Clinical Study Protocol

A Phase 2, partial 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 previously vaccinated with oral polio vaccine (OPV) or inactivated polio vaccine (IPV), compared with historical controls given Sabin OPV2 or placebo.

Product	- nOPV2 candidate 1 (S2/cre5/S15domV/rec1/hifi3) - nOPV2 candidate 2 (S2/S15domV/CpG40)
Protocol Number	UAM4
EudraCT Number	2018-001684-22
Clinical Phase	II
Clinical Indication	Oral polio vaccine immunization
Issue Date	20-Dec-2018
Version	3.1

Sponsor	University of Antwerp (with grant support from the Bill and Melinda Gates Foundation)
Sponsor Representative	Prof Dr Pierre Van Damme, PhD University of Antwerp Campus Drie Eiken, Universiteitsplein 1 2610 Antwerpen (Wilrijk) Belgium Tel + 32-3-2652538, Fax +32-3-2652404

This study will be conducted in compliance with this protocol, the ICH Note for Guidance on Good Clinical Practice (CMPM/ICH/135/95) and with the applicable regulatory requirement(s).

SIGNATURES

Signature of Sponsor Representative

Title: **A Phase 2, partial blind, randomized, placebo-controlled, multicenter study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccine candidates in healthy adults previously vaccinated with oral polio vaccine (OPV) or inactivated polio vaccine (IPV), compared with historical controls given Sabin OPV2 or placebo.**

Name:

Affiliation:

Address:

'This Clinical Study Protocol has been reviewed and approved by the Sponsor in order to ensure compliance with Good Clinical Practice.'

Signature:

Date:

Signature of Statistician

Title: **A Phase 2, partial blind, randomized, placebo-controlled, multicenter study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccine candidates in healthy adults previously vaccinated with oral polio vaccine (OPV) or inactivated polio vaccine (IPV), compared with historical controls given Sabin OPV2 or placebo.**

Name:	Christopher Gast, Ph.D.
Affiliation:	PATH Center for Vaccine Innovation and Access
Address:	2201 Westlake Ave., Suite 200 Seattle, WA 98121 USA

'This Clinical Study Protocol has been reviewed and approved by the study statistician in order to ensure that the protocol and any amendments cover all relevant statistical/pharmacokinetic matters clearly and accurately, using technical terminology as appropriate'

Signature:

Date:

Signature of Investigators

Title: A Phase 2, partial blind, randomized, placebo-controlled, multicenter study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccine candidates in healthy adults previously vaccinated with oral polio vaccine (OPV) or inactivated polio vaccine (IPV), compared with historical controls given Sabin OPV2 or placebo.

Name:

Affiliation:

Address:

'I have read this Clinical Study Protocol and agree that it contains all information necessary for proper conduct of the study. I will carry out the study as outlined herein and will complete the study within the designated time.'

Signature:

Date:

PROTOCOL HISTORY

Protocol History University of Antwerp – UAM4			
Document	**Issue Date**	**Amendment Type**	**Comments**
Initial Clinical Study Protocol	26-Apr-2018	-	
Version 2.1	30Aug2018	-	
Version 3.1	20Dec2018	Substantial	Amendment 1

SUBSTANTIAL AMENDMENT 1: CHANGES TO THE PROTOCOL

In case of extended shedding of the subjects sampling of household contacts will be offered to the subjects' households: this is explained in appendix 4 and also added at section 1.3.1 'Potential Risks'.
Frequency of follow-up samples to be taken in case of extended shedding has been adapted: 'every 3 weeks' has been deleted and subjects will be asked to collect further 3 consecutive stool samples (with a maximum of one sample per day) after the last per-protocol sample as soon as these PCR-positive results are known (anticipated approximately 3 weeks after the last such sample provided for evaluation) and to repeat this until shedding is PCR-negative for type 2 poliovirus on 3 consecutive stool samples, which then determines study end for this person. This is adapted accordingly in the following sections: synopsis 'Study/treatment duration', section 9.1 'Study Completion' and in Appendix 4.
The DSMB will also monitor study enrollment, particularly for IPV-vaccinated subjects, and recommend truncation and/or closure of study groups if enrollment stagnates and when current enrollment is considered sufficient to meet study objectives and no safety signals occurred. The minimum number of IPV-vaccinated subjects agreed on by DSMB per candidate vaccine is 24. With a randomization of 2:1 for placebo the minimum study cohort size for Groups 5, 6 and 7 will be 16. In case of safety signals the DSMB reserves the right to reverse the truncated enrollment. This explanation is added in sections: Synopsis 'Overview of Study Design', section 4.1 'Overview of Study Design' and section 6.6 'Randomization and Blinding'.
Safety endpoint evaluation by age group for IPV- vaccinated subjects has been deleted in synopsis and section 3.1 'Primary endpoints' and section 3.2 'Secondary endpoints'
Affiliation of statistician has been changed on signature page and section of Study Administrative Structure.

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PROTOCOL SYNOPSIS

Study Title	**A Phase 2, partial blind, randomized, placebo-controlled, multicenter study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccine candidates in healthy adults previously vaccinated with oral polio vaccine (OPV) or inactivated polio vaccine (IPV), compared with historical controls given Sabin OPV2 or placebo.**		
Product	- nOPV2 candidate 1 (S2/cre5/S15domV/rec1/ hifi3) - nOPV2 candidate 2 (S2/S15domV/CpG40)	**Clinical Phase**	II
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Objectives:

The primary objectives of the study are

- To assess the safety (serious adverse events [SAEs] and severe* adverse events [AEs]) of novel monovalent live attenuated oral serotype 2 poliovirus vaccine (nOPV2) candidate 1 and novel monovalent live attenuated oral serotype 2 poliovirus vaccine (nOPV2) candidate 2 in healthy OPV-vaccinated adults, relative to historical controls given Sabin OPV2;
- To compare the immunogenicity (seroprotection rate) of novel monovalent live attenuated oral serotype 2 poliovirus vaccine (nOPV2) candidate 1 and novel monovalent live attenuated oral serotype 2 poliovirus vaccine (nOPV2) candidate 2 in healthy OPV-vaccinated adults to historical controls given Sabin OPV2;
- To assess the safety (serious adverse events [SAEs] and severe adverse events [AEs]) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated adults, compared with placebo.

Secondary objectives are

- To assess the safety (any solicited and unsolicited AEs, laboratory assessments) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy OPV-vaccinated adults, compared with historical controls given Sabin OPV2;
- To assess the safety (any solicited and unsolicited AEs, laboratory assessments) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated adults, compared with placebo;
- To compare the immunogenicity (seroconversion rate, median antibody titer (post-vaccination)) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy OPV-vaccinated adults, with historical control of Sabin OPV2;

* List of severe AEs as mentioned in the diary cards: fever > 39°C, headache, fatigue, myalgia, arthralgia, paresthesia, anesthesia, paralysis, or gastrointestinal symptoms (nausea, vomiting diarrhea and/or abdominal pain) that prevent normal activity or any other severe AE that prevents normal activity.

- To assess the immunogenicity (seroprotection rate, seroconversion rate, median post-vaccination antibody titer) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated adults.

Exploratory objectives are

- to compare immunogenicity (geometric mean titer [GMT]) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy OPV-vaccinated adults, with historical control of Sabin OPV2;
- to assess immunogenicity (geometric mean titer [GMT]) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated adults;
- to compare viral shedding following nOPV2 candidate 1 or nOPV2 candidate 2 administration in a pre-specified subset of stool samples in healthy OPV-vaccinated adults, with historical control of Sabin OPV2;
- to assess viral shedding following nOPV2 candidate 1 or nOPV2 candidate 2 administration in a pre-specified subset of stool samples in healthy IPV-only vaccinated adults;

The shedding analyses will initially be conducted using a subset of samples in the 10 days following administration of each dose (e.g. day 0, 3, 5, 7, and 10) and all samples at day 14 and thereafter. The remaining samples will be stored pending evaluation of the initial analyses; PCR and $CCID_{50}$ will be conducted on these stored samples if deemed important for the complete interpretation of the study results.

Exploratory objectives will also include comparison of neurovirulence (as measured in animal model(s)) and assessment of the genetic stability via analysis of genetic sequence, including but not limited to the modified regions of shed virus in a subset of stool samples of all OPV-vaccinated adults, relative to historical control of Sabin OPV2.

Exploratory objectives will also include assessment of neurovirulence (as measured in animal model(s)) and the genetic stability via analysis of genetic sequence, including but not limited to the modified regions of shed virus in a subset of stool samples of all IPV-only vaccinated adults given doses of nOPV2 candidates 1 and 2.

Overview of Study Design:

This will be a multicenter, partial blind, placebo-controlled, randomized study in 200 healthy OPV-vaccinated adults and in 48 to 132 healthy IPV-only vaccinated adults (age range 18 to 50 years), as follows:

- 50 OPV-vaccinated adults to receive 1 dose of nOPV2 candidate 1 (Group 1);
- 50 OPV-vaccinated adults to receive 2 doses of nOPV2 candidate 1 (Group 2), administered 28 days apart;
- 50 OPV-vaccinated adults to receive 1 dose of nOPV2 candidate 2 (Group 3);
- 50 OPV-vaccinated adults to receive 2 doses of nOPV2 candidate 2 (Group 4), administered 28 days apart;
- 16 to 44 IPV-only vaccinated adults to receive 2 doses of nOPV2 candidate 1 (Group 5), administered 28 days apart;
- 16 to 44 IPV-only vaccinated adults to receive 2 doses of nOPV2 candidate 2 (Group 6), administered 28 days apart;
- 16 to 44 IPV-only vaccinated adults to receive placebo (Group 7), administered 28 days apart.

Groups will be enrolled sequentially such that subjects receiving candidate 2 will be enrolled first (IPV and OPV groups simultaneously), followed by subjects receiving candidate 1. IPV-only vaccinated adults will be randomized 2:1 to candidate 2 (Group 6) or placebo (Group 7), respectively, until 16 to 44 Group 6 subjects are enrolled, after which randomization 2:1 to candidate 1 or placebo will commence for Groups 5 and 7. For OPV-vaccinated subjects, randomization to Groups 1 and 2 to receive candidate

1 will ensue following complete randomized enrollment of subjects to Groups 3 and 4. In all cases, block randomization will be used to ensure balanced randomization across time.

The DSMB has established stopping rules for safety prior to study start which will be continuously assessed. The DSMB will also monitor study enrollment, particularly for IPV-vaccinated subjects, and recommend truncation and/or closure of study groups if enrollment stagnates and when current enrollment is considered sufficient to meet study objectives and no safety signals occurred. The minimum number of IPV-vaccinated subjects agreed on by DSMB per candidate vaccine is 24. With a randomization of 2:1 for placebo the minimum study cohort size for Groups 5, 6 and 7 will be 16. In case of safety signals the DSMB reserves the right to reverse the truncated enrollment.

