

nOPV2: Clinical Development and Evidence Summary

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What is nOPV2 and why is it needed?

The oral polio vaccines (OPV) that are used to prevent or interrupt polio outbreaks contain attenuated (weakened) strains of the live poliovirus. The weakened vaccine virus induces protective immunity against paralytic poliomyelitis through the generation of antibodies. Additionally, it replicates in the human gut and is excreted primarily via stool in communities. This replication and excretion in communities is beneficial in most circumstances: as the vaccine virus induces mucosal immunity at the site of viral replication (in the gut) and is shed for several weeks, it can then pass between individuals in the community during this period—providing secondary or “passive” immunization against the poliovirus.

However, in rare circumstances, OPV viruses may mutate in such a way that they regain virulence comparable to wild polioviruses. Because these polioviruses are derived from the attenuated vaccine virus, they are called vaccine-

derived poliovirus, or VDPV. In areas with persistently low population immunity to the poliovirus, VDPVs can circulate between individuals within a community, and are then called **circulating vaccine-derived poliovirus, or cVDPV**.¹²³

The novel oral polio vaccine type 2, or nOPV2, is a modified version of the monovalent oral polio vaccine type 2 (mOPV2), the oral polio vaccine that has been used to respond to outbreaks of type 2 cVDPV following the 2016 global “switch” from the use of trivalent OPV (tOPV) to bivalent OPV (bOPV) in routine immunization.⁴ nOPV2 is similar to mOPV2, but with increased genetic stability conferred through modifications to targeted sites on the vaccine strain’s genome. The increased genetic stability of the vaccine can help reduce the risk of vaccine-associated paralytic poliomyelitis (VAPP) and decrease the likelihood of generating new type 2 cVDPV, while maintaining the immune protection that is provided by the original vaccine. It will ensure that the benefits associated with using an oral polio vaccine (i.e., individual and community protection) can continue while greatly decreasing the likelihood of mutations that lead to virulence in the vaccine virus—meaning that nOPV2 can help reduce the risk of continued cVDPV2 outbreaks.

How was nOPV2 developed?

Early development activities for nOPV2 began over ten years ago, led by a consortium of experts who had previously conducted studies to understand the molecular basis of the attenuation of OPV vaccine strains as well as the genetic mutations that had caused these vaccine strains to become virulent in some cases.⁵ These insights were harnessed to engineer nOPV2 vaccine strains that preserved the essential characteristics of the mOPV2 vaccine (i.e., attenuation, antigenic and immunogenic characteristics) while increasing its genetic stability (i.e., reducing the risk of the vaccine virus losing its attenuation at key sites linked with virulence).

While two candidates were developed, one (often referred to as nOPV2 candidate 1, or c1, henceforth referred to as nOPV2 unless otherwise noted) was selected following a comprehensive assessment of pre-clinical, clinical, and

¹ VDPVs are defined as OPV virus strains that are > 1% divergent (or ≥ 10 nucleotide changes, for types 1 and 3) or > 0.6% divergent (≥ 6 nucleotide changes, for type 2) from the corresponding OPV strain in the complete VP1 genomic region. Source: Global Polio Eradication Initiative. Classification and reporting of vaccine-derived polioviruses (VDPV): GPEI guidelines. Geneva: World Health Organization; 2016 (http://polioeradication.org/wp-content/uploads/2016/09/Reporting-and-Classification-of-VDPVs_Aug2016_EN.pdf, accessed 3 January 2021). Note that the type 2 threshold is lower to enable early detection of cVDPV2 outbreaks (Source: Lopalco PL. Wild and vaccine-derived poliovirus circulation, and implications for polio eradication. *Epidemiol Infect.* 2017 Feb; 145(3):413-419. doi: 10.1017/S0950268816002569).

² Note: This differs from vaccine-associated paralytic polio, or VAPP. VAPP is a rare adverse event associated with polio vaccination that occurs in OPV recipients or their close contacts. Risk of VAPP is highest after the first dose and sharply decreases with the administration of subsequent doses. Source: Lopalco PL, 2017.

³ cVDPVs are defined as VDPV isolates for which there is evidence of person-to-person transmission in the community. More specifically, they are genetically linked VDPVs which are isolated: i) from at least two individuals (not necessarily AFP cases), who are not direct (i.e., household) contacts, ii) from one individual and one or more environmental surveillance (ES) samples, or iii) from two or more ES samples if they were collected at more than one distinct ES collection site (no overlapping of catchment areas), or from one site if collection was more than two months apart. Source: Global Polio Eradication Initiative. Classification and reporting of vaccine-derived polioviruses (VDPV): GPEI guidelines. Geneva: World Health Organization; 2016 (http://polioeradication.org/wp-content/uploads/2016/09/Reporting-and-Classification-of-VDPVs_Aug2016_EN.pdf, accessed 3 January 2021).

⁴ For more on the “switch,” see: World Health Organization. *Weekly Epidem Rec.* 11 Dec 2015; 90(50):681-700 (<https://apps.who.int/iris/bitstream/handle/10665/254536/WER9050.pdf?sequence=1&isAllowed=y>, accessed 26 May 2022). For more on prior poliovirus type 2 outbreak response protocols using mOPV2, please consult previous response protocols such as: Global Polio Eradication Initiative. Standard operating procedures: Responding to a poliovirus event or outbreak. Part 2: Protocol for poliovirus type 2, effective 01 November 2017 until 30 April 2018. Geneva: World Health Organization; 2017 (<https://polioeradication.org/wp-content/uploads/2018/01/pol-sop-responding-polio-event-outbreak-part2-20180117.pdf>, accessed 26 May 2022).

