccination followed by national and subnational nOPV2 campaigns. These nOPV2 immunization rounds resulted in 95% coverage of all children below 6 years and a serological survey in 228 children demonstrated a seroconversion of 77% after two nOPV2 doses with an increase of type2 seroprevalence from 26% at baseline to 83% after 2 doses. ((240), (241))

In addition, even with very low annual numbers now of wild type 1 viruses, as long as circulation is present in even 1 country, risk of importation to another country

with low vaccination coverage is real. This was demonstrated in Feb 2022 in Malawi when the genetic sequence of the virus of a paralytic young child confirmed wild type 1 polio virus, could be linked to a sequence detected in Pakistan in 2020. Africa had been declared wild type polio free only in August 2020, yet 1 year later (the child became paralyzed in Nov 2021) it was imported again. As the family didn't travel to Pakistan and knowing that >90% of polio infections remain asymptomatic it was clear that silent circulation of WP1 was going on. Low vaccination coverage (<80%), absence of recent catch up campaigns and unavailability of IPV between 2016 and 2018 created susceptible populations. In addition, AFP- and environmental surveillance was insufficient although cVDPV2 circulation was recognized in certain areas. A national health emergency was declared immediately and several rounds of immunization campaigns were initiated, also in the neighboring countries to prevent further spread. (242)

Nevertheless, in May 2022 a first case with the same WPV1 strain in Mozambique was reported. In addition to the low immunization coverage and surveillance gaps this country has geographically even more constraints to reach all children in remote areas and several new cases followed despite intense catch up efforts. Up till now Africa's polio-free certification is unchanged because the circulating strain is not indigenous but it is of huge importance to stop further spread as soon as possible, and lower the proportion of susceptible people. (243) As of Aug 2022 no new cases WPV1 in both countries(Malawi and Mozambique) have been detected although cVDPV1 cases still occurred end of 2022.

In 2022 a series of reported events indicated that industrialized countries also cannot lean back and rely on overall high vaccination coverage with IPV. In sequential UK sewage samples collected from the London Beckton sewage treatment works between Feb and July 2022 a substantial number of genetically linked poliovirus isolates related to the serotype 2 Sabin strain were detected. All isolates were recombinants with a species C enterovirus and from May 2022 onwards some of them met the definition of cVDPV2 with 6 to 10 nucleotide changes in the VP1 capsid gene. The increasing number over time indicated ongoing transmission in this area. (244) The UK switched to IPV immunization in 2004 and has an overall coverage of 95% for children of 5 years old, however in some areas in London primary vaccine coverage was well below the WHO recommended coverage and reached only 87,1%, 87,4% and 90.3 % for 1, 2 and 5 years old, respectively, with a pre-school booster uptake of 72.8%. ((245), (246)) In addition, since October 2012 the UK recommends an IPV containing pertussis vaccine to pregnant women which induces higher neutralizing passive antibody

titers in infants at 2 months of age but much lower seroconversion rates at 5 months of age (18-44% vs. 71-92%) and lower seroprotection rates at 13 months of age compared to infants of unvaccinated mothers (32-63% versus 70-91%) due to blunting as showed by Grassly et al. (247) With an uptake of IPV-dTpa of 64% in pregnancy and the next booster in childhood only at 3-4 years an important immunity gap in this young population of children is created. (247)While IPV generates an excellent humoral protection it induces only poor mucosal response and after importation of wild or Sabin polioviruses silent transmission can continue for longtime if no efficient environmental surveillance system is in place as demonstrated already through WP1 circulation detected in sewage under high IPV coverage in 2013 in Israel. (248) Fortunately, in London the surveillance system alerted in time to initiate catch-up campaigns and prevent actual paralytic cases. (249) Since November 2022 no further VDPV2 isolates have been detected in London sewage but catch-up campaigns offering IPV and other childhood immunizations will be continued in 2023 through primary school and community clinics to reach unvaccinated and under-vaccinated children between 1 and 11 years. (250)

