

PART 1 (COUNCIL DECISION 2002/813/EC)

**SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF
GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN
ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC**

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

- | | |
|---|--|
| (a) Member State of notification | Belgium |
| (b) Notification number | B/BE/18/BVW2 |
| (c) Date of acknowledgement of notification | 24/04/2018 |
| (d) Title of the project | A Phase 2, double-blind, randomized, placebo-controlled, multicenter study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines candidates, in healthy adults and adolescents previously vaccinated with oral polio vaccine (OPV) or inactivated polio vaccine (IPV), compared with historical controls given Sabin OPV2 or placebo. |
| (e) Proposed period of release | Recruiting as of Q3 / 2018
Period of release as early as Q4 / 2018 till the end of Q2 / 2019 |

2. Notifier

Name of institution or company: Centre for the Evaluation of Vaccination,
University of Antwerp, Belgium

3. GMO characterisation

- (a) Indicate whether the GMO is a:
- | | |
|----------------|-----|
| viroid | (.) |
| RNA virus | (X) |
| DNA virus | (.) |
| bacterium | (.) |
| fungus | (.) |
| animal | |
| - mammals | (.) |
| - insect | (.) |
| - fish | (.) |
| - other animal | (.) |

specify phylum, class ...

(b) Identity of the GMO (genus and species)

Genus *Enterovirus*
Species *Enterovirus C*

(c) Genetic stability – according to Annex IIIa, II, A(10)

Sabin OPV2 has inherent genetic instability at the attenuating positions which leads to reversion to virulence. The proposed clinical study is part of a development program investigating genetic modifications to improve the genetic stability and thereby produce a more stable vaccine, i.e. less likely to revert to virulence.

Sabin-2 OPV is highly attenuated in both monkeys and transgenic mice carrying the human poliovirus receptor (TgPVR mice). Two mutations are understood to account for the majority of the attenuation as compared to wild-type type 2 virus. The mutation in Sabin-2 OPV which is primarily responsible for attenuation resides in the 5' untranslated region (UTR) (Macadam et al., 1993). This mutation (nucleotide 481A) acts to thermodynamically destabilize an RNA stem-loop structure known as domain V which forms part of the internal ribosome entry site. It is believed that this mutation results in attenuation by reducing the efficiency of the initiation of translation of the viral polyprotein in neural cells, thereby reducing neurovirulence.

After oral administration of the Sabin 2 vaccine, reversion at the 481 site occurs quickly in the human gut, leading to shedding of virus with increased neurovirulence. For example, virus shed only one week after trivalent OPV (tOPV) vaccination in children was shown to contain 33-96% reverted (481G) type 2 virus (Laasri et al., 2005). This reversion results in a domain V structure which is more thermodynamically stable than the attenuated sequence, and rare but serious cases of vaccine-associated paralytic polio have been caused by strains that have reverted at this attenuation determinant.

The other mutation which accounts for some attenuation of the Sabin-2 vaccine is in amino acid 143 in VP1, resulting in a threonine to isoleucine substitution. Selective pressure in the human gut against this mutation appears to be lower than for nucleotide 481, with about half of the VP1-143 codons exhibiting changes in samples isolated 3 weeks after vaccination (Macadam et al., 1993).

Other than these modifications, we are not aware of other factors making the Sabin-2 strain different in genetic stability than other polioviruses or, more broadly, than other group C enteroviruses i.e. modification by recombination and by infidelity during viral replication are both possible.

The GMOs, which are novel type 2 oral polio vaccine (nOPV2) candidates are intended to be more genetically stable than the recipient Sabin OPV2 strain, resulting in a much lower -if any- reversion to a neurovirulent phenotype. Whereas Sabin OPV2 is based on two single nucleotide mutations, the nOPV2 candidate strains include different combinations of 5 distinct modified regions of the Sabin-2 genome, including changes to the RNA sequence in the 5' untranslated region of polio genome (5' UTR), the capsid protein coding region (P1), the non-structural protein 2C, and the polymerase 3D. Of these modifications, only the changes to polymerase 3D result in a change in the amino acid sequence. The rest of the modifications aim to stabilize the genetic sequence against reversion in either the 5' UTR or capsid regions.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (X)

If yes, insert the country code(s) ...

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (.) No (X)

If yes:

- Member State of notification ...
- Notification number B/././...

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:

- Member State of notification ...
- Notification number B/././...

7. Summary of the potential environmental impact of the release of the GMOs.

Polioviruses have a very narrow host range and there are no reports of transmission of the recipient Sabin-2 vaccine strain to organisms other than humans outside of laboratory settings (experimental infection of transgenic mice expressing the human poliovirus receptor).

Like Sabin-2, nOPV2 candidate vaccines may survive for a limited time in the environment, but can only multiply in humans, non-human primates, or laboratory mice which carry the human poliovirus receptor. Their use will be limited to the clinical trial facilities, where measures are in place to ensure that only the intended clinical trial participants will be exposed. Subsequent release via shedding can occur in a broad environment, in which the nOPV2 candidate vaccine can passively spread and from which it eventually will be eliminated. Unless another person ingests the shed nOPV2 candidate vaccine, no subsequent multiplication and further shedding is foreseen.

The potential of the nOPV2 candidate vaccine strains for spread beyond the clinical study participants is considered very low as the high rate of polio vaccination coverage in Belgium due to mandatory routine vaccination (OPV from 1966-2000, IPV from 2001 on) means that there are no large groups of susceptible individuals that could support circulation. Similar conclusions can be drawn for nearby countries, with polio immunization coverage for Belgium and nearby countries in 2016 as follows: Belgium-98%, Netherlands-95%, Germany-94%, France-97%, Luxembourg-99%, United Kingdom-94% (source: WHO Global Health Observatory data repository).