Procedures and processes will be unified across sites through training and additional preparatory activities; demographic and baseline characteristics will be assessed by site in order to ensure data can be pooled. Immunogenicity outcomes will be assessed by site and pooled within group.

Figure 1: Trial overview

Study code/ Country	Group N°	Age (yr)	Immunogenic Background	Candidate	Study Cohort Size	No. of Doses	Dose level
UAM4 Belgium	Group 1	18-50	OPV	nOPV2 candidate 1	50	1	~10^6
UAM4 Belgium	Group 2	18-50	OPV	nOPV2 candidate 1	50	2	~10^6
UAM4 Belgium	Group 3	18-50	OPV	nOPV2 candidate 2	50	1	~10^6
UAM4 Belgium	Group 4	18-50	OPV	nOPV2 candidate 2	50	2	~10^6
UAM4 Belgium	Group 5	18-50	IPV	nOPV2 candidate 1	16-44	2	~10^6
UAM4 Belgium	Group 6	18-50	IPV	nOPV2 candidate 2	16-44	2	~10^6
UAM4 Belgium	Group 7	18-50	IPV	Placebo	16-44	2	Sirupus simplex

One hundred OPV-vaccinated subjects will be evaluated for the 1-dose regimen of nOPV2 candidate 1 (Groups 1 and 2) and 50 of these 100 subjects will be evaluated for the 2-dose regimen of nOPV2 candidate 1 (Group 2).

One hundred OPV-vaccinated subjects will be evaluated for the 1-dose regimen of nOPV2 candidate 2 (Groups 3 and 4) and 50 of these 100 subjects will be evaluated for the 2-dose regimen of nOPV2 candidate 2 (Group 4).

Sixteen to forty-four IPV-only vaccinated subjects will be evaluated for the 2-dose regimen of nOPV2 candidate 1 (Group 5).

Sixteen to forty-four IPV-only vaccinated subjects will be evaluated for the 2-dose regimen of nOPV2 candidate 2 (Group 6).

Sixteen to forty-four IPV-only vaccinated subjects will be evaluated for the 2-dose regimen of oral placebo (Group 7).

The study will be conducted at 2 sites in Belgium.

The assessments performed are summarized per visit in the Time and Events Schedule.

Study Population:

Healthy OPV-vaccinated adults (age range 18 to 50 years)

Healthy IPV-only vaccinated adults (age range 18 to 50 years)

At each visit participants will be reminded of and checked for the inclusion and exclusion criteria, and the necessity of adherence to the criteria will be reiterated.

Inclusion Criteria:

1. For Groups 1, 2, 3 and 4: healthy males or females, from 18 to 50 years of age inclusive, having previously received at least 3 doses of OPV more than 12 months before the start of the study;
2. For Groups 5, 6 and 7: healthy males or females, from 18 to 50 years of age inclusive, having previously received at least 3 doses of IPV more than 12 months before the start of the study;
3. Having residence in Belgium;
4. In good physical and mental health as determined on the basis of medical history and general physical examination performed at Day 0;
5. Female subjects of childbearing potential must agree to the use of an effective method of birth control throughout the study and up to 3 months after last vaccine dose (see Section 7);
6. Willing to adhere to the prohibitions and restrictions specified in this protocol (see Section 7);
7. Informed Consent Form (ICF) and Code of Conduct signed voluntarily by the subject before any study-related procedure is performed, indicating that the subject understands the purpose of any procedures required for the study and is willing to participate in the study.

Exclusion Criteria:

1. A condition that, in the opinion of the Investigator, could compromise the well-being of the subject or course of the study, or prevent the subject from meeting or performing any study requirements;
2. For Groups 5, 6 and 7: ever having received any OPV in the past;
3. Any travel to polio endemic countries or countries with evidence of recent (within last 6 months) wild or vaccine-derived poliovirus circulation during the total duration of the study;†
4. Professional handling of food, catering or food production activities during the total duration of the study;
5. Having Crohn's disease or ulcerative colitis or having had major surgery of the gastrointestinal tract involving significant loss or resection of the bowel;
6. A known allergy, hypersensitivity, or intolerance to the study vaccine or the placebo, or to any of their components or to any antibiotics;
7. Any confirmed or suspected immunosuppressive or immunodeficiency condition (including human immunodeficiency virus [HIV] infection, hepatitis B or C infections or total serum IgA level below laboratory lower limit of normal (LLN));
8. Will have household or professional contact with known immunosuppressed people or people without full polio vaccination (i.e. complete primary infant immunization series), e.g. babysitting during the total duration of the study;
9. Neonatal nurses or others having professional contact with children under 6 months of age during the total duration of the study;
10. Chronic administration (i.e., longer than 14 days) of immunosuppressant drugs or other immune-modifying drugs within 6 months prior to the first vaccine dose or planned use during the study. For instance, for corticosteroids, this means prednisone, or equivalent, ≥ 0.5 mg/kg/day (inhaled and topical steroids are allowed whereas intra-articular and epidural injection/administration of steroids are not allowed);
11. Presence of contraindications to administration of the study vaccine on Day 0: acute severe febrile illness deemed by the Investigator to be a contraindication for vaccination or persistent diarrhea or vomiting;
12. Indications of drug abuse or excessive use of alcohol at Day 0 (males: > 21 units/week; females > 14 units/week);
13. Being pregnant or breastfeeding. Women of childbearing potential will undergo a urine pregnancy test at each vaccination visit. Subjects with a positive pregnancy test will be excluded;
14. Participation in another clinical study within 28 days prior to entry in this study or receipt of any investigational product (drug or vaccine) other than the study vaccine within 28 days prior to the first administration of study vaccine, or planned use during the study period;
15. Administration of any vaccine other than the study vaccine within 28 days prior to the first dose of study vaccine and during the entire study period;
16. Administration of any polio vaccine within 12 months before the start of the study;

17. Having had a transfusion of any blood product or application of immunoglobulins within the 4 weeks prior to the first administration of study vaccine or during the study;
18. Subject is an employee of the Investigator or study site, with direct involvement in the proposed study or other studies under the direction of that Investigator or study site, or is a family member of an employee or the Investigator, or was a study subject in the historical control studies UAM1 or UAT1 or in the study UAM4a;
19. Having a family or household member participating in the study CVIA 065 or being a study subject in the study CVIA 065.

Test Product and Placebo, Dose, Mode of Administration:

nOPV2 candidate 1 and nOPV2 candidate 2 are attenuated serotype 2 polioviruses derived from a modified Sabin 2 infectious clone and propagated in Vero cells.

Each dose of nOPV2 candidate 1 and nOPV2 candidate 2 contains approximately 10^6 50% cell culture infective dose units ($CCID_{50}$).

Sirupus simplex, Propylenglycolum,. European Pharmacopoeia (Ph.Eur.), will be used as placebo. This clear, colorless to pale yellow liquid contains 64% saccharose, 0,08% methylparaben, 0,02% propylparaben and propyleneglycol, presented in a HDPE container of 1 liter.

The vaccines and placebo will be administered orally. One dose of vaccine (0.3 ml) is contained in six drops which are delivered with a spoon from the dropper supplied with the vaccine. For the placebo a pipette will be used to count 6 drops on a spoon to be administered in a similar way to the subject.

Reception of the vaccines, dose preparation and administration will be done by unblinded medical study personnel who will not participate in any safety evaluation.

Study/Treatment Duration:

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

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 or stable or until the event is otherwise explained.

Also, if type 2 virus shedding is detected by PCR on one of the last 3 scheduled stool samples, the study duration for this individual will be extended. As soon as these results are known (anticipated approximately 3 weeks after the last such sample provided for evaluation) the subject will be asked to collect further 3 consecutive stool samples (with a maximum of one sample per day) after the last per-protocol sample, and to repeat this until shedding is PCR-negative for type 2 poliovirus on 3 consecutive stool samples, which then determines study end for this person.

If the last stool sample is missing the subject will be asked to provide a new sample as soon as possible in order to determine their end of study or the need for further stool sample collection according to the collection cadence until shedding is PCR negative on 3 consecutive stool samples.

[†] Updated list will be made available at the start of the study. Study will take in consideration the most recent WHO guidelines.

Criteria for Evaluation:

Assessment of the following endpoints for the 2 candidate vaccines in OPV primed subjects (Groups 1 to 4) will be based on comparison with data obtained in the previously conducted Phase 4 study of Sabin mOPV2 in order to assess immunogenicity and establish an acceptable safety profile in these populations. For the safety endpoint of the IPV-primed subjects (Groups 5, 6) comparison will be made with the data of the placebo group (Group 7).

Primary

Safety: The following endpoints will be evaluated by group and overall:

- incidence, type and causality of SAEs and severe AEs throughout the study period.

Immunogenicity:

- seroprotection rate of type 2 polio antibodies at Day 28, following a single dose of nOPV2 candidate 1 (combined Groups 1 and 2).
- seroprotection rate of type 2 polio antibodies at Day 28, following a single dose of nOPV2 candidate 2 (combined Groups 3 and 4).
- Seroprotection is defined as type 2-specific antibody titers ≥ 1:8.

Secondary

Safety: The following endpoints will be evaluated by group and overall:

- incidence, type, causality and severity of solicited adverse events for days 0-7 in Groups 1 and 2 combined and days 28-35 in Group 2;
- incidence, type, causality and severity of solicited adverse events for days 0-7 in Groups 3 and 4 combined and days 28-35 in Group 4;
- incidence, type, causality and severity of solicited adverse events for days 0-7 and days 28-35 in Groups 5, 6 and 7;
- incidence, type, causality and severity of unsolicited adverse events throughout the study period in all groups;
- incidence, causality and description of deviations from normal safety labs at Day 0, Day 7, Day 14 and Day 28 for Groups 1 through 4, and at Day 35, Day 42 and Day 56 for Groups 2 and 4;
- incidence, causality and description of deviations from normal safety labs at Day 0, Day 7, Day 14, Day 28, Day 35, Day 42 and Day 56 for Groups 5, 6, and 7.

Immunogenicity:

- Median titers of type 2 polio antibodies at Day 28 in Groups 1 and 2 combined;
- Median titers of type 2 polio antibodies at Day 28 in Groups 3 and 4 combined;
- Median titers of type 2 polio antibodies at Day 28 in Groups 5, 6 and 7.