Only few months later, in July 2022 paralytic polio caused by cVDPV2 was diagnosed in an unvaccinated young adult in Rockland County, New York. Overall polio vaccination coverage (IPV since 2000) with 3 doses in US by Aug 2022 was 92.7% but for New York State 79,0%. (251) In addition, some NY counties reach much lower percentages as was the case in Rockland county with an overall coverage of 60.3% in children of 2 years age and in some districts even as low as 37.3%. Wastewater surveillance showed silent spread already for several months, also in 2 neighboring counties and genetic sequencing revealed linkage with the cVDPV2 positive sewage samples detected in the UK as well as in Israel some months earlier. (252) More than 15% of Rockland County population is Jewish and extensive travel between the 3 countries poses a substantial risk for importation of Sabin derived strains. Despite countywide catch-up vaccination and information campaigns to the public the increased vaccine uptake was only temporary with no big improvement in general population coverage (252). As a consequence, in Feb 2023 again cVDPV2 positive sewage samples were detected in Rockland county while Israel recently confirmed 1 paralytic polio case and 3 asymptomatic ones. (253)

None of these countries use OPV in their immunization strategy, so the ongoing cVDPV2 circulation probably originates from a traveling shedding individual. This could only happen because of the low vaccination levels in these communities and

highlights the sustained threat of polio, wild type as well as cVDPV, as long as not every single child in the whole world is sufficiently vaccinated, regardless the country it is born in.

During 2021-2022, 88 active cVDPV outbreaks in 46 countries occurred, of which 76 were caused by cVDPV2. (254) In 2022, 30 WPV1 cases occurred versus 172, 657 and 1 cases caused by cVDPV1, cVDPV2 and cVDPV3 respectively. Compared to the 1082 cases cVDPV2 in 2020 there is a significant decline in number of cases but between 2021 and 2022 the decrease is minimal (682 cases in 2021 versus 657 in 2022). More than 75% of cVDP2 cases were detected in 2 countries, The Democratic Republic of Congo (DCR) and Yemen while DCR is one of the countries that suffers at the same time from cVDPV1 circulation. Strikingly, cVDPV1 cases increased immensely in 2022 compared to the 16 cases in 2021. (255)The covid pandemic interrupted significantly routine immunization and also preventive as well as outbreak response activities were delayed and often of poor quality during a long time period, which is reflected in a decrease of national vaccination coverage in many countries and the continued circulation of cVDPVs. New genetic emergences decreased with 9 and 5 new emergences in 2021 and 2022 respectively, probably due to extensive deployment of nOPV2 in 24 countries. More than 600 million doses of nOPV2 have been administrated already but challenges remain to ensure sufficient supplies to start new outbreak response on time. (254)

Nevertheless, in March 2023 the first cVDPV2 cases derived from nOPV2 have been diagnosed, six cases in DCR and one in Burundi. Genetic sequencing of isolates of these infected children and additionally, of 5 environmental samples in Burundi indicated 2 separate and new emergences of cVDPV2 linked to nOPV2 that originated in DCR. This is not unexpected as nOPV2 has shown a much lower risk to reversion but still contains live attenuated viruses capable to mutate and recombine with other enteroviruses. Two years after start of nOPV2 vaccination in outbreak response almost 600 million doses have been administered in 28 countries. Although these nOPV2 cases are unfortunate, if the same amount of doses would have been applied with Sabin OPV2 on average 30 to 40 new chains of type 2 viruses would have emerged instead of the 2 now. As these 2 cVDPV2 lineages are the only ones that emerged up till now this confirms the enhanced safety and stability of the new oral vaccine. (256) However, it also underlines the importance of high vaccination coverage as key message for polio control. DCR has a history of cVDPV2 outbreaks since 2005 with only a short break with no detections between 2013-2016. Since 2017 more than 19 outbreaks causing more

than 235 paralytic cases demonstrate the low overall vaccination coverage for 3th OPV dose of maximum 78% and often below 70% despite many supplementary immunization activities (SIAs) with mOPV2. As genetic sequencing indicated that most recent outbreaks originated in mOPV2 response to outbreaks conducted in 2018 or later it was decided to initiate in 2022 SIAs with nOPV2 and these will be continued in 2023 to increase type 2 mucosal immunity in the community. (257) At the same time strengthening of routine immunization is essential and second IPV dosage or IPV SIAs should be considered to boost humoral and mucosal immunity in previously OPV-vaccinated children. Enhanced efforts to increase AFP and environmental surveillance should be induced as well as improving of sanitary and hygiene conditions to reduce virus transmissions in these areas.