⁵ nOPV2 program partners include: PT Bio Farma, the University of Antwerp, Fighting Infectious Diseases in Emerging Countries (FIDEC), icddr, PATH, the University of California, San Francisco, the UK National Institute for Biological Standards and Control, the US Centers for Disease Control and Prevention, the US Food and Drug Administration, and the partner agencies of the Global Polio Eradication Initiative (World Health Organization, Rotary, US Centers for Disease Control and Prevention, UNICEF, the Bill & Melinda Gates Foundation, and Gavi).

manufacturing information across vaccine candidates.⁶ The selected strain carries key modifications to the genome (as compared to mOPV2). These modifications were tested through a number of pre-clinical methods (e.g., serial passaging, cell culture assays used to estimate temperature sensitivity, testing in transgenic mice models) before initiating clinical development in 2017. More information on the modifications to the selected nOPV2 strain is provided in Table 1, for reference.⁷

Table 1. Modifications made to the mOPV2 virus genome to generate nOPV2 c1, the nOPV2 candidate strain selected for full clinical development and Emergency Use Listing (EUL) submission

Modification	Scientific Rationale
A restructured, genetically stabilized RNA stem-loop structure in the 5' noncoding region, known as domain V or <i>domV</i> , called S15domV	This site is the major determinant of OPV2 attenuation and often serves as a “gatekeeper” that leads to other mutations. For the mOPV2 virus, there is one specific A-to-G mutation at nucleotide 481 that results in increased tolerance to temperature and makes the virus more neurovirulent. The genetically stabilized S15domV has been designed to avoid loss of attenuation through single point mutations in domain V.
Relocated and modified an essential replication element called the cis-acting replication element within the 5' untranslated region (cre5)	To reduce the frequency of recombination events. Without this mutation, a single recombination event with another virus could potentially result in the replacement of the <i>domV</i> described above with the unattenuated <i>domV</i> from the other virus, making the resulting recombinant virus lose its attenuation and become more fit for viral replication. With this cre relocation and modification, a single recombination event that replaces the nOPV2 <i>domV</i> will also remove cre, inhibiting viral replication and thereby rendering the virus non-viable and non-infectious.
Introduced two amino acid substitutions: the High Fidelity (D53N) and Rec 1 (K38R) substitutions in the viral RNA-dependent RNA polymerase (3Dpol)	To limit the adaptive capacity of the virus by reducing mutation rate and improving replication fidelity (in the case of D53N) and by reducing recombination frequency (in the case of K38R).

⁶ When tested in infants and children in the Phase II studies in Panama (M5a and M5b), the second of the two nOPV2 candidate strains narrowly missed the non-inferiority criterion for immunogenicity (specifically, seroprotection) at the lower dose; and thus, this candidate strain (candidate 2, or nOPV2 c2) was not moved forward for further clinical development and submission to WHO for an Emergency Use Listing (EUL). For more details, see: Sáez-Llorens X, Bandyopadhyay AS, Gast C, De Leon T, DeAntonio R, Jimeno J, Caballero MI, Aguirre G, Oberste MS, Weldon WC, Konopka-Anstadt JL, Modlin J, Bachtiar NS, Fix A, Konz J, Clemens R, Costa Clemens SA, Rüttimann R. Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in children and infants: two clinical trials. *Lancet*. 2021; 397: 27–38. [https://doi.org/10.1016/S0140-6736\(20\)32540-X](https://doi.org/10.1016/S0140-6736(20)32540-X).

⁷ As will be described further in this paper, two nOPV2 vaccine candidates entered clinical development in 2017. The modifications described in Table 1 pertain to nOPV2 vaccine candidate 1, which was ultimately selected for further clinical development and EUL submission. 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. To learn more about the modifications to vaccine candidate 1 described in this table, please consult: Yeh MT, Bujaki E, Dolan PT, Smith M, Wahid R, Konz J, Weiner AJ, Bandyopadhyay AS, Van Damme P, De Coster I, Revets H, Macadam A, Andino R. Engineering the live-attenuated polio vaccine to prevent reversion to virulence. *Cell Host Microbe*. 2020 May 13;27(5):736-751.e8. doi: 10.1016/j.chom.2020.04.003. For more complete details on the modifications to candidate 2, see Konopka-Anstadt JL, Campagnoli R, Vincent A, Shaw J, Wei L, Wynn NT, Smithee SE, Bujaki E, Te Yeh M, Laassri M, Zagorodnyaya T, Weiner AJ, Chumakov K, Andino R, Macadam A, Kew O, Burns CC. Development of a new oral poliovirus vaccine for the eradication end game using codon deoptimization. *NPJ Vaccines*. 2020 Mar 20; 5:26. doi: 10.1038/s41541-020-0176-7. The candidate 1 and candidate 2 modifications are also summarized in Macklin G, Peak C, Eisenhower M, Kurji F, Mach O, Konz J, Gast C, Bachtiar NS, Bandyopadhyay AS, and Zipursky S, on behalf of the nOPV2 Working Group. Enabling accelerated vaccine roll-out for Public Health Emergencies of International Concern (PHEICs): Novel oral polio vaccine type 2 (nOPV2) experience. *Vaccine*. 2022 Mar 17; S0264-410X(22)00195-5. doi: 10.1016/j.vaccine.2022.02.050.