Virus that will be shed from feces of study participants will end up in the waste water system where it will be largely diluted and eventually inactivated by waste water treatment.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:
(select one only)

- viroid (.)
- RNA virus (X)

DNA virus (.)
 bacterium (.)
 fungus (.)
 animal
 - mammals (.)
 - insect (.)
 - fish (.)
 - other animal (.)
 (specify phylum, class) ...

other, specify ...

2. Name

(i) order and/or higher taxon (for animals) ...
 (ii) genus *Enterovirus*
 (iii) species *Enterovirus C*
 (iv) subspecies *Poliovirus*
 (v) strain Sabin Type 2
 (vi) pathovar (biotype, ecotype, race, etc.) ...
 (vii) common name Sabin OPV2

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
 Yes (.) No (X) Not known ()

(b) Indigenous to, or otherwise established in, other EC countries:
 (i) Yes (.)
 If yes, indicate the type of ecosystem in which it is found:
 Atlantic ..
 Mediterranean ..
 Boreal ..
 Alpine ..
 Continental ..
 Macaronesian ..

(ii) No (.)
 (iii) Not known (X)

It is not conclusively known if Sabin-2 derived polioviruses are still circulating at a low level in parts of the EC.

(c) Is it frequently used in the country where the notification is made?
 Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?

Yes (.) No (.)

Unknown. It is likely that a local vaccine manufacturer is storing Sabin-2 (OPV2) materials in Belgium, as the product is still locally registered.

4. Natural habitat of the organism

- (a) If the organism is a microorganism
- water (.)
 - soil, free-living (.)
 - soil in association with plant-root systems (.)
 - in association with plant leaf/stem systems (.)
 - other, specify:

Poliovirus, from which Sabin OPV2 is derived has a highly-restricted host range (humans and, to a lesser degree, non-human primates). Non-primates and their cell cultures lack the human poliovirus receptors and are refractory to natural infection.

- (b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques

Polioviruses isolated through environmental or Acute Flaccid Paralysis surveillance can be readily identified by any Global Poliovirus Laboratory Network lab. Additionally, a sensitive real time PCR method has been developed by the U.S. CDC which is able to detect Sabin-2 virus. It has been successfully applied to detecting shed Sabin-2 and Sabin-2-derived viruses in a number of recent trials, including “A Phase 4 study to evaluate the safety and immunogenicity of monovalent oral polio vaccine type 2 in healthy OPV-vaccinated adults” (EudraCT 2015-003325-33) conducted in Belgium in late 2015, as well as “A Phase 1, blinded, single center study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines, derived from a modified Sabin 2 infectious cDNA clone, in healthy adults previously primed with inactivated polio vaccine (IPV)” (Eudra CT 2017-000908-21).

- (b) Identification techniques

The real-time PCR method for the detection of polio vaccine virus was implemented for analysis in nucleic acid extracts from stool suspensions in the monovalent OPV2 clinical studies. The method has been shown to be 97% sensitive in detecting poliovirus in stool samples which tested positive for Sabin 2 using cell culture isolation methodologies. The specificity was measured at 71% which can be attributed to the increased sensitivity of the assay over cell culture isolation, especially for samples that have low concentrations of virus.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (X) No (.)

If yes, specify

The effective use of Sabin OPV2 has been essential for the eradication of type 2 polio. As such, and in spite of the risk for vaccine-associated paralytic polio (VAPP), it was not classified as a pathogen or raising any other concern for human health and/or the environment: it can only cause disease upon reversion, it poses minimal risk to human health and the environment in regions with well-vaccinated populations, it is unlikely to spread or to establish itself; there is effective prophylaxis.

With the implementation of WHO's Global Action Plan III (GAPIII) for poliovirus risk mitigation, the handling of OPV2 will be restricted to Polio Essential Facilities that meet stringent containment criteria. Formally, no hazard classification has yet been determined.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (X) No (.) Not known (.)

If yes:

(a) to which of the following organisms:

humans (X)

animals (.)

plants (.)

other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

Vaccination with Sabin OPV2 is generally well tolerated; however, fever, vomiting, diarrhea and allergic/anaphylactic reactions (latter presumably due to hypersensitivity to vaccine components or manufacturing process residues, e.g. neomycin, polymyxin) have been described after vaccination with GSK's oral poliomyelitis vaccine (Summary of Product Characteristics for GSK's monovalent OPV2).

On rare occasions, particularly in immunodeficient infants, aseptic meningitis and encephalitis have been reported after OPV (WHO, 2014).

The main reasons for discontinuation of Sabin-2 in tOPV are the risks for vaccine-associated paralytic polio (VAPP) in vaccinees or their close contacts and the emergence of circulating vaccine-derived polioviruses (cVDPVs) that have acquired transmissibility and neurovirulence. As Sabin strains can replicate in the gut of vaccine recipients, there is a possibility that the attenuating mutations in the vaccine strains revert and that virulence of the vaccine strain is restored. This reversion of attenuating mutations during OPV replication in humans is the underlying cause of VAPP and VDPVs. Both are discussed further below.

In extremely rare cases, OPV vaccination has led to paralysis in vaccinees or in their unimmunized or immunodeficient close contacts. Onset of VAPP usually occurs 4-30 days following receipt of OPV or within 4-75 days after contact with a recipient of OPV.