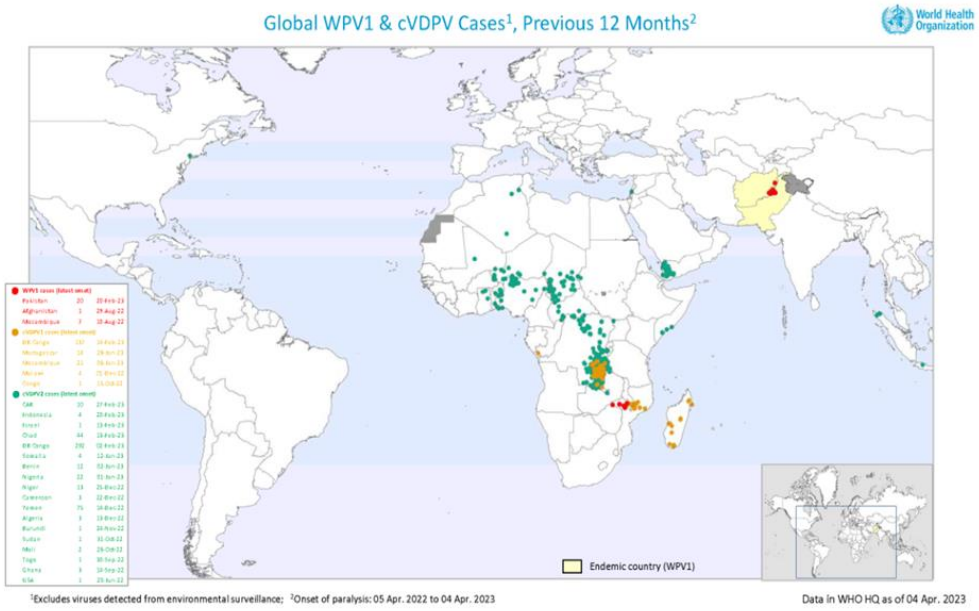
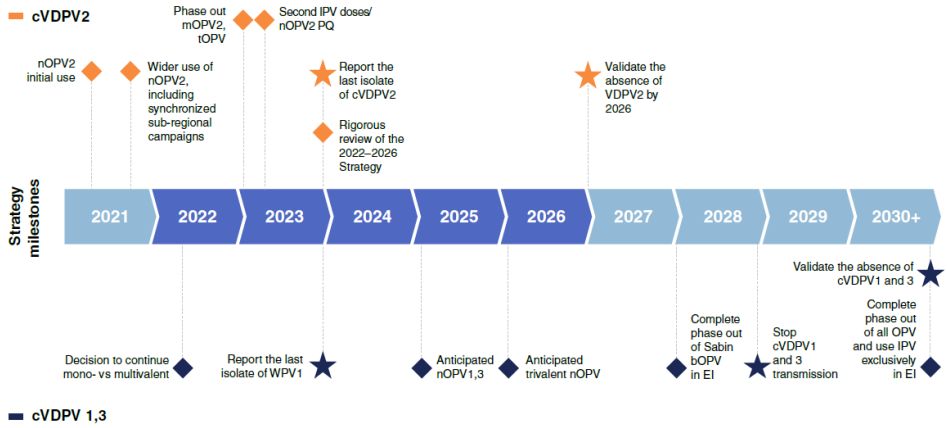


Fig. 2 GPEI-Polio Now, https://polioeradication.org/polio-today/polio-now/, consulted 9Apr2023 (258)

Next steps:

Currently, a large phase 3 nOPV2 study is going on in The Gambia to increase the data set for safety, immunogenicity and lot-to- lot consistency necessary for WHO prequalification and licensure by 2024. In the meantime, first studies have been conducted with nOPV1 and nOPV3 candidates in previously OPV- or IPV-primed adults in the US (NCT04529538) and a pediatric study with nOPV1 in children and infants is running in Bangladesh (NCT05644184). ((259), (260))

Recently, GPEI launched the 2022-2026 Strategic Plan for Polio Eradication. The first goal remains halting all WPV1 transmission in the last 2 endemic countries by end of 2023 with a projected certification of WPV1 eradication by 2027. Secondly, the program aims to stop all cVDPV transmission in order to gradually make a global switch to IPV-only immunization. To achieve both objectives the strategic plan will have to be diverse and include several action areas: political advocacy with increased ownership by national and provincial governments, improvement of community information to tackle vaccine hesitancy, implementation of new approaches to reach less accessible areas, wider use of nOPV2 with monitoring of quality vaccine delivery in all areas, increasing the capacity for good environmental and AFP surveillance and timely outbreak response and strong coordination with the routine immunization programs to identify zero-dose and under-immunized children. By implementing these intensified efforts one seeks to report the last isolate of cVDPV2 by the end of 2023, develop novel OPV vaccines for types 1 and 3 by 2026 and stop all cVDPV transmission in 2028. If successful, transition to IPV-exclusive essential immunization can start by 2030. Of course, this strategy contains many risks and progress as well as hurdles will be continuously monitored to implement corrective and mitigation measures as appropriate. (261)