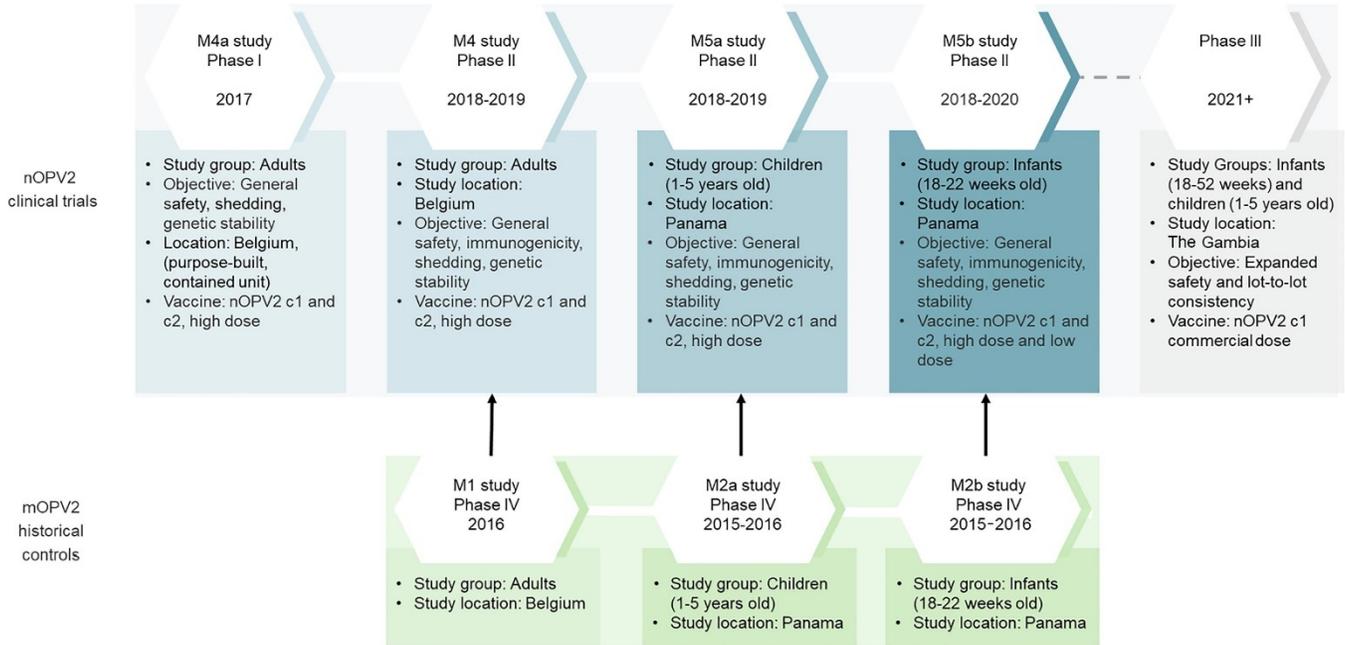
What clinical research framework was used to test nOPV2 and compare it to mOPV2?

nOPV2’s clinical development framework was designed to not only evaluate the safety, immunogenicity, and genetic stability of nOPV2 in different populations, but to also provide a comparative evaluation of nOPV2 to mOPV2. The framework evaluated nOPV2 in different populations, culminating in study populations that most closely represent nOPV2’s target population: children and infants with both OPV and IPV vaccination histories. In the trials that compare mOPV2 to nOPV2, nOPV2 is compared to historical control groups that received licensed mOPV2 vaccine, using similar study designs. Rather than concurrent trials, historical control trials were conducted with mOPV2 in 2015-2016 for future nOPV2 comparison. This was done in anticipation of mOPV2 containment guidelines that would come into effect in 2016 due to the withdrawal of the vaccine from national immunization schedules, which would preclude mOPV2 from being used in clinical trials.⁸

The number of subjects included in each study for the comparison group was determined to ensure sufficient statistical power to draw conclusions about whether criteria for non-inferiority compared to mOPV2 had been met. A unique feature of the trials is the extensive follow up and stool sampling: infants were followed for approximately 6 months after vaccination for long-term safety evaluation. Additionally, between 14 and 28 stool samples per subject were collected across different studies, enabling detailed assessment of shedding and genetic stability. This unprecedented scale of sampling and level of rigor for studies of this kind was implemented to help generate the most robust evidence base possible and ensure confidence in the studies’ conclusions.

The table below provides a snapshot of the most relevant aspects of the clinical framework.

Figure 1. Snapshot of the Key Trials in the nOPV2 Clinical Development Framework



⁸ The containment guidelines went into effect in 2016, following the “switch” from tOPV to bOPV. See: WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use. Geneva: World Health Organization; 2014 (https://apps.who.int/iris/bitstream/handle/10665/208872/WHO_POLIO_15.05_eng.pdf?sequence=1&isAllowed=y, accessed 26 May 2022).

Source: Macklin G, Peak C, Eisenhower M, Kurji F, Mach O, Konz J, Gast C, Bachtiar NS, Bandyopadhyay AS, and Zipursky S, on behalf of the nOPV2 Working Group. Enabling accelerated vaccine roll-out for Public Health Emergencies of International Concern (PHEICs): Novel oral polio vaccine type 2 (nOPV2) experience. *Vaccine*. 2022 Mar 17; S0264-410X(22)00195-5. doi: 10.1016/j.vaccine.2022.02.050. Fig. 1. nOPV2 Clinical Development Plan.

Additional phase II studies and control trials that were not prioritized for EUL data submission are not shown on this figure but are described in the text of that article and later in this paper.

What have nOPV2 clinical trials to date demonstrated in terms of the vaccine's safety, immunogenicity, and genetic stability?

Key conclusions across clinical studies are summarized below.

Safety: The data from these studies indicate that nOPV2 is well-tolerated in adults, young children, and infants. No safety concerns were identified from the available data.