In industrialized countries, VAPP occurs mainly in early infancy associated with the first dose of OPV and decreases sharply with subsequent OPV doses. A review of VAPP cases in the United States from 1990-1999 was performed by Alexander et al. (2004). The rate of VAPP for vaccine recipients is estimated as 1 case per 6.4 million doses (1 case per 1.4 million first doses and 1 case per 35.4 million subsequent doses). For contacts of vaccine recipients, the rate is estimated at 1 case per 13.3 million doses OPV given (1 case per 4.5 million first doses and 1 case per 23.6 million subsequent doses). A review of VAPP cases in Hungary, which used monovalent OPVs almost exclusively from 1961-1981, estimates the VAPP risk for Sabin-2 at 0.56 per million doses given (Estívariz et al., 2011).

Persons with primary immunodeficiency disorders are at much higher risk of VAPP (approximately 3000-fold) than the general population, but VAPP is rare even in this group.

The Sabin-2 strain was found to be the cause of 30% of cases of VAPP following OPV vaccination (Platt et al., 2014).

Through prolonged replication in either individuals with primary immunodeficiency disorders or in a community with low polio vaccine coverage, vaccine-derived polioviruses (VDPVs) can emerge. These are characterized by a VP1 sequence divergence of >1% from the parental strain for type 1 and 3 and >0.6% for type 2, indicating prolonged replication (or transmission) of the vaccine virus. Though the definition of VDPVs is based on the estimated duration of replication, it is likely that many of these have re-acquired the neurovirulence and transmissibility characteristics of wild-type poliovirus. Especially among isolates of type 2 VDPVs, the mutations controlling neurovirulence are frequently found to have reverted (Macadam et al., 1991; Macadam et al., 1993; Minor, 2009).

In regions with low vaccination coverage rates, where competing wild-type poliovirus has been eliminated and where epidemiologic conditions (e.g. low socioeconomic status, poor hygiene/sanitation and crowding) favor poliovirus transmission, VDPVs have the potential for sustained circulation. When there is evidence of person-to-person transmission in the community these are called circulating VDPVs (cVDPVs). Strikingly, the majority of reported cases of VAPP following cVDPV outbreaks that have occurred since 2000 have been associated with type 2 (Burns et al., 2014). While wild-type poliovirus type 2 has been eradicated since 1999, type 2 Sabin virus accounts for more than 95% of cVDPV outbreaks detected in recent years (Bandyopadhyay et al., 2015).

In a small number of individuals with primary immunodeficiency, OPV immunization can lead to infections which persist for prolonged periods, resulting in chronic shedding of VDPVs. These immunodeficiency-associated VDPVs (iVDPV) show increased neurovirulence. No iVDPV is known to have generated secondary cases with paralysis (WHO, 2016). The occurrence of iVDPVs is very rare. Since the introduction of OPV in 1961 to March 2015, approximately 100 persons with primary immunodeficiencies worldwide have been found to be excreting iVDPVs. Like cVDPVs, type 2 iVDPVs are the most prevalent (65%) (Diop et al., 2015).

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Sabin OPV2 relies on a primate host for replication. When Sabin OPV2 is administered orally, the attenuated poliovirus attaches to the poliovirus receptor (CD155) on the cytoplasmic membrane of the cells within the host's gastrointestinal system. Individual replication cycles occur over about 10 hours, but can continue until the infection is controlled by the host immune response.

(b) Generation time in the ecosystem where the release will take place: ...See (a) above

(c) Way of reproduction: Sexual .. Asexual X

(d) Factors affecting reproduction:

The vaccination history of the clinical study participant will impact the extent and duration of viral shedding. All study participants will be fully vaccinated against poliovirus type 2 with OPV or IPV, thereby limiting the anticipated extent and duration of shedding. Further details are provided in item 10.(b).

9. Survivability

(a) ability to form structures enhancing survival or dormancy: Not applicable

- (i) endospores (.)
- (ii) cysts (.)
- (iii) sclerotia (.)
- (iv) asexual spores (fungi) (.)
- (v) sexual spores (funghi) (.)
- (vi) eggs (.)
- (vii) pupae (.)
- (viii) larvae (.)
- (ix) other, specify ...

(b) relevant factors affecting survivability:

Polioviruses are resistant to inactivation by many common detergents and disinfectants, including soaps, but are rapidly inactivated by exposure to ultraviolet light (WHO, 2016). Also dilute solutions of formaldehyde or free residual chlorine can inactivate polioviruses (Dowdle & Birmingham, 1997).

Poliovirus from infected stool has been reported to have survived in fresh water for 188 days at 4°C under laboratory conditions. However, in nature survival will depend on physical, chemical and biological factors in the environment. The estimation as used by WHO is that at ambient temperatures a 90% decrease in infectivity is expected every 5.5 days in fresh water and every 2.5 days in seawater (Dowdle & Birmingham, 1997). Duizer *et al.* (2016) use a more conservative estimate of 90% decrease in infectivity every 17.5 days in fresh water and every 7 days in seawater at 18.5°C. Sewage treatment as commonly practiced will substantially reduce virus concentrations. A reduction by 0.7-2 log₁₀ (5 to 100 times) is assumed (Duizer *et al.*, 2016).

Poliovirus may survive in soil for weeks or months, often longer than in water. In temperate climates, poliovirus infectivity in soil was found to decrease by 90% every 20 days in winter and every 1.5 days in summer (Dowdle & Birmingham, 1997). At ambient temperatures a 90% decrease in infectivity occurs in sewage every 26 days, in freshwater every 5.5 days, and in seawater every 2.5 days (WHO, 2003).

10. (a) Ways of dissemination

Polioviruses can be spread by fecal-oral or oral-oral routes.

(b) Factors affecting dissemination

Prior polio vaccination has been shown to reduce the extent and duration of shedding (oral and in feces). Proper hygiene and availability of waste treatment facilities minimize the risk of dissemination.