bOPV = bivalent oral polio vaccine; cVDPV1 = circulating vaccine-derived poliovirus type 1; cVDPV2 = circulating vaccine-derived poliovirus type 2; cVDPV3 = circulating vaccine-derived poliovirus type 3; EI = essential immunization; IPV = inactivated polio vaccine; mOPV2 = monovalent oral polio vaccine type 2; nOPV1,3 = novel oral polio vaccine types 1 and 3; nOPV2 = novel oral polio vaccine type 2; OPV = oral polio vaccine; PQ = prequalification; tOPV = trivalent oral polio vaccine; VDPV2 = vaccine-derived poliovirus type 2; WPV1 = wild poliovirus type 1.

Source: WHO.

Fig.3 GPEI_Polio Eradication Strategy 2022-2026, https://polioeradication.org/gpei-strategy-2022-2026/ (261)

7.4 Conclusion:

In this thesis I demonstrate that both nOPV2 candidates are safe and show non-inferior immunogenicity in comparison with mOPV2. In addition, in the presented studies both candidates demonstrated enhanced genetic stability of shed viruses with low neurovirulence in animal testing and no reversion of domain V, the most dominant mutation site. These results have led to further testing in children and infants with ultimate selection and roll-out of the current nOPV2 vaccine.

Currently, WPV2 and WPV3 are eradicated and WPV1 circulation is reduced to sub-areas of 2 endemic countries. Yet, due to waning type 2 immunity and insufficient vaccination coverage, many countries have struggled in the last few years with increasing outbreaks of cVDPV2. These communities could only rely on mOPV2 use for outbreak response, although the risk of seeding new cVDPVs exist when insufficient number of children are reached. The development and fast distribution of nOPV2 in many countries affected by cVDPV2s can change this. In addition, the development and EUL (Emergency Use Listing) process of this novel vaccine has paved the way for much faster development of other more genetically stable vaccines for polio type 1 and 3. Only by eliminating cVDPV outbreaks we

will eventually be able to stop OPV use and move on to IPV use only. The ideal IPV vaccine would also induce mucosal immunity and we investigated one possible candidate adjuvanted with dmLT, though with negative results and further research will be needed.

The nOPV2 has proven to be a very important vaccine that due to its enhanced genetic stability and safety profile can be one of the final keys to global polio eradication. If cVDPVs can be strongly reduced (eliminated) the transition phase to IPV only vaccination can be further continued in a much safer way. Yet, a vaccine is only effective when administered. The risk of reversion is lower than with mOPV2 but the longer the vaccine viruses can circulate chances to reversion and recombination with other enteroviruses increase. Therefore, enhanced efforts to reach sufficiently high national vaccination coverage with specific attention to communities hard to reach and underserved remain key priority in our goal to global polio eradication.

Dankwoord

Graag wil ik iedereen bedanken die heeft bijgedragen tot het tot stand komen van dit proefschrift.

Nieuwe vaccins kunnen enkel ontwikkeld worden als vrijwilligers bereid zijn in onze studies te stappen. Daarom wil ik graag alle deelnemers bedanken die aan deze studies hebben deelgenomen. Tijd maken en zich inzetten om iets bij te dragen aan de maatschappij is sowieso niet evident in onze vaak jachtige samenleving maar deze studies in het bijzonder, vroegen nog net dat tikkeltje meer. Ik wil dan ook zeker onze deelnemers aan de quarantainestudie extra in de spotlights zetten. Dank jullie wel voor de interesse en het enthousiasme waarmee jullie hebben deelgenomen. Niet alleen tijdens jullie verblijf in Poliopolis maar ook tijdens de (soms zeer lange) opvolgtermijn voelden wij ons gesteund door jullie onverminderde inzet en begrip voor onverwachte resultaten. Bedankt voor de tientallen stalen die elk van jullie ons bezorgde, het zijn waardevolle bijdragen geweest.

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