Immune protection (immunogenicity): Immune responses are measured through seroprotection rates, seroconversion responses, and analysis of levels of neutralizing antibodies. Non-inferiority for seroprotection was established in clinical trials for both low-dose and high-dose potencies of nOPV2 (i.e., there was no significant difference in seroprotection rates between nOPV2 and mOPV2). Across all clinical studies, nOPV2 demonstrated robust immune responses with high seroconversion rates that were comparable with mOPV2.

Genetic Stability: Data to date indicate nOPV2's increased genetic stability compared to mOPV2. To assess genetic stability, researchers used next-generation sequencing (NGS) to analyze poliovirus genome from both vaccine lots and from shed stool following poliovirus vaccination and to describe the presence and nature of any polymorphisms (i.e., variations in the nOPV2 vaccine virus genome) that were identified. Results to date have shown that nOPV2's modifications have remained intact, and further details on NGS methodology may be found in a [2022 *npj Vaccines* paper](#), which describes how NGS is used to analyze stool, as well as a [2021 *Vaccine* paper](#), which describes how NGS can be used to identify and analyze polymorphisms in manufactured vaccine lots. To evaluate phenotypic stability, researchers used a common method for assessing loss of vaccine virus attenuation: mouse models derived from the WHO OPV lot-release assay that are used to evaluate the neurovirulence of polioviruses (i.e., to measure paralysis rates in transgenic mice after intraspinal inoculation of the amplified shed virus). Using this mouse model, the vaccine viruses isolated from the stool of participants after vaccination with mOPV2 or nOPV2 were able to be compared. In participants who receive mOPV2, the shed vaccine virus typically causes high paralysis rates in the mouse model after approximately 7 days. In contrast, nOPV2 shows limited mouse paralysis associated from stool samples collected across the clinical studies, regardless of the age of the study participants.

Shedding: Data from adult studies showed comparable rates to mOPV2 within the first week of administration, and then significantly lower levels of shedding within 28 days after vaccination. Data from the M5a study published in the *Lancet* demonstrated that in children aged 1-5, similar proportions of children shed whether they received mOPV2 or one high dose of either of the nOPV2 candidates, with a comparable rate of shedding to nOPV2 by day 28; however, children had significantly higher peak shedding titers after one high dose of either nOPV2 candidate than after one dose of mOPV2. In infants where the lower dose that is being used in the field was evaluated (M5b and the Bangladesh study in vaccine-naïve, newborn infants), the rate of nOPV2 shedding was comparable to mOPV2 at the peak of shedding (first 2 weeks); however, the proportion of infants that shed nOPV2 was lower than mOPV2 historic controls by week 4, indicating a likely shorter duration of shedding. Regarding the amount of virus shed in infants, analysis of M5b cohorts published in *The Journal of Infectious Diseases* showed that the amount of infectious dose

shed in infants was significantly lower for all nOPV2 groups at Day 28. And in the Bangladesh study on newborn infants, overall amounts of nOPV2 excreted were low.

Table 2. Key nOPV2 Trials: Summary of Results for Each Clinical Trial

Clinical Trial	Summary and Key Results
<p>Phase I nOPV2 trial Belgium, adults (M4a Study) <u>Overall trial results published in <i>The Lancet</i></u> <u>Additional immunogenicity data on intestinal antibody responses (including IgA) published in the <i>Journal of Infectious Diseases</i></u> <u>Additional data on genetic stability published in <i>npj Vaccines</i> (nOPV2 Phase I/M4a data was combined with Phase II/M4 data for this analysis; see further details in the Phase II/M4 section, below)</u></p>	<p>Study Details: In 2017, 30 adult subjects with a history of IPV-only vaccination were vaccinated with a high dose (10⁶ cell culture infectious dose 50% units, or CCID50) of either one of the two nOPV2 candidates (n=15 for each of the two groups).</p> <p>Safety: Both nOPV2 candidates were well tolerated among adults with a prior history of IPV vaccination, and no serious adverse events⁹ were reported.</p> <p>Immunogenicity: Both nOPV2 vaccine candidates were immunogenic. 28 days after vaccination, there were high seroconversion rates in both groups and all participants had seroprotective antibody titers. Additionally, an evaluation conducted by the US Centers for Disease Control and Prevention and published in <i>the Journal of Infectious Diseases</i> showed a modest but detectable rise in total poliovirus specific as well as IgA antibodies for both candidates following the direct measure of antibody titers in participants’ stool samples.</p> <p>Viral Excretion (Shedding): Vaccine virus was detected in the stools of all 15 subjects who received candidate 1 and in 13 (87% of) those who received candidate 2. Shedding stopped at a median of 23 days following candidate 1 administration and 12 days after candidate 2 administration.</p> <p>Genetic Stability: Testing of participant stool samples for neurovirulence in mouse models showed no evidence of increased virulence in domain V of the 5’-untranslated region, the site of the primary determinant of attenuation for Sabin OPV2 (nucleotide 481). Additionally, among the 15 samples from participants given candidate 1, all genetic modifications engineered into candidate 1 were retained.</p>

⁹ Note: A serious adverse event is defined as any untoward medical occurrence that at any dose: results in death; requires inpatient hospitalization or prolongation of existing hospitalization; results in persistent or significant disability or incapacity; is life-threatening; results in a congenital anomaly or birth defect. The term “severe” is not synonymous with serious. In the English language, “severe” is used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe); the event itself, however, may be of relatively minor medical significance (such as severe headache). Seriousness (not severity), which is based on patient/event outcome or action criteria, serves as guide for defining regulatory reporting obligations. Source: Safety Monitoring of Medical Products: Reporting system for the general public. Geneva: World Health Organization; 2012 (<https://apps.who.int/iris/bitstream/handle/10665/336225/9789241503198-eng.pdf?sequence=1&isAllowed=y>, accessed 26 May 2022).