- Seroprotection rate and median titers of type 2 polio antibodies at Day 56 in Groups 2 and 4;
- Seroprotection rate of type 2 polio antibodies at Day 28 and at Day 56 in Groups 5, 6 and 7.

- Seroconversion rate of type 2 polio antibodies at Day 28 for Groups 1 and 2 combined and at Day 56 in Group 2;
- Seroconversion rate of type 2 polio antibodies at Day 28 for Groups 3 and 4 combined and at Day 56 in Group 4;
- Seroconversion rate of type 2 polio antibodies at Day 28 and at Day 56 for Groups 5, 6 and 7.

Seroconversion is defined as a change from seronegative to seropositive and antibody titers of ≥1:8, and in seropositive subjects, as an antibody titer increase of ≥ 4-fold over baseline titers.

Exploratory

- GMT of type 2 polio antibodies at Day 28 in Groups 1 and 2 combined and at Day 56 in Group 2;
- GMT of type 2 polio antibodies at Day 28 in Groups 3 and 4 combined and at Day 56 in Group 4;
- GMT of type 2 polio antibodies at Day 28 and at Day 56 in Groups 5, 6 and 7.

- Viral shedding positivity rate (as determined using quantitative PCR) will be assessed at a pre-specified subset of the stool collection time points
- each stool sample collection time point
- Median 50% cell culture infective dose ($CCID_{50}$; titer) of shed virus after viral extraction from PCR-positive stool samples will be assessed at a pre-specified subset of the stool collection time points
- Time-to-cessation of viral shedding will be assessed following each dose
- The extent of shedding, including quantity and duration, will be assessed among pre-specified stool collection time points following each dose.

Exploratory endpoints will include assessment for neurovirulence of shed virus (as measured in animal model(s)) and will also include assessment of the genetic stability of the modified regions of shed virus in a subset of stool samples.

Due to observed transient and asymptomatic elevated values of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) in the first-in-human phase 1 study, UAM4a, conducted in IPV-vaccinated adults in a contained environment, CK, ALT, AST, GGT, bilirubin and albumin have been designated for further evaluation in this study. Should any anomaly be detected, this can be further explored by additional laboratory testing (e.g.CK subtypes, electrophoresis, auto-antibodies (e.g. ANA, AMA, LKM-1, SLA/LP, other viral pathogens)

Statistical Methods:

Sample size

Groups 1 through 4:

Sample size is chosen here to mirror that of the historical control study, UAM1. The serotype 2 Day 28 seroprotection rate among OPV-vaccinated individuals is expected to be 95% for subjects receiving a single dose of either investigational vaccine or the type 2 Sabin OPV control vaccine (historical control). 77 evaluable subjects per vaccine group are required to achieve 80% power for a test of non-inferiority to the historical control with a type I error rate of $\alpha = 0.025$ and a non-inferiority margin of 10%. 45 subjects are required to have 90% probability of observing at least one occurrence of an adverse event, when the true adverse event rate is 5%. Allowing for a 10% non-evaluability/dropout rate requires 50 subjects for the safety evaluations. For each vaccine candidate, both 1- and 2-dose groups may be used to assess the Day 28 immunogenicity endpoint, providing 100 subjects for the primary immunogenicity comparison. Therefore, 50 subjects will be allocated to each dose group, to also collect adequate safety data for each group, as defined above.

Groups 5 through 7:

Due to observed transient and asymptomatic elevated values of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) in the first-in-human phase 1 study, UAM4a, conducted in IPV-vaccinated adults in a contained environment, CK, ALT, AST, GGT, bilirubin and albumin have been designated for further evaluation in this study. Data from OPV-primed individuals in study UAM1 suggest the background rate of spurious elevations with the safe mOPV2 vaccine may be approximately 6%. If the true rate with either candidate vaccine is 4 times the expected rate, 44 subjects per group (each vaccine, plus control) would be required to have 80% power to detect a significant increase in the rate (lower one-sided 95% confidence bound for risk difference > 0) relative to the placebo group. If, upon review of interim safety data, the DSMB agrees that a) despite all reasonable efforts, recruitment has stagnated, and b) sufficient data have accrued to satisfy safety objectives relevant to these groups, group sizes can be truncated.

Comparative Analyses

The study UAM1, conducted in a pre-OPV2 cessation period (2015-2016), was specifically designed to provide active control comparator data for both safety and immunogenicity for the OPV-vaccinated adults given the novel vaccine candidates in this study. For all endpoints, summaries of data from this study will be presented alongside corresponding summaries from UAM1. Summaries of demographics, baseline characteristics, and immunogenicity data from this study will be prepared by site and overall, to assess comparability of sites. Comparative analyses will be limited to comparison of each vaccine to the UAM1 Sabin 2 control.

Safety endpoints will be compared descriptively, using rates for adverse events and laboratory deviations, and continuous summaries for safety laboratory values.

The primary immunogenicity endpoint, seroprotection after a single dose of each vaccine candidate, will be formally compared to the corresponding endpoint from UAM1 via a non-inferiority test of each of the novel candidates to the UAM1 Sabin 2 control, each using $\alpha = 0.025$ and a non-inferiority margin of 10%. Secondary endpoints will be compared to corresponding endpoints from UAM1 in the same manner, using $\alpha = 0.025$ and margin = 10% for binary endpoints (seroconversion), and 2/3 $\log_2$ for median antibody titer and GMT ratios. GMTs will be estimated with likelihood-based methods to accommodate censoring at assay ULOQ and LLOQ, and ANCOVA methods will be used to address imbalances in baseline immunity. Non-inferiority comparisons will be conducted by computing the difference in endpoint between each candidate vaccine and the UAM1 Sabin 2 control (novel minus comparator), with corresponding two-sided $\alpha = 0.05$ confidence intervals, with inference drawn from comparison of the lower confidence limit to the non-inferiority margin. Score-based confidence intervals will be used for binary comparisons, asymptotic normal-based methods will be used for GMTs, and bootstrap methods will be used for median titers and other continuous endpoints. Statistical methods will be further detailed in a Statistical Analysis Plan (SAP).

The primary comparison will be based on the combined data from each site in this study compared to the single-site data from UAM1. In the event that between-site comparisons within this study indicate that significant differences in outcomes (two-sided tests for differences at $\alpha = 0.05$) exist between sites,

additional comparisons for all immunogenicity endpoints will be added to compare vaccine candidates using only data at the site in common between both studies.

Descriptive Summaries

Immunogenicity

Neutralizing Type-Specific Poliovirus Antibody Titers

At each time point where neutralizing antibody titers are obtained:

- Seroprotection rate with 95% CIs will be tabulated.
- Seroconversion rate with 95% CIs will be tabulated for post-vaccination time points
- Median $\log_2$ antibody titers with accompanying 95% CIs will be computed.
- GMT with accompanying 95% CIs will be computed.
- Plots of the reverse cumulative distribution of antibody titers will be generated.

Viral Shedding

For each subject, viral shedding PCR positivity will be determined at a pre-specified subset of the stool collection time points , and the rate of positivity (percent positive of those samples provided which are positive for type 2) will be computed. Additionally, the $CCID_{50}$ of shed virus will be obtained for each PCR-positive sample and summarized. Also, a viral shedding index will be calculated as the average of $\log_{10}$-transformed values of viral titer in stool samples as determined using quantitative PCR (viral identity) and $CCID_{50}$ (titer) from select stool samples taken following each vaccine dose. Each of these measures will be summarized by group, and combined for Groups 1 and 2 following the first dose, and for Groups 3 and 4 following the first dose.

Safety

Safety parameters and abnormalities will be tabulated and analyzed descriptively.

Adverse Events

Analyses described below will be performed for solicited and unsolicited AEs as well as for SAEs and severe AEs. The original terms used in the designated sections of the eCRFs by Investigators to identify AEs will be fully described and coded according to the current Medical Dictionary for Regulatory Activities (MedDRA). For comparison with the M1 study M1 data will be recoded if necessary.

All AEs will be summarized by group (combined within vaccine group, where relevant), occurrence in relation to vaccination, and overall.

Separate tables and listings will be created for those subjects who died, discontinued the study vaccine due to an AE, or experienced a severe or serious AE. Summaries, listings, and narratives may be provided, as appropriate.

Clinical Laboratory Tests

Each continuous biochemistry and hematology laboratory test will be evaluated by means of descriptive statistics (i.e., number of subjects, mean, SD, median, minimum, and maximum) on the actual values, at each assessment time point and by group. Changes from baseline will also be summarized by assessment time point and by group, and combined groups, wherever relevant.

Clinical laboratory test values will be evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (toxicity grades) or in accordance with the normal ranges of the clinical laboratory (below, within, or above normal range) for parameters for which no toxicity grades are defined.

A listing of subjects with any clinical laboratory test result outside the reference ranges will be provided.

While all safety lab values, including abnormalities, will be summarized, for the specific safety lab parameters listed below, the rate of elevated values will be presented at the subject level overall, by timepoint, by group, and by abnormality grade, and accompanied by a two-sided 90% confidence interval for the risk difference of each vaccine candidate vs. the placebo group.

Safety lab values of OPV-vaccinated subjects will be compared with those of the historical control UAM1 study where possible (e.g. ALT/AST), and in addition, all safety lab values (ALT, AST, CK, GGT, bilirubin and albumin) of OPV-vaccinated subjects and of IPV-vaccinated subjects (Groups 5 and 6) will be compared to the placebo group (Group 7).

Parameters: ALT, AST, CK, GGT, bilirubin and albumin plus routine other lab. Eventual further testing to include CK subtypes, electrophoresis, auto-antibodies (e.g. ANA, AMA, LKM-1, SLA/LP) and other viral pathogens can be done if any parameter is found to be consistently abnormal.

Vital Signs

Pulse rate, SBP, DBP and body temperature will be evaluated by means of descriptive statistics (actual values and changes from baseline and frequency tabulations of abnormalities at each assessment time point and by group, and combined groups, where relevant.

The percentage of subjects with values beyond clinically important limits will be summarized.

Physical Examination

Abnormal findings in physical examination will be listed.

Data Safety Monitoring Board:

A Data Safety Monitoring Board (DSMB) will monitor the benefit-risk and data integrity of this trial, as well as review interim immunogenicity data, to inform the sponsor about recommendations for future clinical trial phases.

