

nOPV2: Clinical Development Summary

What is nOPV2 and why is it needed?

The oral polio vaccines (OPV) that are used to prevent polio in polio-affected and at-risk countries 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 (shed) 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 vaccine viruses may mutate in such a way that they become virulent polioviruses, different from the wild form of the poliovirus in their genetic makeup but causing disease just like wild poliovirus.¹² Because these polioviruses are derived from the 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**.³

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 is currently used to respond to outbreaks of type 2 cVDPV. nOPV2 is similar to mOPV2, but with increased stability conferred through modifications to targeted sites on the vaccine strain's genome. The increased genetic stability of the vaccine is likely to reduce the risk of vaccine-associated paralytic poliomyelitis (VAPP) and may also decrease the likelihood of disease caused by type 2 cVDPV, all while maintaining the immune protection that is provided by the vaccine. It will ensure that the benefits associated with using an oral polio vaccine (i.e., passive immunization) 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 nearly 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.⁴ 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).

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. Epub 2016 Nov 21. PMID: 27866483.)

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. There is little evidence of virus circulation after VAPP cases. Source: Lopalco PL, 2017.

³ cVDPV 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 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).

One nOPV2 vaccine strain (often referred to as nOPV2 candidate 1, or c1) was selected following a comprehensive assessment of pre-clinical, clinical, and manufacturing information across vaccine candidates. The selected strain carries five key modifications to the genome (as compared to the mOPV2 vaccine strain). 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 below, for reference.⁵

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

Modification	Scientific Rationale
1: 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.
2 and 3: Relocated and modified the cre (cis-acting replication element), called cre5 , within the 5' untranslated region	To prevent the replacement of the modified, attenuating nOPV2 <i>domV</i> described above with the unattenuated domV from another virus through a single recombination event.
4 and 5: Introduced two amino acid substitutions (D53N and K38R) in the viral RNA-dependent RNA polymerase (3Dpol)	To limit the adaptive capacity of the virus by reducing mutation rate and recombination frequency.

What was the clinical research framework 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.⁶

⁵ As will be described further in this paper, two nOPV2 vaccine candidates entered clinical development in 2017. The modifications described in this table 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. Epub 2020 Apr 23. PMID: 32330425; PMCID: PMC7566161. 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. PMID: 32218998; PMCID: PMC7083942.

⁶ The containment guidelines went into effect in 2016, following the "switch" from tOPV to bOPV. See: ⁶ World Health Organization. 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. 2014. http://polioeradication.org/wp-content/uploads/2016/12/GAPIII_2014.pdf

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 and describes additional ongoing/upcoming studies that are yet to be initiated and/or completed.

Table 2. Snapshot of the nOPV2 Clinical Development Framework

2017	Phase I, Belgium: Safety, immunogenicity, viral excretion, and genetic stability of two nOPV2 vaccine candidates in adults with IPV-only vaccination histories under containment
2018-2019	Phase II, Belgium: Safety, immunogenicity, viral excretion, and genetic stability of two nOPV2 vaccine candidates in adults with vaccination histories that include both IPV and OPV, as compared to mOPV2 historical control groups (OPV subjects) or placebo (IPV subjects)
2018-2019	Phase II, Panama: Safety, immunogenicity, viral excretion, and genetic stability of two nOPV2 vaccine candidates in children and infants with vaccination histories that include both IPV and OPV, as compared to mOPV2 historical control groups
2020-2021	Phase II, Bangladesh:* Safety and immunogenicity of the selected nOPV2 vaccine candidate in vaccine-naïve infants
	Phase II, Bangladesh:* Safety and immunogenicity of the selected nOPV2 candidate co-administered with bOPV in infants
2021	Phase III, Gambia:* Safety, immunogenicity, and lot-to-lot consistency of the selected nOPV2 candidate

*Note: An asterisk in the above table indicates that the study is ongoing or has not yet begun.

What have these clinical trials demonstrated in terms of nOPV2's safety, immunogenicity and genetic stability?