Clinical Trial	Summary and Key Results
<p>Phase II Belgium nOPV2/mOPV2 comparator trial All groups adults nOPV2 recipients (M4 study) compared to mOPV2 Phase IV historical controls (M1 Study) Overall trial results published in <i>The Lancet</i></p> <p>Additional data on genetic stability published in <i>npj Vaccines</i></p>	<p>Study Details: In 2018-19, adults with OPV vaccination histories were administered either one or two high doses of one of the two nOPV2 vaccine candidates (n=50 for each of the four groups). Adults with IPV-only vaccination histories were administered either two high doses of nOPV2 candidate 1 (n=17), two high doses of nOPV2 candidate 2 (n=16), or a placebo (n=17). To establish non-inferiority to mOPV2, the results from the study groups were compared to 100 adults that had been vaccinated in 2016 with either one or two standard doses of mOPV2. Like the nOPV2 study groups, these mOPV2 control groups had vaccination histories that included both IPV and OPV.</p> <p>Safety: mOPV2 and both nOPV2 candidates were well tolerated by participants, with no serious adverse events or withdrawals that were determined to be related to vaccination.</p> <p>Immunogenicity: Seroprotection rates were high at baseline and following vaccination for both nOPV2 candidates, and demonstrated non-inferiority to mOPV2. Median seroprotective antibody titers were similar across all vaccinated cohorts, whether nOPV2 or mOPV2.</p> <p>Viral Excretion (Shedding): mOPV2 and both nOPV2 candidates were shed in the stool at a similar rate among participants with a history of prior OPV vaccination, and virtually all study participants had stopped shedding by the end of the 28-day follow-up period. Shedding was observed to be higher in IPV-only vaccinated participants, particularly after the first dose (as expected, because IPV induces little to no primary intestinal immunity). After the second dose, the number of vaccine recipients shedding and the magnitude of viral excretion were lower than after the first dose, indicating that one dose of either of the nOPV2 candidates had induced intestinal immunity in recipients who had previously only been vaccinated with IPV.</p> <p>Genetic Stability: Consistent with the results of the Phase I study, sequencing of the vaccine virus from participant stool samples showed no reversion at the genetically stabilized primary attenuation site (domain V) for either nOPV2 candidate. For nOPV2 c1, no mutations in the relocated cis-acting element were observed, and no variants consistent with reversion in the Rec1/K38R or Hifi/D53N modification locations of the 3D polymerase were observed.</p> <p>In addition to genetic stability results published in <i>The Lancet</i>, which compared M4 to M1 cohorts, an analysis comparing both M4a and M4 cohorts to M3 cohorts (child mOPV2 controls with prior IPV-only vaccination histories) was published in <i>npj Vaccines</i>. This study allowed the genetic stability of the two nOPV2 candidates to be assessed in adults with both IPV and OPV vaccination histories, and then compared with mOPV2. Next-generation sequencing (NGS) of stool samples showed no evidence of reversion in domain V, and neurovirulence testing through mouse models indicated that both nOPV2 candidates are considerably less likely than Sabin-2 to evolve towards significant cVDPV2 virulence.</p>
<p>Phase II Comparator trial, Panama M5a and M5b (nOPV2 children and infants), compared to</p>	<p>Study Details: In 2018-19, studies were done to compare nOPV2 candidates to mOPV2 in children and infants. The vaccination history of both the nOPV2 groups and the mOPV2 historical control groups was complete polio immunization with either tOPV or IPV. In the nOPV2 groups, both nOPV2 candidates were administered in high and low dosages to encompass the range of dosage experienced in practical use, from first uses (high dose) to the end of shelf life (low dose). <i>Children:</i> 101 1- to 5-year-olds were vaccinated with two high doses of one of the two nOPV2 candidates. Results from these groups were compared to a historical control group of 50 1- to 5-year-olds vaccinated with two standard doses of mOPV2 in 2015-2016.</p>