TIME AND EVENTS SCHEDULE-GROUPS 1 AND 3

Assessments					
Visit	**1**	**2**	**3**	**4**	**5**
Time of Visit (days) **Visit window**	**D0**	**D7** **(+/- 2D)**	**D14** **(+/- 3D)**	**D28[a]** **(+/- 2D)**	**D 42** **(+/- 4D)**
Informed consent[b]	X				
In-/exclusion criteria	X				
Medical history/concomitant diseases	X				
Medication history[c]	X				
Demographic data	X				
Physical examination[d]	X[e]	X	X	X	X
Vital signs[f]	X[e,f]	X	X	X	X
Clinical laboratory tests[g]	X[e]	X	X	X	
Pregnancy test[h]	X[e]				X
Serology[i]	X[e]				
Randomization	X				
Administration of vaccine[j]	X				
Serum sample for polio antibodies	X[e]			X	
Stool sample for viral culture/quantitative PCR, stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay[l]	X----------X[l]		X	X[l]	X
Solicited systemic AEs (Diary)[k]	X----------X				
Remote contact for safety follow-up[m]					
Concomitant therapies[n]	X	X	X	X	X
Adverse events[n]	X	X	X	X	X
Reminder Code of Conduct[o]	X	X	X	X	X

a. In case of early termination the subjects will be asked to return to the clinical site within 14 days after discontinuation for assessments as outlined on Day 28.
b. No study-related assessment is to be carried out before signing of the informed consent form.
c. Including polio vaccination history.
d. Includes weight and height at Day 0. After Day 0, symptom-directed physical examination.
e. Prior to vaccination.
f. Blood pressure and heart rate (supine) and oral body temperature. On Day 0, vital signs will be assessed prior to and 30 min after vaccination.
g. For a list of assessments, please see Appendix 1: Overview of Laboratory assessments.
h. In women of childbearing potential, a urine pregnancy test will be performed.
i. Includes HBsAg, anti-HCV and anti-HIV antibodies and IgA.

j. The subjects will be kept under medical supervision for at least 30 min after vaccination.
k. Solicited AEs will be collected for Days 0-7.
l. Subjects will be asked to collect the first stool of every day in the provided container and to store this in the provided thermal bag with cooling elements: daily collection on Days 0 to 10 plus collection on Days 14, 21, V4 minus 1 or 2 days and V5 minus 1 or 2 days. At Day 0, the storage procedure will be explained and the collection material provided. Subjects who are still shedding virus in one of the 3 last stool samples will be asked to collect 3 consecutive stool samples every 3 weeks till end of shedding.
m. Remote contact (text message by sms, WhatsApp or email) on day 3 and on Day 21 for safety follow up and reminder stool collection scheme .
n. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity. The subjects can record unsolicited AEs on their diary card.
o. After signing on screening visit, weekly reminder Code of Conduct by email or sms and on each visit to emphasize the need and importance of hygienic measures and respecting the inclusion/exclusion criteria until end of study.

TIME AND EVENTS SCHEDULE – GROUPS 2, 4, 5, 6 AND 7

Assessments								
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Time of Visit (days) **Visit window**	**D0**	**D7** **(+/- 2D)**	**D14** **(+/- 3D)**	**D28** **(+/- 2D)**	**D35** **(+/- 2D)**	**D42** **(+/- 3D)**	**D56[a]** **(+/- 2D)**	**D 70** **(+/- 4D)**
Informed consent[b]	X							
In-/exclusion criteria	X							
Medical history/concomitant diseases	X							
Medication history[c]	X							
Demographic data	X							
Physical examination[d]	X[e]	X	X	X[e]	X	X	X	X
Vital signs[f]	X[e,f]	X	X	X[e]	X	X	X	X
Clinical laboratory tests[g]	X[e]	X	X	X[e]	X	X	X	
Pregnancy test[h]	X[e]			X[e]				X
Serology[i]	X[e]							
Randomization	X							
Administration of vaccine[j]	X			X				
Serum sample for polio antibodies	X[e]			X[e]			X	
Stool sample for viral culture/quantitative PCR, stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay[l]	X----------X[l]		X	X[e]-------X[l]		X	X[l]	X
Solicited systemic AEs (Diary)[k]	X----------X			X----------X				
Remote contact for safety follow-up[m]								X
Concomitant therapies[n]	X	X	X	X	X	X	X	X
Adverse events[n]	X	X	X	X	X	X	X	X
Reminder Code of Conduct[o]	X	X	X	X	X	X	X	X

a. In case of early termination the subjects will be asked to return to the clinical site within 14 days after discontinuation for assessments as outlined on Day 56.
b. No study-related assessment is to be carried out before signing of the informed consent form.
c. Including polio vaccination history.
d. Includes weight and height at Day 0. After Day 0, symptom-directed physical examination.
e. Prior to vaccination.
f. Blood pressure and heart rate (supine) and oral body temperature. On Day 0 and Day 28 vital signs will be assessed prior to and 30 min after vaccination.
g. For a list of assessments, please see Appendix 1: Overview of Laboratory assessments.

h. In women of childbearing potential, a urine pregnancy test will be performed.
i. Includes HBsAg, anti-HCV and anti-HIV antibodies, and IgA.
j. The subjects will be kept under medical supervision for at least 30 min after vaccination.
k. Solicited AEs will be collected for Days 0-7 and Days 28-35.
l. Subjects will be asked to collect the first stool of every day in the provided container and to store this in the provided thermal bag with cooling elements: after each vaccine dose, daily collection on Days 0 to 10 plus collection on Days 14, 21,V4 minus 1 or 2 days (=V7 minus 1 or 2 days after 2nd vaccination) and V8 minus 1 or 2 days. At Day 0 the storage procedure will be explained and the collection material provided. Subjects who are still shedding virus in one of the 3 last stool samples will be asked to further collect 3 consecutive stool samples every 3 weeks till end of shedding.
m. Remote contact (text message by sms, WhatsApp or email) on Day 3 and on Days 21, 31 and 49 for safety follow-up and reminder stool collection scheme.
n. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity. The subjects can record unsolicited AEs on their diary card.
o. After signing on screening visit, weekly reminder Code of Conduct by email or sms and on each visit to emphasize the need and importance of hygienic measures and respecting the inclusion/exclusion criteria until end of study.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

List of Abbreviations

AE	Adverse event
ALT	Alanine Aminotransferase
AMA	Anti-Mitochondrial antibodies
ANA	Anti-Nuclear antibodies
AST	Aspartate Aminotransferase
bpm	Beats per minute
$CCID_{50}$	50% cell culture infective dose
CI	Confidence interval
CK	Creatine phosphokinase (including isozymes)
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
cVDPVs	Circulating vaccine-derived polioviruses
cVDPV2	Circulating vaccine-derived poliovirus type 2
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
GMT	Geometric mean titer
GPEI	Global Polio Eradication Initiative
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDPE	High Density Polyethylene
HIV	Human immunodeficiency virus
HR	Heart rate
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IME	Important medical event
IMP	Investigational medicinal product
IPV	Inactivated polio vaccine
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
LKM-1	Liver-kidney microsomal type 1 (antibodies)
LSLV	Last Subject Last Visit
MedDRA	Medical Dictionary for Regulatory Activities
mOPV2	Monovalent oral polio vaccine type 2
nOPV2	Novel oral polio vaccine type 2
OPV	Oral polio vaccine
PD50	50% paralytic dose
PP	Per-protocol

RNA	Ribonucleic acid
SAE	Serious adverse event
SAGE	Strategic Advisory Group of Experts on immunization
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SD	Standard deviation
SLA/LP	Soluble liver antigen/liver-pancreas (antibodies)
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
tOPV	Trivalent oral polio vaccine
TgPVR	Transgenic mice expressing the cell receptor for poliovirus
TMF	Trial Master File
VAPP	Vaccine-associated paralytic poliomyelitis
WHO	World Health Organization
WPV	Wild poliovirus

STUDY ADMINISTRATIVE STRUCTURE

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1. INTRODUCTION

1.1 BACKGROUND INFORMATION

In 2013 the Global Polio Eradication Initiative (GPEI) launched the Polio Eradication and Endgame Strategic Plan with the objective to end all polio disease globally. The 4 main objectives of the Polio Eradication and Endgame Strategic Plan are: to detect and interrupt all poliovirus transmission, to strengthen immunization systems and withdraw the historic oral polio vaccine (OPV), to contain poliovirus and certify interruption of transmission and legacy planning.

The global effort to eradicate polio has made significant progress with only 3 countries remaining with endemic wild-type poliovirus transmission—Nigeria, Afghanistan and Pakistan. In 2017, only 22 cases of paralytic poliomyelitis due to wild poliovirus (all type 1) have been reported globally, compared with 37 for 2016. Conversely, 95 cases of circulating vaccine-derived poliovirus (cVDPV) cases have been reported in 2017 – all due to the type 2 component of OPV.

For a long time, trivalent oral polio vaccine (tOPV, incorporating poliovirus types 1, 2, and 3) was the preferred vaccine for polio control and eradication. Global use of this vaccine has enabled the eradication of wild-type poliovirus type 2. Despite these advantages, most industrialized countries have transitioned to inactivated polio vaccine (IPV), primarily because OPV has the major disadvantage of causing paralytic disease in rare cases. It can cause vaccine-associated paralytic poliomyelitis (VAPP) in vaccine recipients and close contacts at an estimated rate of about 4.7 per million births (range: 2.4-9.7) globally. Moreover, in settings of poor immunization coverage, the live vaccine virus can mutate in ways that confer the transmissibility and neurovirulence properties of wild viruses, leading to polio outbreaks caused by these altered viruses known as circulating vaccine-derived polioviruses (cVDPVs). Most cVDPVs are due to the type 2 component of OPV. Several outbreaks of cVDPV2 have been documented since 2000 and most are controlled by means of focused immunization campaigns using tOPV, and more recently, also using monovalent OPV2 with or without inactivated polio vaccine (IPV). While OPV is more effective than IPV in interrupting transmission in settings of poor sanitation and hygiene, as long as the type 2 Sabin is in use, the risk for cVDPV exists and polio cannot be entirely eradicated from susceptible populations.

As part of the Polio Eradication and Endgame Strategic Plan, the Strategic Advisory Group of Experts on immunization (SAGE) called for a globally synchronized switch from tOPV to bOPV in routine immunization programs (i.e., withdrawal of OPV2) as the first step towards complete withdrawal of all oral polio vaccines. To mitigate the risks associated with this switch, SAGE recommended the addition of at least a single dose of inactivated polio vaccine (IPV) to routine immunization programs prior to withdrawal of OPV2. Adding IPV should result in a reduction of the risk of paralytic poliomyelitis if exposure to a type 2 virus occurred after OPV2 withdrawal, an improved response to any future use of a monovalent type 2 polio vaccine in the case of an outbreak, a reduction of transmission of a reintroduced type 2 virus; and boosting of immunity to the remaining wild poliovirus serotypes 1 and 3.