Once administered to a human subject, the Sabin-2 virus can replicate. During the first 4-6 weeks following vaccination, the majority of non-immune vaccine recipients shed OPV in nasopharyngeal secretions and feces (WHO, 2016). Shedding is reduced when the vaccine is administered to individuals who have previously received OPV or IPV (Fine & Carneiro, 1999).

Fewer than 5% of children who have received 3 or more doses of OPV shed virus from the oropharynx following a challenge OPV dose. Fecal shedding is also reduced to 22-

37% of recipients who shed the challenge virus for a mean of 5-7 days at titers that are approximately 3 log₁₀ lower than non-immune OPV recipients (Horstmann et al., 1959; Ghendon & Sanakoyeva, 1961; Henry et al., 1966; Onorato et al., 1991).

Prior vaccination with at least 2 IPV doses reduces oropharyngeal excretion of the OPV challenge virus to <5% and also reduces the rate of fecal excretion compared with non-immune subjects, but magnitude of the effect is less than among naturally immune and OPV immune subjects. Across studies, 63-100% of IPV vaccinated children demonstrate fecal excretion at 7-10 days after the OPV challenge (Horstmann et al., 1959; Ghendon & Sanakoyeva, 1961; Henry et al., 1966; Onorato et al., 1991; Mohammed et al., 2008). The effect of IPV vaccination on duration and titer of virus shed is greater (mean 12 days, 50% shorter and 10^{4.1} tcid₅₀/gram, 1 log₁₀ lower than non-immune OPV recipients, respectively). (Ghendon & Sanakoyeva, 1961; Hird & Grassly, 2012).

All trial participants will be fully vaccinated with OPV or IPV.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers) ..., B/./././...
Not applicable.

C. Information relating to the genetic modification

1. Type of the genetic modification

- | | | |
|-------|-------------------------------|-----|
| (i) | insertion of genetic material | (X) |
| (ii) | deletion of genetic material | (X) |
| (iii) | base substitution | (X) |
| (iv) | cell fusion | (.) |
| (v) | others, specify ... | |

2. Intended outcome of the genetic modification

The GMOs are intended to be more genetically stable than the recipient Sabin OPV2 strain, resulting in a much lower (if any) reversion to a neurovirulent phenotype. Whereas Sabin OPV2 attenuation is based primarily on two single nucleotide mutations, the nOPV2 candidate strains include different combinations of five distinct modified regions of the Sabin OPV2 genome. These changes are designed to stabilize the genome, including changes to the primary determinant of attenuation in Sabin OPV2, which reduce the potential for loss of attenuation.

3. (a) Has a vector been used in the process of modification?
Yes (X) No (.)

If no, go straight to question 5.

- (b) If yes, is the vector wholly or partially present in the modified organism?
Yes () No (X)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

- (a) Type of vector
 - plasmid (.)
 - bacteriophage (.)
 - virus (.)
 - cosmid (.)
 - transposable element (.)
 - other, specify ...
- (b) Identity of the vector
- (c) Host range of the vector
...
- (d) Presence in the vector of sequences giving a selectable or identifiable phenotype
 - Yes (.) No (.)
 - antibiotic resistance (.)
 - other, specify ...
 - Indication of which antibiotic resistance gene is inserted
- (e) Constituent fragments of the vector
...
- (f) Method for introducing the vector into the recipient organism
 - (i) transformation (.)
 - (ii) electroporation (.)
 - (iii) macroinjection (.)
 - (iv) microinjection (.)
 - (v) infection (.)
 - (vi) other, specify ...

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

- (i) transformation (.)
- (ii) microinjection (.)
- (iii) microencapsulation (.)
- (iv) macroinjection (.)
- (v) other, specify ...

6. Composition of the insert

- (a) Composition of the insert

For nOPV2 candidate 1

Table 1 lists the five modifications that were introduced in the Sabin-2 cDNA clone to establish the nOPV2 candidate 1 (S2/cre5/S15domV/rec1/hifi3).

Table 1 Overview of the modifications made in nOPV2 candidate 1 (To) in comparison with the recipient Sabin OPV2 strain (From). Nucleotide position is based on mOPV2 numbering

Name of modification	Type of modification	Nucleotide Position	Sequence changes
cre5	Insertion	Between 120 and 121	CAUUAACAAUUAGGUACAGCUCUAGAGCAAAC ACCGUAUAGAGCCAGUACCUUUGUUAGUA
S15 domain V	Substitution	468-535	From UCCUAACCACGGAACAGGGCGGUCGCGAACCAG UGACUGGCUUGUCGUAACGCGCAAGUCUGUGG CGGA To UUCUAACCAUUGAGCAGGCAGCUGCAACCCAG CAGCCAGCCUGUCGUAAC GCGCAAGUCA AUGGCGAA
cre knock-out SL3	Substitution	4447-4499	From AAUAAUUACGUACAGUUCAAAUCCAAGCACCG UAUUGAGCCAGUAUGUUUGUU To AAUAAUUAUGUCCAAUUUAAAUCCAAGCACCG UAUCGAGCCAGUAUGUUUGUU
Rec 1	Substitution	6097-6099; 3Dpol - 38	From AAG; K (lysine) To AGA; R (arginine)
HiFi 3	Substitution	6142-6144; 3Dpol - 53	From GAC; D (aspartate) To AAU; N (asparagine)

The modifications are:

- A new 61 nucleotide sequence was inserted into the 5' UTR to generate a new *cre* element. The sequence inserted originated from the Sabin-2 *cre* (located in protein 2C) but includes changes to increase thermodynamic stability of the secondary structure and to introduce STOP codons if the new *cre* somehow was returned to its position in protein 2C (for example by recombination).
- Modifications were made to the domain V to eliminate the potential for further stabilization of the structure by a single nucleotide change. The modifications are shown in comparison to Sabin-2 domain V. More specifically, the sequence of RNA structural domain V in the 5'UTR (nucleotides 468-535) has been replaced with the equivalent region of the virus S15 (nucleotides 471-538) (Macadam et al., 2006).
- Substitutions were made to the sequence of the original *cre* element in the protein 2C gene in order to eliminate its function. These substitutions all involve synonymous codon usage and therefore do not impact protein 2C amino acid sequence.
- Two codons were modified to result in two amino acid changes in the polymerase which improve fidelity (hifi3) and reduce recombination (rec1).
Apart from the modifications in the polymerase gene (3D) there were no amino acid changes deliberately introduced into the Sabin-2 polyprotein.