Clinical Trial	Summary and Key Results
<p>M2a and M2b (mOPV2 Phase IV historical controls, children and infants) Overall trial results published in <i>The Lancet</i></p> <p>Additional shedding analysis for the infant cohorts published in the <i>Journal of Infectious Diseases</i></p> <p>Additional genetic stability and preliminary recombination analysis for the child cohorts published in <i>npj Vaccines</i></p> <p>Additional genetic and phenotypic stability analysis for the infant cohorts published in <i>The Lancet Microbe</i></p>	<p>Infants: 574 18- to 22-week-old infants were vaccinated with one low dose or one high dose of one of the two nOPV2 candidates; a subset of each of these groups (n=50 in each group, whether low- or high- dose, totaling 200 all together) received a second dose. Results from these groups were compared to 110 18- to 22-week-old infants vaccinated in 2015-2016 with one dose of mOPV2, a subset of which (n=50) received a second dose of mOPV2.</p> <p>Safety: mOPV2 and both nOPV2 candidates were well tolerated across study cohorts, and no serious adverse events were determined to be causally associated with vaccination.</p> <p>Immunogenicity: The established non-inferiority criterion for seroprotection in infants at day 28 was met for all low-dose and high-dose novel OPV2 candidates after one dose, except for the low dose of nOPV2 candidate 2. Following a second vaccine dose, both seroprotection and seroconversion rates were uniformly high across nOPV2 candidate groups.</p> <p>Viral Excretion (Shedding): Analysis of stool samples was ongoing at the time of the <i>Lancet</i> study publication; in-depth analysis of the infant samples was later published in the <i>Journal of Infectious Diseases</i>. Estimated rates of shedding, measured as viral RNA and infectious virus, were either similar or more commonly lower for nOPV2 than mOPV2 across post-vaccination sampling days for both high and low dosages. While viral shedding of mOPV2 and nOPV2 was similar in the first week after vaccination, the proportion shedding on day 28 was significantly lower for both nOPV2 candidates than for mOPV2. Additionally, shedding rates were lower after the second doses of both mOPV2 and nOPV2, indicating that the first doses of mOPV2 as well as each of the nOPV2 candidates induced intestinal immunity.</p> <p>Genetic Stability: Stool samples from children aged 1-5 in the M2 and M5 trials were further analyzed for genetic stability of shed virus, and results were published in <i>npj Vaccines</i>. Modified transgenic mouse neurovirulence tests (mTgmNVT) indicated significantly reduced odds of mouse paralysis from virus obtained from nOPV2-c1 recipients compared to mOPV2 recipients (adjusted odds ratio [aOR]=0.001, 95% CI < 0.001, 0.121, p=0.0060). Next-generation sequencing (NGS) was also performed on full-length poliovirus genome shed in stool, to assess stability of the genome. NGS analysis indicated that the nOPV2 modifications were retained as the virus replicated in the intestine: no polymorphisms consistent with increased virulence were detected in the S15 domain V of shed nOPV2-c1, and no mutations impacting the modified K38R/Rec1 or D53N/Hifi3 locations of the 3D polymerase were observed.</p> <p>Similarly, stool samples from infants aged 18-22 weeks in the M2 and M5 trials were analyzed for genetic and phenotypic stability, with results published in <i>The Lancet Microbe</i>. mTgmNVT again indicated significantly reduced odds of mouse paralysis from virus obtained from nOPV2 recipients compared with mOPV2 recipients (estimated aOR at 4.5 log₁₀ = 0.007 (95% CI 0.002–0.023; p<0.0001)). Following NGS performed on viral RNA in nOPV2 stool samples, no reverting polymorphisms were observed in the domain V region of shed nOPV2. This contrasts with the mOPV2 samples, which saw consistent reversion at domain V nucleotide 481, the primary attenuation site.</p> <p>Recombination: Coverage maps were used to map readings from the child cohorts' stool samples to key viral references (a Sabin 2 reference virus as well as Sabin 1 virus, Sabin 3 virus, and non-polio enteroviruses). No recombinant viruses were observed in either the child or the infant cohort analyses.</p>

Clinical Trial	Summary and Key Results
<p>Phase II Study comparing nOPV2 to placebo in a key subpopulation: vaccine-naïve newborn infants in Bangladesh Overall trial results published in The Lancet</p>	<p>Study Details: In 2020-2021, newborn infants were randomly assigned (2:1) to receive either two doses of nOPV2 $10^{5+/-0.5}$ CCID50 (n=220) or a placebo (n=110), administered at age 0–3 days and at 4 weeks.</p> <p>Safety: nOPV2 was found to be as well tolerated as the placebo, with only mild solicited adverse events. No serious adverse events or adverse events of special interest related to the study were reported.</p> <p>Immunogenicity: Two doses of nOPV2 elicited a 90% seroconversion rate, which resulted in 99% of nOPV2 recipients having seroprotective titers at week 8. Additionally, of the 16 nOPV2 recipients who initially had no detectable poliovirus type 2 antibody titers at birth, 8 (50%) had seroprotective titers at week 4 and 16 (100%) had seroprotective titers at week 8.</p> <p>Viral Excretion (Shedding): Mothers were asked to collect samples of approximately 8g of stool at birth and at weeks 2, 4, 6, 8, 10, and 12. As expected, no RT-PCR-detectable shedding of poliovirus types 1, 2, or 3 was observed in baseline stool samples. However, shedding was observed at week 2 in nOPV2 recipients (52%) as well as weeks 4 and 6 (40% and 64%, respectively) before gradually decreasing (n=1 nOPV2 recipient shedding at week 12). In addition to duration of shedding, the amount of shedding was also measured. Amounts of virus excreted by the nOPV2 group were found to be low: only 17% at week 2 (2 weeks after dose 1) and 14% at week 6 (2 weeks after dose 2) shed sufficient virus to be measurable by culture (i.e., type 2 RT-PCR positive or at least CCID50 nOPV2 virus per gram of stool). This indicates that there is no evidence of any increased risk of transmitting the nOPV2 vaccine virus for those who receive nOPV2 as their first poliovirus vaccination compared to those who receive IPV as their first polio vaccination prior to receiving nOPV2¹⁰.</p>

Which other studies provide useful information on nOPV2 (in addition to the studies associated with nOPV2’s clinical research framework)?

In addition to the studies that are part of nOPV2’s clinical framework, there are other notable studies that address key nOPV2 attributes. Please see Table 3 for a summary of studies that demonstrate nOPV2’s effectiveness in the field, and Table 4 for a detailed list of publications to consult.