All countries in the world that used tOPV switched to bOPV in April 2016. Following this switch, mOPV2 is only reserved as a stockpile option for outbreak response. However,

with on-going outbreaks of type 2 cVDPV in Syria and DRC more than a year and half from the global cessation of routine OPV2 use and the small but important risk of seeding new vaccine-derived circulation with outbreak response use of mOPV2 pose a challenge to long-term and complete eradication of any type 2 circulation. The novel OPV2, with reduced risk of generating new vaccine-derived circulation offers promise in this regard and could be an important tool to sustain eradication.

1.2 RATIONALE FOR THE STUDY AND STUDY DESIGN

1.2.1 *Rationale for the study*

Sabin 2 vaccine component has been withdrawn from routine use globally from April 2016 per the current SAGE recommendations. Following this OPV2 cessation, stockpiles of mOPV2 will be maintained for potential use in outbreak response. However, there is a risk of cVDPV2 from Sabin type 2 in settings of low population immunity. Research has been ongoing to develop vaccines that are genetically more stable than the currently available Sabin 2 containing OPVs.

Two nOPV2 vaccine candidates have been developed as attenuated serotype 2 polioviruses derived from a modified Sabin 2 infectious cDNA clone. nOPV2 Candidate 1 (S2/cre5/S15domV/rec1/hifi3) and nOPV2 Candidate 2 (S2/S15domV/CpG40) were generated by modifying the Sabin-2 RNA sequence to improve phenotypic stability and make the strains less prone to reversion to virulence. The two nOPV2 candidate vaccine strains have been used as an investigational medicinal product (IMP) in a first-in-human (FIH) Phase 1 containment trial in 2 groups of 15 healthy IPV-only vaccinated adults in Belgium, to evaluate the general safety and immunogenicity of both of the nOPV2 candidate vaccine strains (EudraCT number 2017-000908-21, ClinicalTrials.gov: NCT03430349).
Preliminary data from the study have been evaluated. Most of the volunteers in the study were men (25 of the 30), and the average age was 32.8 years (ranging from 21 to 50 years of age). The two groups of volunteers were similar in terms of the number of men and women and their age. Most of the volunteers had five or six vaccinations with IPV in the past. All volunteers completed the study.

In general, the two vaccine candidates were well tolerated. 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.

Some volunteers in both groups had abnormal blood tests. None of the volunteers experienced symptoms associated with these laboratory test changes, and it is not clear whether these were due to vaccine or due to other causes, such as intense exercise (during the weeks of being restricted to the research facility, volunteers had exclusive access to a gym in the study facility). The next study will include blood tests to look into this further.

Blood tests to assess whether the vaccine candidates resulted in the desired protective response to poliovirus were encouraging. Volunteers demonstrated clear immune response to both vaccine candidates.

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. 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 (more detailed data are reported in the IB). None of the volunteers demonstrated any illness associated with longer duration of shedding of vaccine virus.

As an exploratory analysis, samples from subjects that shed the candidate vaccines in stools were tested for neurovirulence as well as by deep sequencing. Data collected to date support the phenotypic stability (lack of significant reversion) of the nOPV2 candidates. No meaningful increases in neurovirulence could be detected and sequencing confirmed that there were no reverting mutations in the main site of attenuation which are analogous to the domain V A481G reversion in Sabin OPV2.

1.2.2 *Rationale for the study design*

1.2.2.1 *OVERALL STUDY DESIGN*

Due to the withdrawal of Sabin mOPV2 and prohibition of its use from April 2016 onwards, well before the availability of nOPV2 for clinical testing, Phase 4 trials have been conducted with Sabin mOPV2 to provide control data on safety, immunogenicity, against which data for nOPV2 in subsequent Phase I and II studies will be evaluated and compared. The Phase 4 trials of Sabin mOPV2 were designed to parallel the expected design of the Phase 1 and 2 nOPV2 studies with respect to overall design, inclusion of similar study cohorts. As for these reasons head to head comparison of nOPV2 and mOPV2 is not possible, the overall clinical development plan with the Phase I and II studies was designed taking into consideration the unique situation of OPV2 cessation in April 2016, and the global public health need of a vaccine with lower risk of VAPP and VDPV for outbreak response in the post-cessation era.
The Phase 2 study is designed to evaluate the safety and immunogenicity of both nOPV2 vaccines in adults before testing in young children and then infants. The primary objectives of the Phase 2 study include the general safety and immunogenicity of the two candidate vaccines, primarily based on comparison with historical data obtained in the Phase 4 study of Sabin mOPV2 for OPV-vaccinated subjects, in order to establish non-inferior immunogenicity and acceptable safety profile. Assessment of the general safety of the 2 candidate vaccines in IPV-only vaccinated subjects will be based on comparison with data from a placebo group.

The novel vaccine will eventually be licensed based on 3 criteria: a similar safety profile to the currently licensed mOPV2 of the Sabin strain, non-inferior immunogenicity, and reduced reversion to virulence.

The Phase 2 study will include both OPV-vaccinated and IPV-vaccinated adults to provide safety and immunogenicity data relevant to the decision to advance to future studies with testing in children who have not been exposed to OPV2.

1.2.2.2 *SEQUENTIAL TESTING OF STUDY VACCINES*

The globally coordinated cessation of trivalent OPV implemented two years ago was largely successful, as noted by rapid disappearance of the type 2 vaccine virus from most part of the world. However, a major, evolving concern for the global eradication program is the continuation of type 2 circulating vaccine-derived (cVDPV2) outbreaks in specific geographies with poor immunization coverage. More importantly, some of the more recent outbreaks of cVDPV2 have been attributed to the use of the current outbreak response tool, monovalent OPV2. Although benefits of using mOPV2 is considered to outweigh the risks of seeding new VDPV2s, there is an urgent, global need to explore all possible options to accelerate the development of novel OPV2 (nOPV2) vaccines so that there is less risk of continuation of such transmission of VDPV2s following an outbreak response. For the clinical development process for nOPV2, a sequential enrollment with candidate vaccines for earlier results availability on at least one of the candidates will be important given the on-going type 2 epidemiology globally. With promising results from the first-in-human study conducted in Antwerp along with supportive pre-clinical data for both the candidates, and understanding of availability of clinical trial materials for the subsequent use in infant trials, candidate 2 is considered to be first in the sequence, with candidate 1 following behind as rapidly as possible for enrollment in the proposed clinical trial. We have a historic opportunity to meaningfully contribute to the efforts to eradicate all types of polioviruses, and the approach to generate data on nOPV2 candidates earlier could be a game changer by providing a potentially more genetically stable alternative to the current outbreak response tool.

1.3 RISK BENEFIT ANALYSIS

1.3.1 *Potential Risks*

The nOPV2 vaccine candidates have been developed as attenuated serotype 2 polioviruses derived from a modified Sabin 2 infectious cDNA clone. nOPV2 Candidate 1 (S2/cre5/S15domV/rec1/hifi3) and nOPV2 Candidate 2 (S2/S15domV/CpG40) were generated by modifying the Sabin-2 RNA sequence to improve phenotypic stability and make the strains less prone to reversion to virulence. Therefore, the modifications made to the nOPV2 candidate vaccine strains are not expected to change host range or tropism of the virus, nor to cause any additional adverse effects compared to Sabin-2. Development of VAPP or cVDPV is not expected as volunteers are OPV or IPV-primed. However, taking into account the nature of the parental organism, the potential adverse effects that the nOPV2 candidate vaccine strains may exert on human health by conducting the clinical trial are:

- Direct effects of the transmission of the nOPV2 candidate vaccine strains to an unintended human recipient.
- Indirect effects of the transmission of a genetic variant of the nOPV2 candidate vaccine strains to an unintended human recipient. These indirect effects could be immediate (upon transmission of a variant from a study subject to an unintended human recipient) or delayed (in case a genetic variant would be able to start circulating in the population).

The potential risks posed by transmission of the nOPV2 candidate vaccine strains or their genetic variants to an unintended human recipient are estimated by combining the likelihood of transmission with the magnitude of the consequences if transmission occurs. For the groups in which transmission is most likely to occur (close contacts of the study subjects and study site personnel), these consequences are negligible due to previous vaccination, therefore also the overall risk is negligible. The likelihood of transmission to other unintended recipients is considered to be negligible for the general population and low for those in close contact with family members of the study participants. As polio vaccination has been mandatory in Belgium since 1966 and the rate of vaccination of the inhabitants is >95%, practically the entire population is effectively protected from disease caused by the poliovirus, including VAPP. Therefore, the magnitude of the consequences of the nOPV2 candidate vaccine strains or their genetic variants is considered negligible for this group although for those who are unvaccinated or immunocompromised consequences of low magnitude are expected (taking into account the extremely low frequency of occurrence of the serious adverse event of VAPP).

Preliminary data obtained with the candidate nOPV2 vaccines in a first-in-human Phase 1 clinical study in Belgium with IPV-only vaccinated adults (>18 years old) (EudraCT number 2017-000908-21, ClinicalTrials.gov: NCT03430349), confirm that in IPV primed subjects, fecal shedding occurs following administration of the nOPV2 candidate vaccines, with median durations of fecal shedding of about 2 and 4 weeks for the two candidates, yet no nasopharyngeal shedding was observed in this study. This is generally in line with expectations for individuals with an IPV-only vaccination history who are given Sabin OPV.

Presence of vaccine virus in stool was observed somewhat more for vaccine candidate #1 than #2. 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 (more detailed data are reported in the IB). None of the volunteers demonstrated any illness associated with longer duration of shedding of vaccine virus.

As an exploratory analysis, samples from subjects that shed the candidate vaccines in stools were tested for neurovirulence as well as by deep sequencing. Data collected to date support the phenotypic stability (lack of significant reversion) of the nOPV2 candidates. No meaningful increases in neurovirulence could be detected and sequencing confirmed that there were no reverting mutations in the main site of attenuation which are analogous to the domain V A481G reversion in Sabin OPV2.
To reduce the likelihood that nOPV2 candidate vaccine strains or their genetic variants could be transmitted to unintended recipients who are not vaccinated for polio or who are immunocompromised, study subjects will only be eligible for inclusion in the study if:

- They have received at least three doses of OPV or IPV in the past.
- They have their residence in Belgium
- They are willing to sign a code of conduct referring to the inclusion/exclusion criteria, rules of the protocol and hygienic measures as well as travel restrictions.
- They do not have any confirmed or suspected immunosuppressive or immunodeficiency condition, or have been treated with immunosuppressant drugs or

other immune-modifying drugs for longer than 14 days within 6 months prior to the first vaccine dose or have such use planned during the study.
- They will not have household or professional contact with known immunosuppressed people or people without full polio vaccination during the whole study duration, or have professional contact with children under 6 months old during the whole study duration.
- Any travel to polio endemic countries or countries with evidence of recent (within last 6 months) wild or vaccine-derived poliovirus circulation during the total duration of the study;‡
- Professional handling of food, catering activities (e.g. working in restaurant kitchen, bakery, ..) during the total duration of the study;

If type 2 virus shedding is detected by PCR on one of the 3 last stool samples the study duration for this individual will be extended and subject will be asked to further collect 3 consecutive stool samples every 3 weeks after the last per protocol sample until shedding is PCR negative on 3 consecutive stool samples. Travel/other restrictions described above will continue to apply until end of shedding is reached.