Key conclusions across studies are outlined below. A summary of results for each trial is featured in Table 3, for reference.

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 for both low dose and high dose potencies of nOPV2 (i.e., there was no significant difference in seroconversion rates between nOPV2 and mOPV2). Across all studies, nOPV2 demonstrated robust immune responses with high seroconversion rates that were comparable with mOPV2.

Vaccine Candidate Selection: 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) was not moved forward for further clinical development and submission to WHO for an Emergency Use Listing (EUL).

Genetic Stability: Data to date indicates nOPV2's increased genetic stability as compared to mOPV2. To assess 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, the selected nOPV2 candidate, candidate 1, shows limited mouse paralysis associated from stool samples collected across the studies, regardless of the age of the study participants (adults and children, with additional data from infants forthcoming).

Shedding: In 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.

Table 3. Key nOPV2 Trials: Summary of Results for Each Trial

Trial	Summary and Key Results
<p>nOPV2 Phase I, Belgium <i>Overall trial results published in The Lancet</i> <i>Additional data on intestinal antibody (IgA) responses published in the Journal of Infectious Diseases</i></p>	<p>Study Details: In 2017, 30 adult subjects with a history of IPV-only vaccination were vaccinated with a high (10^6 CCID₅₀) dose of either one of the two nOPV2 candidates.</p> <p>Safety: Both nOPV2 candidates were well tolerated among adults with a prior history of IPV vaccination, and no serious adverse events⁷ were reported.</p> <p>Immunogenicity: Both of the nOPV2 vaccine candidates were immunogenic. 28 days after vaccination, there were high seroconversion rates in both groups and all participants had seroprotective antibody titres. Additionally, for both candidates, a modest but detectable rise in total as well as poliovirus-specific and IgA antibodies was observed following direct measure of antibody titres 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 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).</p>
<p>nOPV2 Phase II, Belgium, compared to mOPV2 Phase IV historical controls (all groups adults) <i>Results published in The Lancet</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. Similar to 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 titres 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, due to the fact that 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 nOPV2 candidate 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.</p>

⁷ 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: World Health Organization. Safety Monitoring of Medical Products: Reporting system for the general public. 2012.

https://www.who.int/medicines/areas/quality_safety/safety_efficacy/ConsumerReporting.pdf

<p>nOPV2 Phase II, Panama (children and infants), compared to mOPV2 Phase IV historical controls (children and infants) Results published in <i>The Lancet</i></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.</p> <p><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> <p><i>Infants:</i> 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 subset (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 study publication, but preliminary data indicated that while the proportions of shedding at day 7 were similar for monovalent OPV2 and both novel OPV2 candidates, a significantly lower rate of shedding for nOPV2 candidates compared with monovalent OPV2 in infants was evident by day 28.</p> <p>Genetic Stability: Analysis of stool samples was ongoing at the time of manuscript submission and publication; complete data for both shedding and genetic stability will be published separately and this table will then be updated.</p>
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What have the trials conducted as part of nOPV2's clinical development demonstrated about mOPV2?

In addition to demonstrating comparable safety, immunogenicity, as well as enhanced genetic stability of nOPV2 compared to mOPV2, these trials also added significant evidence to the body of data showing that mOPV2 is a safe and effective vaccine. Additional mOPV2 studies that are part of nOPV2's clinical development framework but are not discussed in this paper because they were not used in comparator trials have also reinforced mOPV2's safety and immunogenicity. One example is a study in Lithuania which challenged IPV-vaccinated children with mOPV2 and demonstrated mOPV2's ability to induce intestinal immunity and provide seroprotection against type 2 poliovirus.⁸

Next Steps and More Information

This paper will be updated as studies are completed and/or as more information becomes publicly available. In the meantime, to consult all scientific research and data on nOPV2, including all peer-reviewed publications and 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.

⁸ 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.* 2020 Jul 4: jiaa390. doi: 10.1093/infdis/jiaa390. Epub ahead of print. PMID: 32621741.