Assessing the immunogenicity of nOPV2 administered in outbreak response campaigns: Tajikistan 2021
Table 3: Studies Demonstrating nOPV2’s Effectiveness in the Field

<p>To assess poliovirus type 2 seroprevalence in children under 5, a community serosurvey was conducted in 7 districts in Tajikistan with detection of cVDPV2 isolates. Dried blood spot specimens were collected at 3 specific time points during visits to health facilities in the district that were selected through simple random sampling without replacement using Epi Info. Visit 1 was in the days before the first nOPV2 campaign, visit 2 was 1 month after the first nOPV2 campaign (just before the second nOPV2 campaign), and visit 3 was 1 month after the second</p>
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¹⁰ See also: Gast C, Bandyopadhyay AS, Sáez-Llorens X, De Leon T, DeAntonio R, Jimeno J, Aguirre G, McDuffie LM, Coffee E, Mathis DL, Oberste MS, Weldon WC, Konopka-Anstadt JL, Modlin J, Bachtiar NS, Fix A, Konz J, Clemens R, Costa Clemens SA, Rüttimeann R. Fecal Shedding of 2 Novel Live Attenuated Oral Poliovirus Type 2 Vaccine Candidates by Healthy Infants Administered Bivalent Oral Poliovirus Vaccine/Inactivated Poliovirus Vaccine: 2 Randomized Clinical Trials. J Infect Dis. 2022 Sep 1; 226(5): 852–861. doi: [10.1093/infdis/jiab507](https://doi.org/10.1093/infdis/jiab507)

campaign concluded. Participation in the nOPV2 campaigns was reported through parental recall. Children <6 months were omitted due to potential interference with maternal antibodies, and children who did not complete all 3 study visits were also excluded.

Seropositivity for each serotype was defined as the reciprocal titer of poliovirus neutralizing antibodies of 8 or more. In terms of seroprevalence in the study population, after one dose of nOPV2, the seroprevalence against type 2 poliovirus increased to 77% (95% CI 70 to 82; 161/210) and after two doses of nOPV2 the seroprevalence increased to 83% (77 to 88; 174/209). This increase in seroprevalence was statistically significant between baseline and after one nOPV2 dose (51 percentage points [42 to 59]; $p < 0.0001$), but not between the first and second doses (6 percentage points [-2 to 15]; $p = 0.12$).

Seroconversion was defined as the change from seronegative to seropositive (from reciprocal titer of < 8 to ≥ 8) in children with antibodies at baseline. The proportion of children that seroconverted between visit 1 and 2 was 67% (89/132; 95% CI 59–75), between visit 2 and 3 was 44% (20/45; 30–60), and between visit 1 and 3 was 77% (101/132; 68–83; table 2). The first nOPV2 dose was more immunogenic than the second (seroconversion 67% vs 44%; $p = 0.010$). Notably, seroconversion varied across the 7 districts.

Assessing the immunogenicity of nOPV2 administered in outbreak response campaigns: Liberia 2021 [Results published in The Lancet Global Health](#)

To measure type 2 poliovirus seroprevalence and antibody titers among children living in areas targeted by two nOPV2 outbreak response campaigns in Liberia, a cross-sectional, community-based, seroprevalence survey among children under 5 was conducted more than four weeks after the second campaign concluded. Clustered sampling methodology was used in four geographic regions of Liberia, and simple random sampling of households within the clusters was then used to identify eligible participants. Participation in nOPV2 campaigns was reported through parental recall, and dried blood spot specimens were collected and analyzed. Children <6 months were omitted due to potential interference with maternal antibodies, and children who acutely or chronically ill or requiring hospitalization at the time of enrolment were also excluded from the study.

Out of 436 participants with analyzable data, 85% reported receiving two nOPV2 doses, 10% one dose, and 5% no doses. No significant difference was observed between type 2 seroprevalence in children who reported receiving two doses of nOPV2 (42% [95% CI: 36.8, 47.5%]), one dose (28% [95% CI: 12.1, 49.4]), or no doses (37.5% [95% CI: 8.5, 75.5]). A history of IPV vaccination was not significantly associated with type 2 seroprevalence.

Potential explanations for the lower-than-expected seroprevalence findings (given results from other studies) may include the high prevalence of chronic intestinal infections in resource-limited settings, which can compromise the immunogenicity of nOPV2,¹¹ and/or low nOPV2 actual campaign coverage despite high reporting from parental recall.

Evaluating the genetic stability of nOPV2 during its initial use period: March-October 2021 (Multi-country) [Results published in the CDC MMWR](#)

During nOPV2's initial use period (March-October 2021), 128 nOPV2 isolates were identified from stool samples collected through routine AFP surveillance conducted in 6 different countries, and 123 isolates were identified from 39 different environmental surveillance samples from 7 countries. Whole-genome sequences were generated for these 251 nOPV2 isolates, and each isolate sequence was then compared with that of the nOPV2 vaccine strain. Isolates were classified into one of 9 categories based on their risk profile and loss of key attenuating nOPV2 mutations. Among the 251 isolates, 32 (13%) were classified as category 9 (meaning no changes from nOPV2), and 213 (85%) were classified as category 8 (showing no reverting mutations in domain V, no recombination, and 0–5

¹¹ Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis. 1991 Sep-Oct;13(5):926-39. doi: 10.1093/clinids/13.5.926

VP1 substitutions). None of the isolates had changes in the primary attenuation site (domain V) that would be predicted to increase neurovirulence. The most frequent mutations were noted at nucleotide positions that have been shown or inferred to slightly decrease attenuation when present individually.

In addition to these nOPV2 isolates, whole-genome sequences of 331 cVDPV2 isolates from outbreaks in countries geographically associated with nOPV2 were analyzed as part of this study. None were found to contain any of the three nOPV2-specific nucleotides in the capsid-coding region at positions 814, 817, and 1,375, which indicates that none of the cVDPV2 isolates sequenced were derived from an nOPV2-recombinant virus.