Study site personnel is considered to have a low likelihood of exposure to and hence transmission of the nOPV2 virus either by accidental contact during administration or when samples from study participants are processed. To minimize the risk of potential transmission to the study staff, study site personnel will be checked for history of polio vaccination and will be offered a booster IPV vaccination at least one month before the start of the study.

Based on the currently available information, the overall risk posed by transmission of nOPV2 candidate vaccine virus or a genetic variant to an unintended human recipient is considered to be negligible. As an additional risk assessment measure household contact monitoring will be offered (see appendix 4) in case of extended shedding (PCR positive of at least one of the follow-up stool samples after D42 following last vaccination). Monitoring activities will be in place during the study to detect possible adverse events caused by the nOPV2 candidates as well as provide information to address uncertainties in the available data. An OPV2 specific PCR developed by US CDC is available for rapid use should unanticipated or unusual clinical events occur during the study that require diagnostic testing.

In the phase 1, first-in-human study, performed under containment at the University of Antwerp, the two vaccine candidates were well tolerated. Two cohorts of 15 adult participants each were enrolled sequentially, with all participants in each cohort receiving the same vaccine candidate to avoid cross-contamination between the two candidates. All participants had previously received at least 3 doses of IPV, and none had a history of receiving OPV (per eligibility criteria). The assignment of product to the cohorts was

‡ Updated list will be made available at the start of the study. Study will take in consideration the most recent WHO guidelines.

performed randomly, and neither study staff nor participants were aware of which product was administered to which cohort.

In general, the safety data were reassuring. There were no remarkable clinical events. The primary clinical endpoints were serious (SAE) and severe adverse events: there were no SAEs; none of the solicited adverse events (pre-specified parameters during the 7 days following vaccination) were severe, and most were mild; and the severe unsolicited AEs were predominantly transient, asymptomatic abnormalities in monitoring laboratory assessments. Most unsolicited clinical AEs were mild, and all self-resolving. No severe AEs were assessed as probably related to the vaccines.

Two participants (one from each cohort) exhibited transient, asymptomatic, severe elevations of AST, both associated with mild or moderate elevation of ALT, severe elevation of CK, no elevation of either GGT or bilirubin, and with complete resolution to normal values. Other AEs on the basis of AST elevations were asymptomatic and generally mild and self-resolving, and also associated with elevated CK levels but normal GGT and bilirubin levels. All AEs on the basis of ALT elevations were transient and asymptomatic, with only one moderate AE and the balance mild. Half of the participants exhibited asymptomatic severe elevations of CK, most often at the day 7 assessment, with resolution of values elevated in the first couple of weeks post-vaccination to normal or close to normal.

Such patterns of enzyme changes have been seen with intermittent, intense exercise, which could provide an explanation for these participants suddenly restricted to a contained unit with an exercise facility for 28 days or more. However, in the absence of a control group in this study, a relationship to vaccine cannot be ruled out. As such, the subsequent study has been redesigned to further assess enzyme changes in additional participants who have not received OPV in the past, and to randomize these participants to receive either one of the vaccine candidates or placebo.

1.3.2 *Potential Benefits*

Subjects who previously completed the childhood polio vaccination schedule and receive a dose of nOPV2 in this study may have a boosted immunity for poliovirus type 2.

In the FIH study immune responses to both candidates were clearly evident, despite the high number of previously administered doses of IPV and the inability to detect a 4-fold rise in some participants due to baseline titers within 4-fold of the upper limit of quantitation for the assay. Only one participant did not have a seroprotective level of neutralizing antibodies to type 2 poliovirus at baseline (<1:8), and most participants demonstrated seroconversion (4-fold rise in antibody from baseline to 28 days post-vaccination). Median fold-rise in titer was 12.7 for Cohort 1 and 8.0 for Cohort 2.

This study is of major importance for global public health, contributing to the search of an improved OPV2 vaccine with less chance of reversion to neurovirulence.

2. STUDY OBJECTIVES

Assessment of the general safety and immunogenicity of the 2 candidate vaccines in OPV vaccinated subjects will be primarily based on comparison with data obtained in the previously conducted Phase 4 study of Sabin mOPV2 in order to assess immunogenicity and establish an acceptable safety profile in these populations. Assessment of the general safety of the 2 candidate vaccines in IPV-only vaccinated subjects will be based on comparison with data of the placebo group.

2.1 PRIMARY OBJECTIVE

The primary objectives of the study are

- to assess the safety (serious adverse events [SAEs] and severe§ adverse events [AEs]) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy OPV-vaccinated adults, relative to historical controls given Sabin OPV2;
- To compare the immunogenicity (seroprotection rate) of novel monovalent live attenuated oral serotype 2 poliovirus vaccine (nOPV2) candidate 1 and novel monovalent live attenuated oral serotype 2 poliovirus vaccine (nOPV2) candidate 2 in healthy OPV-vaccinated adults to historical controls given Sabin OPV2;
- to assess the safety (serious adverse events [SAEs] and severe adverse events [AEs]) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated adults, compared with placebo.

2.2 SECONDARY OBJECTIVES

Secondary objectives are :

- to assess the safety (any solicited and unsolicited AEs, laboratory assessments) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy OPV-vaccinated adults, compared with historical controls given Sabin OPV2;
- to assess the safety (any solicited and unsolicited AEs, laboratory assessments) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated older adults, compared with placebo;
- to compare the immunogenicity (seroconversion rate, median antibody titer (post-vaccination)) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy OPV-vaccinated adults, with historical control of Sabin OPV2.
- To assess the immunogenicity (seroprotection rate, seroconversion rate, median post-vaccination antibody titer) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated adults.

§ List of severe AEs as mentioned in the diary cards: fever > 39°C, headache, fatigue, myalgia, arthralgia, paresthesia, anesthesia, paralysis, or gastrointestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain) that prevent normal activity or any other severe AE that prevents normal activity.

2.3 EXPLORATORY OBJECTIVES

Exploratory objectives are

- to compare immunogenicity (geometric mean titer [GMT]) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy OPV-vaccinated adults, with historical control of Sabin OPV2;
- to assess immunogenicity (geometric mean titer [GMT]) of nOPV2 candidate 1 and nOPV2 candidate 2 in healthy IPV-only vaccinated adults;
- to compare viral shedding following administration of nOPV2 candidate 1 and nOPV2 candidate 2 in a pre-specified subset of stool samples in healthy OPV-vaccinated subjects, with historical control of Sabin OPV2;
- to assess viral shedding following administration of nOPV2 candidate 1 and nOPV2 candidate 2 in a pre-specified subset of stool samples in healthy IPV-only vaccinated adults;

The shedding analyses will initially be conducted using a subset of samples in the 10 days following administration of each dose (e.g. day 0, 3, 7, and 10) and all samples at day 14 and thereafter. The remaining samples will be stored pending evaluation of the initial analyses; PCR and CCID50 will be conducted on these stored samples if deemed important for the complete interpretation of the study results.

Exploratory objectives will also include comparison of neurovirulence (as measured in animal model(s)) and assessment of the genetic stability via analysis of genetic sequence, including but not limited to the modified regions of shed virus in a subset of stool samples of all OPV-vaccinated adults, relative to historical control of Sabin OPV2.

Exploratory objectives will also include assessment of neurovirulence (as measured in animal model(s)) and the genetic stability via analysis of genetic sequence, including but not limited to the modified regions of shed virus in a subset of stool samples of all IPV-only vaccinated adults given doses of nOPV2 candidates 1 and 2.

3. STUDY ENDPOINTS

Assessment of the following endpoints for the 2 candidate vaccines in OPV-vaccinated subjects (Groups 1 and 2) will be based on comparison with data obtained in the previously conducted Phase 4 study of Sabin mOPV2 in order to assess immunogenicity and establish an acceptable safety profile in these populations. For the safety endpoint of the IPV-vaccinated adults (Groups 5, 6) comparison will be made with the data of the placebo group (Group 7).

3.1 PRIMARY ENDPOINTS

Safety: The following endpoints will be evaluated by group and overall:

- incidence, type and causality of SAEs and severe[§] AEs throughout the study period.

Immunogenicity:

- seroprotection rate of type 2 polio antibodies at Day 28, following a single dose of nOPV2 candidate 1 (combined Groups 1 and 2) compared with the seroprotection rate of type 2 polio antibodies at Day 28 in the historical control mOPV2 study (combined group 1 and 2) (see annex).
- seroprotection rate of type 2 polio antibodies at Day 28, following a single dose of nOPV2 candidate 2 (combined Groups 3 and 4) compared with the seroprotection rate of type 2 polio antibodies at Day 28 in the historical control mOPV2 study (combined Groups 1 and 2) (see annex).

Seroprotection is defined as type 2-specific antibody titers ≥ 1:8.

3.2 SECONDARY ENDPOINTS

Safety: The following endpoints will be evaluated by group and overall:

- Incidence, type, causality and severity of solicited AEs for Days 0-7 in Groups 1 and 2 combined and days 28-35 in Group 2;
- Incidence, type, causality and severity of solicited AEs for Days 0-7 in Groups 3 and 4 combined and days 28-35 in Group 4;
- Incidence, type, causality and severity of solicited AEs for Days 0-7 and Days 28-35 in Groups 5, 6 and 7;
- Incidence, type, causality and severity of unsolicited AEs throughout the study period in all groups;
- Incidence, causality and description of deviations from normal safety labs at Day 0 , Day 7 , Day 14 and Day 28 for Groups 1 through 4 and at Day 35, Day 42 , and Day 56 for Groups 2 and 4;
- Incidence, causality and description of deviations from normal safety labs at Day 0, Day 7, Day 14, Day 28, Day 35, Day 42 and Day 56 for Groups 5, 6 and 7;

Immunogenicity:

- Median titers of type 2 polio antibodies at Day 28 in Groups 1 and 2 combined;
- Median titers of type 2 polio antibodies at Day 28 in Groups 3 and 4 combined;
- Median titers of type 2 polio antibodies at Day 28 in Groups 5, 6 and 7.