For nOPV2 candidate 2

Table 2 lists the two modifications that were introduced in Sabin-2 cDNA clone to establish nOPV2 candidate 2 (S2/S15domV/CpG40).

Table 2 Overview of the modifications made in nOPV2 candidate 2 (To) in comparison with the recipient Sabin OPV2 strain (From). Nucleotide position is based on mOPV2 numbering

Name of modification	Type of modification	Nucleotide Position	Sequence changes
S15 domain V	Substitution	468-535	From UCCUAACCACGGAACAGGCGGUCGCGAACCAG UGACUGGCUUGUCGUAACGCGCAAGUCUGUGG CGGA To UUCUAACCAUUGAGCAGGCAGCUGCAACCCAG CAGCCAGCCUGUCGUAACGCGCAAGUCA AUGG CGAA
CpG40	Substitution	748-3384; P1	87 N-G mutations at 3 rd base of codons; 7 AGY–UCG codon substitutions; All substitutions are synonymous

The modifications are:

- Modifications were made to the S15 domain V (identical to candidate 1 above).
 - The proportion of CpG dinucleotides in the P1 (capsid) region of the genome was increased to 40%. A total of 95 codons were changed resulting in an increase of 94 CpG₂₋₃ and a loss of 7 CpG₃₋₁, yielding a net increase of 87 CpG over Sabin-2. All substitutions are synonymous (i.e., not resulting in an amino acid change) so that surface antigens remain unaltered.
- (b) Source of each constituent part of the insert
The new sequences described in (a) above were all produced synthetically from in silico designed sequences. No genetic material came from another source.
- (c) Intended function of each constituent part of the insert in the GMO
The rationale for the modified regions of the two candidates is described in Table 3. All the modifications are intended to reduce the risk of reversion to a more neurovirulent form.

Table 3 Genetic modifications of Sabin-2 in nOPV2 candidates and their purposes.

Modification (references)	1	2	Purpose
S15 dom V (Macadam et al., 2006)	x	X	<ul style="list-style-type: none"> • Improved stability of attenuated phenotype. Specifically, improve genetic stability of the domain V attenuating mutation to avoid reversion by single nucleotide changes. • Lack of reversion may reduce shedding and transmission risk.
Cre relocation (Toyoda et al., 2007)	x		<ul style="list-style-type: none"> • Reduce frequency of recombination events. Specifically, a single recombination event replacing dom V will also remove cre, making virus non-viable and non-infectious.
Polymerase (higher fidelity) (Vignuzzi et al., 2006)	x		<ul style="list-style-type: none"> • Improved stability of attenuated phenotype. Specifically, improve fidelity of replication leading to less genetic drift and reversion. • Additional attenuation.
Polymerase (rec) (Xiao & Andino, in preparation.)	x		<ul style="list-style-type: none"> • Reduce frequency of recombination events, thereby reducing ability of population to improve replication fitness. • Additional attenuation.

P1 codon de-optimization (Burns et al, 2006, Burns et al, 2009)		X	<ul style="list-style-type: none"> • Improved stability of attenuated phenotype. • May also reduce transmission (less infectious per particle). • May enhance innate immune response against vaccine. • May increase attenuation.
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- (e) Location of the insert in the host organism
- on a free plasmid (.)
 - integrated in the chromosome (X)
 - other, specify ...

- (f) Does the insert contain parts whose product or function are not known?
- Yes (.) No (X)
- If yes, specify ...

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

- viroid (.)
 - RNA virus (.)
 - DNA virus (.)
 - bacterium (.)
 - fungus (.)
 - animal
 - mammals (.)
 - insect (.)
 - fish (.)
 - other animal (.)
- (specify phylum, class) ...

other, specify

Inserted sequences were developed in silico to improve genetic stability

2. Complete name. Not applicable. Inserted sequences developed in silico.

- (i) order and/or higher taxon (for animals) ...
- (ii) family name for plants ...
- (iii) genus ...
- (iv) species ...
- (v) subspecies ...
- (vi) strain ...
- (vii) cultivar/breeding line ...
- (viii) pathovar ...
- (ix) common name ...

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Not applicable, sequences developed in silico.

Yes (.) No (.) Not known (.)

If yes, specify the following:

(c) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.) No (X) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

...

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (X)

If yes, specify ...

5. Do the donor and recipient organism exchange genetic material naturally?

Not applicable. There is no donor organism.

Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes (.) No (X) Not known (X)

Specify

Environmental stability studies have not been conducted; however, product stability appears similar to Sabin-2 strains, as anticipated since the sequence of the viral structural proteins are unchanged.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (.) No (X) Unknown (.)

Specify

Mode of replication not changed from Sabin-2. Rate of replication in cell culture is similar to Sabin-2.

(d) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (X) Not known (.)

Specify

Dissemination between humans has not been studied. It is likely that GMOs will have similar or less risk of dissemination than Sabin-2 due to genetic stabilization.