Table 4: Detailed List of nOPV2 Publications to Consult

Note: studies summarized in this paper are in **bold**

Topic	Publication Title and Link (if available)
Description of the modifications made to the nOPV2 virus genome to generate nOPV2 candidate 1 (selected for full clinical development and WHO Emergency Use Listing)	Engineering the Live-Attenuated Polio Vaccine to Prevent Reversion to Virulence
Description of the modifications made to the nOPV2 virus genome to generate nOPV2 candidate 2	Development of a new oral poliovirus vaccine for the eradication end game using codon deoptimization
Overall Phase I Trial Results (From M4a trial conducted in healthy adults, Belgium)	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
Additional immunogenicity data on intestinal antibody responses including IgA (From M4a trial conducted in healthy adults, Belgium)	Intestinal Antibody Responses to 2 Novel Live Attenuated Type 2 Oral Poliovirus Vaccines in Healthy Adults in Belgium
Overall Phase II Trial Results (Comparing M4 to M1, healthy adults, Belgium)	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
Overall Phase II Trial Results (Comparing M5a and M5b to M2a and M2b, healthy infants and children, Panama)	Safety and immunogenicity of two novel type 2 oral poliovirus vaccine candidates compared with a monovalent type 2 oral poliovirus vaccine in children and infants: two clinical trials
Additional viral shedding analysis (From the infant cohorts of the Phase II trial comparing M5a and M5b to M2a and M2b, healthy infants and children, Panama)	Fecal Shedding of 2 Novel Live Attenuated Oral Poliovirus Type 2 Vaccine Candidates by Healthy Infants Administered Bivalent Oral Poliovirus Vaccine/Inactivated Poliovirus Vaccine: 2 Randomized Clinical Trials
Genetic stability (Comparing the child cohorts of the mOPV2 Phase IV M3 trial in Lithuania to the adult cohorts of the nOPV2 Phase I M4a and Phase II M4 trials in Belgium)	Assessment of genetic changes and neurovirulence of shed Sabin and novel type 2 oral polio vaccine viruses
Genetic stability and preliminary recombination analysis (From the child cohorts of the Phase II trial comparing M5a and M5b to M2a and M2b, healthy infants and children, Panama)	Evaluating stability of attenuated Sabin and two novel type 2 oral poliovirus vaccines in children
Genetic and phenotypic stability analysis (From the infant cohorts of the Phase II trial comparing M5a and M5b to M2a and M2b, healthy infants and children, Panama)	Genetic and phenotypic stability of poliovirus shed from infants who received novel type 2 or Sabin type 2 oral poliovirus vaccines in Panama: an analysis of two clinical trials

Topic	Publication Title and Link (if available)
Overall Clinical Trial Results (From Phase II trial, Bangladesh, vaccine-naïve newborns)	Evaluation of the safety, immunogenicity, and faecal shedding of novel oral polio vaccine type 2 in healthy newborn infants in Bangladesh: a randomised, controlled, phase 2 clinical trial
Study assessing the immunogenicity of nOPV2 administered in outbreak response campaigns: Tajikistan 2021	Assessment of serological responses following vaccination campaigns with type 2 novel oral polio vaccine: a population-based study in Tajikistan in 2021
Study assessing the immunogenicity of nOPV2 administered in outbreak response campaigns: Liberia 2021	Poliovirus antibodies following two rounds of campaigns with a type 2 novel oral poliovirus vaccine in Liberia: a clustered, population-based seroprevalence survey
Study evaluating the genetic stability of nOPV2 during its initial use period: March-October 2021 (Multi-country)	Genetic Characterization of Novel Oral Polio Vaccine Type 2 Viruses During Initial Use Phase Under Emergency Use Listing – Worldwide, March–October 2021
Assessment of nOPV2's genetic heterogeneity through the application of next-generation sequencing (NGS) in vaccine lot testing	Evaluation and validation of next-generation sequencing to support lot release for a novel type 2 oral poliovirus vaccine
Summary of the pathway used to roll out nOPV2 under EUL	Enabling accelerated vaccine roll-out for Public Health Emergencies of International Concern (PHEICs): Novel Oral Polio Vaccine type 2 (nOPV2) experience
Summary of nOPV2's clinical development and global roll out	A novel tool to eradicate an ancient scourge: the novel oral polio vaccine type 2 story

What has nOPV2 research demonstrated about mOPV2?

In addition to demonstrating comparable safety, immunogenicity, and enhanced genetic stability of nOPV2 compared to mOPV2, nOPV2 trials also added significant evidence to the body of data showing that mOPV2 is a safe and immunogenic vaccine. In addition to the studies discussed in this paper, mOPV2 studies that have been conducted but not used in the comparator trials have also reinforced mOPV2's safety and immunogenicity. One example is the M3 study in Lithuania which evaluated IPV-vaccinated children with a challenge dose of mOPV2 and demonstrated mOPV2's ability to induce intestinal immunity and provide seroprotection against type 2 poliovirus.¹²

Next Steps and More Information

This summary will be updated as studies are completed and/or as more information becomes publicly available. In the meantime, to consult all information available on nOPV2, including the nOPV2 EUL recommendation assessment report, please visit the nOPV2 web page of the GPEI website: <http://polioeradication.org/nOPV2>. You can also write to nOPV2@who.int with any questions.

¹² For more details, see: Bandyopadhyay AS, Gast C, Brickley EB, Rüttimann R, Clemens R, Oberste MS, Weldon WC, Ackerman ME, Connor RI, Wieland-Alter WF, Wright P, Usonis V. A randomized phase 4 study of immunogenicity and safety following monovalent oral type 2 Sabin polio vaccine challenge in IPV-vaccinated children in Lithuania. *J Infect Dis.* 2021; 223(1):119-127. doi: 10.1093/infdis/jiaa390.