- Seroprotection rate of type 2 polio antibodies at Day 56 in Groups 2 and 4;
- Seroprotection rate of type 2 polio antibodies at Day 28 and at Day 56 in Groups 5, 6 and 7.

- Seroconversion rate of type 2 polio antibodies at Day 28 for Groups 1 and 2 combined and at Day 56 in Group 2;
- Seroconversion rate of type 2 polio antibodies at Day 28 for Groups 3 and 4 combined and at Day 56 in Group 4;
- Seroconversion rate of type 2 polio antibodies at Day 28 and at Day 56 for Groups 5, 6 and 7.

  Seroconversion is defined as a change from seronegative to seropositive and antibody titers of ≥1:8, and in seropositive subjects, as an antibody titer increase of ≥ 4-fold over baseline titers.

3.3 EXPLORATORY ENDPOINTS:

- GMT of type 2 polio antibodies at Day 28 in Groups 1 and 2 combined and at Day 56 in Group 2;
- GMT of type 2 polio antibodies at Day 28 in Groups 3 and 4 combined and at Day 56 in Group 4;
- GMT of type 2 polio antibodies at Day 28 and at Day 56 in Groups 5, 6 and 7.

- Viral shedding positivity rate (as determined using quantitative PCR) will be assessed at a pre-specified subset of the stool collection time points
- Median 50% cell culture infective dose ($CCID_{50}$; titer) of shed virus after viral extraction from PCR-positive stool samples will be assessed at a pre-specified subset of the stool collection time points
- Time-to-cessation of viral shedding will be assessed following each dose
- The extent of shedding, including quantity and duration, will be assessed at pre-specified stool collection time points following each dose.

Exploratory endpoints will include assessment for neurovirulence of shed virus (as measured in animal model(s)) and will also include assessment of the genetic stability of modified regions of shed virus in a subset of stool samples.

4. STUDY DESIGN

4.1 OVERVIEW OF STUDY DESIGN

This will be a multicenter, partial blind, placebo-controlled, randomized study in 200 healthy OPV-vaccinated adults and in 48 to 132 healthy IPV-only vaccinated adults (18 to 50 years), as follows:

- 50 OPV-vaccinated adults to receive 1 dose of nOPV2 candidate 1 (Group 1);
- 50 OPV-vaccinated adults to receive 2 doses of nOPV2 candidate 1 (Group 2), administered 28 days apart;
- 50 OPV-vaccinated adults to receive 1 dose of nOPV2 candidate 2 (Group 3);
- 50 OPV-vaccinated adults to receive 2 doses of nOPV2 candidate 2 (Group 4), administered 28 days apart;
- 16 to 44 IPV-only vaccinated adults to receive 2 doses of nOPV2 candidate 1 (Group 5), administered 28 days apart;
- 16 to 44 IPV-only vaccinated adults to receive 2 doses of nOPV2 candidate 2 (Group 6), administered 28 days apart.
- 16 to 44 IPV-only vaccinated adults to receive 2 doses of placebo (Group 7), administered 28 days apart.

Groups will be enrolled sequentially such that subjects receiving candidate 2 will be enrolled first (IPV and OPV groups simultaneously), followed by subjects receiving candidate 1. IPV-only vaccinated adults will be randomized 2:1 to candidate 2 (Group 6) or placebo (Group 7), respectively, until all 16 to 44 Group 6 subjects are enrolled, after which randomization 2:1 to candidate 1 or placebo will commence for Groups 5 and 7. For OPV-vaccinated subjects, randomization to Groups 1 and 2 to receive candidate 1 will ensue following complete randomized enrollment of subjects to Groups 3 and 4. In all cases, block randomization will be used to ensure balanced randomization across time.

The DSMB has established stopping rules for safety prior to study start which will be continuously assessed. The DSMB will also monitor study enrollment, particularly for IPV-vaccinated subjects, and recommend truncation and/or closure of study groups if enrollment stagnates and when current enrollment is considered sufficient to meet study objectives and no safety signals occurred. The minimum number of IPV-vaccinated subjects agreed on by DSMB per vaccine candidate is 24. With a randomization of 2:1 for placebo the minimum study cohort size for Groups 5, 6 and 7 will be 16. In case of safety signals the DSMB reserves the right to reverse the truncated enrollment.

Procedures and processes will be unified across sites through training and additional preparatory activities; demographic and baseline characteristics will be summarized by site and pooled across site. Immunogenicity outcomes will be assessed by site and pooled within group.

Figure 1: Trial overview

Study code/ Country	Group N°	Age (yr)	Immunogenic Background	Candidate	Study Cohort Size	No. of Doses	Dose level
UAM4 Belgium	**Group 1**	**18-50**	**OPV**	**nOPV2 candidate 1**	**50**	**1**	**. ~10^6**
UAM4 Belgium	**Group 2**	**18-50**	**OPV**	**nOPV2 candidate 1**	**50**	**2**	**. ~10^6**
UAM4 Belgium	**Group 3**	**18-50**	**OPV**	**nOPV2 candidate 2**	**50**	**1**	**. ~10^6**
UAM4 Belgium	**Group 4**	**18-50**	**OPV**	**nOPV2 candidate 2**	**50**	**2**	**. ~10^6**
UAM4 Belgium	**Group 5**	**18-50**	**IPV**	**nOPV2 candidate 1**	**16 to 44**	**2**	**. ~10^6**
UAM4 Belgium	**Group 6**	**18-50**	**IPV**	**nOPV2 candidate 2**	**16 to 44**	**2**	**. ~10^6**
UAM4 Belgium	**Group 7**	**18-50**	**IPV**	**placebo**	**16 to 44**	**2**	**Sirupus simplex**

- One hundred OPV-vaccinated subjects will be evaluated for the 1-dose regimen of nOPV2 candidate 1 (Groups 1 and 2) and 50 of these 100 subjects will be evaluated for the 2-dose regimen nOPV2 candidate 1 (Group 2).
- One hundred OPV-vaccinated subjects will be evaluated for the 1-dose regimen of nOPV2 candidate 2 (Groups 3 and 4) and 50 of these 100 subjects will be evaluated for the 2-dose regimen nOPV2 candidate 2 (Group 4).
- Sixteen to forty-four IPV-only vaccinated subjects will be evaluated for the 2-dose regimen of nOPV2 candidate 1 (Group 5).
- Sixteen to forty-four IPV-only vaccinated subjects will be evaluated for the 2-dose regimen of nOPV2 candidate 2 (Group 6).
- Sixteen to forty-four IPV-only vaccinated subjects will be evaluated for the 2-dose regimen of oral placebo (Group 7).

The study will be conducted at 2 sites in Belgium, recruiting each approximately 50% of 248 to 332 subjects per site, in a randomized way.

The assessments performed are summarized per visit in the Time and Events schedule.

4.2 DISCUSSION OF STUDY DESIGN

Randomization will be used to avoid bias in the assignment of subjects to a vaccine dose regimen, to increase the likelihood that known and unknown subject attributes (e.g.,

demographic and baseline characteristics) are evenly balanced across groups, and to enhance the validity of future statistical comparisons across groups.

5. SELECTION OF STUDY POPULATION

For details on the sample size calculation, please refer to Section 10.2.

At each visit participants will be reminded and checked for the inclusion and exclusion criteria, and the necessity of adherence to the criteria will be reiterated.

5.1 INCLUSION CRITERIA

1. For Groups 1, 2, 3 and 4: healthy males or females, from 18 to 50 years of age inclusive, having previously received at least 3 doses of OPV more than 12 months before the start of the study;
2. For Groups 5, 6 and 7: healthy males or females, from 18 to 50 years of age inclusive, having previously received at least 3 doses of IPV more than 12 months before the start of the study;
3. Having residence in Belgium;
4. In good physical and mental health as determined on the basis of medical history and general physical examination performed at Day 0;
5. Female subjects of childbearing potential must agree to the use of an effective method of birth control throughout the study and up to 3 months after last vaccine dose (see Section 7);
6. Willing to adhere to the prohibitions and restrictions specified in this protocol (see Section 7);
7. Informed Consent Form (ICF) and Code of Conduct signed voluntarily by the subject before any study-related procedure is performed, indicating that the subject understands the purpose of any procedures required for the study and is willing to participate in the study.

5.2 EXCLUSION CRITERIA

Subjects meeting any of the following criteria are excluded from participation in this study:

1. A condition that, in the opinion of the Investigator, could compromise the well-being of the subject or course of the study, or prevent the subject from meeting or performing any study requirements;
2. For Groups 5, 6 and 7: ever having received any OPV in the past;
3. Any travel to polio endemic countries or countries with evidence of recent (within last 6 months) wild or vaccine-derived poliovirus circulation during the total duration of the study[**]
4. Professional handling of food, catering or food production activities during the total duration of the study;
5. Having Crohn's disease or ulcerative colitis or having had major surgery of the gastrointestinal tract involving significant loss or resection of the bowel;

[**] Updated list will be made available at the start of the study. Study will take in consideration the most recent WHO guidelines.

6. A known allergy, hypersensitivity, or intolerance to the study vaccine or the placebo, or to any of their components or to any antibiotics;
7. Any confirmed or suspected immunosuppressive or immunodeficiency condition (including human immunodeficiency virus [HIV] infection, hepatitis B or C infections or total serum IgA level below lab lower limit of normal (LNN));
8. Will have household or professional contact with known immunosuppressed people or people without full polio vaccination (i.e. complete primary infant immunization series), e.g. babysitting during the total duration of the study;
9. Neonatal nurses or others having professional contact with children under 6 months old during the total duration of the study;
10. Chronic administration (i.e., longer than 14 days) of immunosuppressant drugs or other immune-modifying drugs within 6 months prior to the first vaccine dose or planned use during the study. For instance, for corticosteroids, this means prednisone, or equivalent, ≥ 0.5 mg/kg/day (inhaled and topical steroids are allowed whereas intra-articular and epidural injection/administration of steroids are not allowed);
11. Presence of contraindications to administration of the study vaccine on Day 0: acute severe febrile illness deemed by the Investigator to be a contraindication for vaccination or persistent diarrhea or vomiting;
12. Indications of drug abuse or excessive use of alcohol at Day 0 (males: > 21 units per week (m); females > 14 units per week);
13. Being pregnant or breastfeeding. Women of childbearing potential will undergo a urine pregnancy test at each vaccination visit. Subjects with a positive pregnancy test will be excluded;
14. Participation in another clinical study within 28 days prior to entry in this study or receipt of any investigational product (drug or vaccine) other than the study vaccine within 28 days prior to the first administration of study vaccine, or planned use during the study period;
15. Administration of any vaccine other than the study vaccine within 28 days prior to the first dose of study vaccine and during the entire study period;
16. Administration of any polio vaccine within 12 months before the start of the study;
17. Having had a transfusion of any blood product or application of immunoglobulins within the 4 weeks prior to the first administration of study vaccine or during the study;
18. Subject is an employee of the Investigator or study site, with direct involvement in the proposed study or other studies under the direction of that Investigator or study site, or is a family member of an employee or the Investigator, or was a study subject in the historical control studies UAM1 or UAT1 or in the study UAM4a;
19. Having a family or household member participating in the study CVIA 065 or being a study subject in the study CVIA 065.