(e) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (X) No (.) Not known (.)

Specify

It is expected that these candidates are less pathogenic than Sabin-2, which itself is considered generally safe and well tolerated. The modifications introduced in the 2 nOPV2 candidate vaccines are aimed at reducing the possibility for reversion of the attenuated virus to a more virulent form, which is the main concern with Sabin-2 OPV. Both nOPV2 candidate vaccines were well tolerated in the preceding Phase 1 study.

2. Genetic stability of the genetically modified organism

The absence of a relevant animal model that includes replication and shedding of polioviruses following oral dosing makes a definitive assessment of genetic stability *in vivo* difficult prior to human clinical trials; however, culture of Sabin-2 in animal cells at physiological temperatures has been shown to result in reversion in a manner similar to that seen in human shedding samples (Macadam et al., 2006).

Both nOPV2 candidates are much more phenotypically stable than Sabin-2 following ten passages in Vero (African green monkey) cells. Specifically, Sabin-2 increased in virulence more than 1000-fold, as defined by the greater than 3 log₁₀ reduction in the dose that paralyzed 50% of a susceptible transgenic mouse strain, whereas candidate 2 showed a marginal (<10-fold) loss of attenuation, and candidate 1 showed no apparent loss of attenuation. These results suggest that reversion of the candidate strains in the human gut should be significantly reduced relative to the Sabin-2 vaccine.

The first trial using these two vaccine candidates was performed at the University of Antwerp in 2017 (“A Phase 1, blinded, single center study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines, derived from a modified Sabin 2 infectious cDNA clone, in healthy adults previously primed with inactivated polio vaccine (IPV)” [Eudra CT 2017-000908-21]). The trial was conducted under contained conditions and enrolled a total of 30 healthy, IPV-vaccinated volunteers (15 volunteers for each of the two vaccine candidates).

Candidate vaccine viruses isolated from a subset of the volunteers’ stool samples were tested in mice susceptible to paralysis from polio, in order to see if they had changed after reproducing in the gut. No meaningful changes in ability to paralyze mice were detected in any of these samples. The method used to test for susceptibility to paralysis was designed to readily detect the changes which occur after administration of Sabin OPV2; therefore, these results provide further reassurance that the candidates are likely to be even safer than the licensed Sabin OPV2 vaccines.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (X) No (.) Unknown (.)

(a) to which of the following organisms?

humans (X)

animals (.)

plants (.)

other ...

- (b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

Vaccines are intended to protect the population and the 2 nOPV2 candidate vaccines fit in the polio-eradication effort by providing safer and more stable alternatives for existing Sabine OPV2.

In general, the two vaccine candidates were well tolerated in the Phase 1 clinical study. There were no serious adverse events reported, and no severe illnesses thought to be due to the vaccine candidates. Most health events reported during the study were generally mild, and all resolved. There were some changes in clinical laboratory tests, but were without clinical symptoms. Volunteers demonstrated clear immune response to both vaccine candidates.

Fecal shedding was observed for both candidate vaccines, while nasopharyngeal was not. The stool samples of most of the volunteers tested positive for the vaccine candidates. These observations were anticipated based on experience with Sabin OPV2 from which these candidates were derived. Presence of vaccine virus was observed somewhat more for vaccine candidate #1 than #2. Most volunteers did not have evidence of vaccine virus for more than a month. Some volunteers had more prolonged shedding of vaccine virus in stool, with the longest period for vaccine candidate #1 being almost 3 months and for vaccine candidate #2 just over a month-and-a-half. Additional experiments looking at the shed virus were done to see if the shed virus has changed from the original vaccine candidate virus (see also above). None of the volunteers demonstrated any illness associated with shedding of vaccine virus.

4. Description of identification and detection methods

- (a) Techniques used to detect the GMO in the environment

The Polio and Picornavirus Laboratory in CDC's Division of Viral Diseases has developed a real-time reverse transcriptase polymerase chain reaction (RT-rtPCR) method to detect and differentiate between Sabin-2 and the two nOPV2 candidate vaccines.

- (b) Techniques used to identify the GMO

The identity of either of these candidates can be readily identified by the unique sequence elements introduced, which were described above. Therefore, any method based on sequencing can be used to identify these strains.

On this basis, the Polio and Picornavirus Laboratory in CDC's Division of Viral Diseases has developed a real-time reverse transcriptase polymerase chain reaction (RT-rtPCR) method to detect and differentiate between Sabin-2 and the two nOPV2 candidate vaccines.

The nOPV2 multiplex RT-rtPCR assay is conducted according to a SOP developed and evaluated by the CDC Population Immunity team. All samples are run using nOPV2 Multiplex RT-rtPCR kit containing six primers and three probes. Extracted viral RNA is used as a positive control for Sabin-2 and nOPV2 candidate 1 and candidate 2 RNA, while nuclease-free water is used as a negative control. The PCR reaction is achieved using AB 7500 equipment and software. After the completion of the run, the data are

analyzed using a manual threshold setting following as per the SOP developed by the CDC.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The purpose of the release is to evaluate the safety and immunogenicity of the two vaccine candidates. The deliberate release covers the Phase 2 multicenter, double-blinded, placebo-controlled, randomized study in 200 healthy OPV-primed adults (age range 18 to 50 years) and in 132 healthy IPV- only-primed adults and adolescents (15 to 50 years). There are no expected environmental benefits from this release; however, if vaccine development is successful, use of these vaccines in lieu of the Sabin OPV2 strain may reduce the risk of circulation of vaccine-derived polioviruses.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (X) No ()

If yes, specify

Sabin-2 OPV usage was discontinued globally in 2016, except in response to outbreaks. In Belgium, Sabin-2 OPV was used for routine vaccination until 2000 and has been used in clinical studies in 2015 and 2016.

3. Information concerning the release and the surrounding area

- (a) Geographical location (administrative region and where appropriate grid reference):

The clinical trial centres are:

- Centre for Evaluation of Vaccination, University of Antwerp
- CEVAC - Center for Vaccinology, UGent

At these locations, exposure and release into the environment will be limited (administration, follow-up of participants and handling of samples). The actual release will occur at the moment of the application by the patient and subsequent shedding.

- (b) Size of the site (m²): not relevant

(i) actual release site (m²): ... m²

(ii) wider release site (m²): ... m²

- (c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

The primary release is the moment where the vaccine is administered to the participant. Since this is done via an oral application, the involved clinical staff can apply the prepared dose without further manipulation. All waste related to the application that have been used for the nOPV2 candidate vaccines, will be collected on the site and will be treated as hazardous medical waste.

While the location of the clinical trial centres will be known, the identity and coordinates of the participants will not be known to the notifier. In addition, shedding may occur during the evacuation of stool. This is not necessarily limited to the home of the participant. As a consequence of release via shedding, the proximity of

significant biotopes, protected areas or drinking water supplies cannot be excluded. However, the only route for exposure would be via the disposal of stool, which would in any event not be expected to reach such areas because of standard waste water treatment.

- (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

Not relevant

4. Method and amount of release

- (a) Quantities of GMOs to be released:

The nOPV2 candidate vaccines are provided to the sites in vials filled in 1.1 ml aliquots, sufficient for 3 doses per vial, and presented as an aqueous yellow-red solution for oral use.

Both candidate vaccines will be administered orally (6 drops of study vaccine). One dose of vaccine (0.3 ml) is contained in six drops which are delivered via a spoon. Each dose of the nOPV2 vaccine candidate 1 and nOPV2 vaccine candidate 2 contains approximately 10^6 CCID₅₀ (cell culture infectious doses).

Following administration, the nOPV2 candidate vaccine will multiply for a limited period in the host and viruses then will be shed.

- (b) Duration of the operation:

Recruitment of the first participants is expected to start in the second half of 2018 (Q3/Q4). Completion will depend on availability of participants fulfilling all selection criteria and could take up 3 to 5 months..

Study duration will be approximately 6 weeks for subjects receiving 1 dose of vaccine and 10 weeks for subjects receiving 2 doses of vaccine, including the 6-week safety follow-up period after last vaccine administration.

A subject will be considered to have completed the study when:

- he or she has completed all study related procedures 42 days after the last study vaccination,
- shedding is PCR negative on 3 consecutive stool samples (with a maximum of one sample per day), and
- no AE or SAE, including clinically significant abnormalities in laboratory safety testing are observed.

- (c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

At the clinical trial centers there will be appropriate handling of all clinical trial material. Staff will wear a lab coat and disposable gloves. Disposable wipes will be used when handling samples. All waste material will be handled as hazardous medical waste. After the last visit of the last subject in the study, any used and unused study vaccine will be destroyed. For any handling in the clinical trial centers during preparation, administration or follow-up, either chemical inactivation (Umonium spray, 5 min. exposure) or collection as hazardous medical waste for heat inactivation/incineration will be used.

All participants will be healthy without any immune system disorders, will have been previously immunized with either OPV or IPV, as will all close contacts. In addition, specific participant exclusion criteria are included to limit the residual risk for exposure of the broader population to shed candidate vaccines. Standard hygienic practices used by the subjects should be sufficient to limit or prevent significant exposure via fecal-oral routes.

Shed material is expected to be eliminated naturally from the environment, similar to any other OPV and wildtype poliovirus. Stools of study participants are expected to be eliminated via standard waste collection and treatment processes and/or in septic tanks of houses that are not connected to standard waste elimination routes.

5. Short description of average environmental conditions (weather, temperature, etc.)

Not relevant. Polio virus and the Sabin 2 have a wide distribution throughout all climatic zones. Environmental conditions which may affect survival outside of the host are expected to be the same as for the recipient Sabin 2 organism. Multiplication in and dissemination by the host are expected to be influenced by the prior vaccination status and immune status of the host, as for the recipient Sabin 2 organism.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

Previous use of these candidate vaccines was under contained use conditions.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable):

Not applicable

- (i) order and/or higher taxon (for animals) ...
- (ii) family name for plants ...
- (iii) genus ...
- (iv) species ...
- (v) subspecies ...
- (vi) strain ...
- (vii) cultivar/breeding line ...
- (viii) pathovar ...
- (ix) common name ...

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

NA

3. Any other potentially significant interactions with other organisms in the environment

NA

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (.) Not known (.)

Give details

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

...

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

- (i) order and/or higher taxon (for animals) ...
- (ii) family name for plants ...
- (iii) genus ...
- (iv) species ...
- (v) subspecies ...
- (vi) strain ...
- (vii) cultivar/breeding line ...
- (viii) pathovar ...
- (ix) common name ...

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

...

(b) from other organisms to the GMO:

...

(c) likely consequences of gene transfer:

...

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

...

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

...

H. Information relating to monitoring

1. Methods for monitoring the GMOs

A quantitative PCR method has been developed which uniquely identifies both nOPV2 candidate vaccines. In principle, the PCR method can be used on material extracted from any type of sample, provided the sample matrix is not hindering the extraction or interfering with detection. Since it is based on the presence of specific sequences, it is possible that fragments of the vaccines also are detected and thereby providing an overestimation of the presence of the GMOs.

The method will be used to evaluate and monitor shedding of type 2 poliovirus in stool samples of study participants.

The primary effect is the immunization of the exposed person. Neutralizing antibodies against type 2 poliovirus can be determined using a sero-neutralization assay. The clinical study protocol schedules specific time points when blood samples will be taken from the participants. Similarly, blood samples can be taken –if necessary- from other persons that might be exposed.

2. Methods for monitoring ecosystem effects

There are no anticipated ecosystem impacts. Monitoring will be focused on study participants.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

The potential for recombination with other C enteroviruses has been indicated. The likelihood has been deemed to be very low, given the absence of C enteroviruses in the potentially exposed population (Personal communication from Prof. M. Van Ranst, Rega Institute, KULeuven, national reference laboratory on enterovirus typing for the period 2011-2016/2017).

The main concern would be that the nOPV2 candidate vaccines revert to neurovirulence by recombination of specific sequences with a C enteroviruses that is present at the same time in the same cell. In case such an event is suspected to have occurred, it is possible to investigate the exact nature by using molecular tools.

4. Size of the monitoring area (m²)

NA. Monitoring will be focused on study participants.

5. Duration of the monitoring

The participants will visit the clinical trial centre at regular times for monitoring.

Participants are expected to visit the clinical trial centres 5 times for groups 1 and 3 (Day 0, Day 7, Day 14, Day 28 and Day 42) and 8 times for all other groups (Day 0, Day 7, Day 14, Day 28, Day 35, Day 42, Day 56 and Day 70). All visits are ambulatory. At specific times, blood samples will be taken for the determination of neutralizing type 2 poliovirus antibodies using a sero-neutralization assay. Stool samples will be collected and shedding of the nOPV2 candidate vaccines in the stools will be evaluated.

A participant will be considered to have completed the study if he or she has completed all study related procedures 42 days after the last study vaccination and shedding is PCR negative on 3 consecutive stool samples (with a maximum of one sample per day). However, if any AE or SAE, including clinically significant abnormalities in laboratory safety testing are observed subjects will continue to be followed until these are resolved or determined to be chronic and stable or until the event is otherwise explained.

Also, if type 2 virus shedding is detected by PCR on last stool sample (V5 minus 1 or 2 days for Groups 1 and 3 and V8 minus 1 or 2 days for Groups 2, 4, 5, 6 and 7), subjects will be asked to further collect 3 consecutive stool samples at least once per week for four weeks after the last per protocol samples and then once per month until shedding is PCR negative on 3 consecutive stool samples.

6. Frequency of the monitoring

See Section H 5 above.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
According to standard practices at the clinical trial centres, all waste is collected and treated as hazardous medical waste, i.e. collected in dedicated and certified bins, which are hermetically sealed and transported by a certified shipper to a specialized incineration facility. Surfaces and non-disposable materials will be chemically decontaminated with Umonium^{38®}.
2. Post-release treatment of the GMOs
No treatment is envisaged of shed viruses. Participants will be asked to observe good hygienic practices. Material shed via feces will be discharged into the sewage system, in which it will be immediately diluted, and subsequent waste water treatment will substantially reduce virus concentrations.
3. (a) Type and amount of waste generated
Two types of waste that are expected to carry nOPV2 candidate vaccines are identified:
 - Materials at the clinical trial centres that contain or have been exposed to the nOPV2 candidate vaccines (e.g. residual doses, empty containers, equipment used during visits of and sampling of participants)
 - Materials that contain or that may have been exposed to viruses shed via feces (e.g. tissues, hygienic wipes)

The amount of waste generated at the clinical trial centres is not expected to be significant and will be within the normal handling capacity.

Based on the Phase 1 results, it is not expected that any material will be shedded via saliva. Material shed via feces will be discharged in the sewage system, in which it is immediately diluted.
3. (b) Treatment of waste
According to standard practices at the clinical trial centres, all waste is collected and treated as hazardous medical waste, i.e. collected in dedicated and certified bins, which are hermetically sealed and transported by a certified shipper to a specialized incineration facility. Surfaces and non-disposable materials will be chemically decontaminated with Umonium^{38®}.

No treatment is envisaged of shed viruses. Participants will be asked to observe hygienic practices such as flushing toilet with toilet lid closed, hand washing after toilet use, hand washing before handling food and no sharing of cutlery.

Stools will be disseminated via standard waste water treatment.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
The proposal takes into consideration a potentially very broad release of the nOPV2 candidate vaccines.

Unexpected spread would mainly be limited to accidental opening of the packaged doses. Even if all the doses are spilled, the quantity remains limited (3ml per doses) and can easily be handled via a spill procedure.

2. Methods for removal of the GMO(s) of the areas potentially affected

nOPV2 candidate vaccines are dependent on humans for multiplication. They are expected to survive in the environment for a limited period. If needed, and depending on the affected area, chemical disinfection can be used in a liquid or gas form.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Material that has been exposed to the nOPV2 candidate vaccines during administration will be either disinfected or inactivated as hazardous medical waste. In other cases, it may suffice to wait until the viruses have been eliminated naturally and foresee that during this period no contact is made with humans as they are the only host. No specific sanitation measures are foreseen.

4. Plans for protecting human health and the environment in the event of an undesirable effect

The main undesirable effect would be the exposure of participants and the broader public to viruses that have reverted to neurovirulence. The 2 nOPV2 candidate vaccines are designed to reduce -if not eliminate- the possibility for reversion to a more virulent form. The data, including those from the Phase 1 study, confirm the improved safety of the nOPV2 candidate vaccines.

Given the general vaccination status of the population this is not expected to induce any effect. In any case, in line with the GAPIII, this would require a broad follow-up in order to ensure that the polio eradication programme can be maintained.

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