5.3 CRITERIA FOR ELIMINATION FROM THE PER-PROTOCOL POPULATION

Subjects meeting the following criteria will be excluded from the Per-protocol analysis population (see Section 10.1):

- Any disease or therapy that, in the investigator's opinion, could significantly affect the subject's immune status.
- positive hepatitis panel (including hepatitis B surface antigen [HBsAg] and anti-hepatitis C virus [HCV] antibodies), positive HIV antibody screens or total serum IgA below lab lower limit of normal (LNN).
- administration of any vaccine other than the study vaccine within 28 days of the first dose of study vaccine and during the entire study period. Immunogenicity data from subjects receiving a non-study vaccine will be included in per-protocol population analyses up to the time of receipt of the non-study vaccine.

These subjects will be included in the Total Vaccinated population (see section 10.1) and will continue in the study for safety follow-up and will not receive further vaccine doses.

5.4 CONTRAINDICATIONS TO VACCINATION

The following AEs constitute temporary contraindications to administration of the study vaccine:

- acute severe febrile illness on the day of vaccination deemed by the Investigator to be a contraindication for vaccination;
- persistent diarrhea or vomiting.

If any of the above-listed AEs occur on the day of second vaccination, the subject should not receive OPV at that time and vaccination should be postponed. OPV may be administered at a later date but no more than 7 days after the original scheduled date.

The following AEs constitute absolute contraindications to further administration of the study vaccine:

- vaccine-related SAE after vaccination;
- known hypersensitivity to any component of the vaccine or placebo or severe reaction following previous administration of the vaccine;
- known hypersensitivity to antibiotics.

If any of the above-listed AEs occur during the study, the subject should not receive additional doses of the study vaccine but may continue other study procedures at the discretion of the Investigator.

Any subject who becomes pregnant during the study must also be withdrawn from further vaccination (see Section 11.8).

5.5 ADDITIONAL CONSTRAINTS

Female subjects of childbearing potential must agree to the use of an effective method of contraception throughout the study and up to 3 months after the last vaccine dose.

Information on prohibited therapies can be found in Section 7.

6. VACCINE

6.1 GENETIC MODIFICATIONS

Novel OPV2 candidates 1 and 2 are attenuated serotype 2 polioviruses derived from a modified Sabin 2 infectious clone and propagated in Vero cells.

The two candidate strains include different combinations of 5 distinct modifications of the Sabin-2 genome, including changes to the RNA sequence in the 5' UTR (5' untranslated region of polio genome), the capsid protein coding region (P1), the non-structural protein 2C, and the polymerase 3D (Table 1).

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.

Table 1: Genetic modifications of Sabin-2 in nOPV2 candidates and their purposes

Modification (references)	1	2	Purpose
S15 dom V	x	x	-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	x		-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)	x		-Improved stability of attenuated phenotype. Specifically, improve fidelity of replication leading to less genetic drift and reversion. -Additional attenuation.
Polymerase (rec)	x		-Reduce frequency of recombination events, thereby reducing ability of population to improve replication fitness. -Additional attenuation.
P1 codon deoptimization		x	-Improved stability of attenuated phenotype. -May also reduce transmission (less infectious per particle). -May enhance innate immune response against vaccine. -May increase attenuation.

6.2 PHYSICAL DESCRIPTION OF THE STUDY VACCINE

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 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 with a spoon from the dropper supplied with the vaccine. Each dose of the nOPV2 vaccine candidate 1 and nOPV2 vaccine candidate 2 contains approximately 10^6 $CCID_{50}$.

6.3 OTHER MEDICATION ADMINISTERED IN THE STUDY

In the control group (Group 7) Sirupus simplex, Propylenglycolum, European Pharmacopoeia (Ph.Eur.), will be used as placebo. The clear, colorless to pale yellow liquid contains 64% saccharose, 0,08% methylparaben, 0,02% propylparaben and propyleneglycol, presented in a HDPE container of 1 liter. The placebo will be administered orally, in the same way as the study vaccine. One dose of placebo will consist of 6 drops, delivered with a spoon from the pipette supplied with the placebo.

6.4 PACKAGING AND LABELING

Both vaccine candidates are labelled and packed according to local law and regulatory requirements.

Detailed information on the packaging and labeling will be specified in the IMP manual.

6.5 STORAGE AND VACCINE ACCOUNTABILITY

The Investigator (or his/her designee) is responsible for the safe storage of all study vaccine assigned to the clinical site, in a locked, secure storage facility with access limited to those individuals authorized to dispense the study vaccine, and maintained within the appropriate ranges of temperature. All study vaccine must be stored as specified at delivery and in the original packaging.

The nOPV2 candidate vaccines should be stored in a freezer at –20°C. After thawing, the vaccine can be stored at +2 to +8°C for 2 weeks.

The placebo should be stored at room temperature (20 +/- 5°C) in a well-closed container, protected from light.

Regular temperature logging of the study vaccine storage room at the clinical site should be performed. In case a deviation in storage conditions should occur, the clinical site must not further dispense the affected study vaccine and notify the Sponsor.

The Investigator is responsible for ensuring that all study vaccine received at the clinical site are inventoried and accounted for throughout the study.

Study vaccine should be dispensed under the supervision of the Investigator, a qualified member of the clinical staff, or by a hospital/clinic pharmacist. The Investigator must maintain accurate records demonstrating date and amount of vaccine administered to whom and by who. Study vaccine will be supplied only to subjects participating in the study.

The Sponsor's designated site monitor will periodically check the supplies of study vaccine held by the Investigator or pharmacist to ensure accountability and appropriate storage conditions of all study vaccine used.

Unused study vaccine must be available for verification by the site monitor during on-site monitoring visits.

After the last visit of the last subject in the study (LSLV), any used and unused study vaccine will be returned to the Sponsor, or destroyed at the clinical site with the Sponsor's written permission (in this case a certificate of destruction will be provided and filed in the Trial Master File [TMF]).

6.6 RANDOMIZATION AND BLINDING

All OPV-vaccinated subjects will receive one of the nOPV2 candidates in a single blind manner and all IPV- vaccinated subjects will receive one of the nOPV2 candidates or placebo in a double-blinded manner.

For the whole study duration all subjects and blinded study staff responsible for safety evaluation of IPV-subjects will not have any information of what has been administered. As the placebo can be distinguished from the vaccine candidates in packaging and color, reception of the vaccines, dose preparation and administration will have to be done by a team of unblinded study personnel. Appropriate measures will be taken at the site to ensure blinding of subjects and blinded team for the whole duration of the study.

Groups will be enrolled sequentially such that subjects receiving candidate 2 will be enrolled first (IPV and OPV groups simultaneously), followed by subjects receiving candidate 1. IPV-only vaccinated adults will be randomized 2:1 to candidate 2 (Group 6) or placebo (Group 7), respectively, until all 16 to 44 Group 6 subjects are enrolled, after which randomization 2:1 to candidate 1 or placebo will commence for Groups 5 and 7. For OPV-vaccinated subjects, randomization to Groups 1 and 2 to receive candidate 1 will ensue following complete randomized enrollment of subjects to Groups 3 and 4. In all cases, block randomization will be used to ensure balanced randomization across time. The target will be to enroll 200 eligible OPV-vaccinated subjects and 48 to 132 eligible IPV-only vaccinated subjects. The DSMB has established stopping rules for safety prior to study start which will be continuously assessed. The DSMB will also monitor study enrollment, particularly for IPV-vaccinated subjects, and recommend truncation and/or closure of study groups if enrollment stagnates and when current enrollment is considered sufficient to meet study objectives and no safety signals occurred. The minimum number of IPV-vaccinated subjects agreed on by DSMB per vaccine candidate is 24. With a randomization of 2:1 for placebo the minimum study cohort size for Groups 5, 6 and 7 will be 16. In case of safety signals the DSMB reserves the right to reverse the truncated enrollment.

Allocation of each subject to a given group will be described in a computer-generated randomization schedule prepared prior to start of the study by Assign Data Management and Biostatistics GmbH, Stadlweg 23, 6020 Innsbruck using nQuery Advisor®. Assign will also provide the site with randomization and emergency envelopes.

6.7 DOSE AND ADMINISTRATION

One dose of vaccine (0.3 ml) is contained in six drops which are delivered from the polyethylene dropper supplied with the multidose container.

One dose of placebo consists of 6 drops administered by the pipette supplied with the placebo container.

The vaccinees will remain under medical supervision for at least 30 min following the administration of vaccine.

6.8 COMPLIANCE

All study vaccine administrations will be supervised by the Investigator or his/her designee.

7. PRIOR AND CONCOMITANT THERAPY

The use of concomitant therapies should be kept to a minimum throughout the study. All therapies (prescriptions and over-the-counter medications) other than the study vaccine administered from informed consent until the last study visit must be recorded in the source documents and in the concomitant therapy section of the electronic case report form (eCRF; name of the drug, dosage, route and dates of administration).

The Sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

Female subjects of childbearing potential must agree to the use of an effective method of contraception throughout the study and up to 3 months after the last vaccine dose, as outlined in Section 5.1. Non-child-bearing potential is defined as pre-menarche, hysterectomy, oophorectomy or post-menopause (after 1 year without menses with an appropriate clinical profile at the appropriate age > 45 years). Effective methods of contraception are: oral, vaginal, injectable, and implantable hormonal contraceptives, intra-uterine device, true abstinence, condom with spermicide, male partner with vasectomy, and tubal ligation. Adequate contraception does not apply to subjects of child bearing potential with same sex partners, when this is their preferred and exclusive lifestyle.

Contraception is to be recorded in the source documents and in the concomitant therapy section of the eCRF.

There will be no restrictions in using concomitant therapies except for any medication that has a potential effect on the immune system in the opinion of the Investigator and any vaccine other than the study vaccine throughout the study.

8. ASSESSMENTS

8.1 TIMING OF ASSESSMENTS

An overview of the timing of treatment(s) and assessments is given in the Time and Events Schedules below.

Subjects will be given a full explanation of the nature of the study and written informed consent (approved by the local ethics committee) will be obtained according to local requirements before any study-related assessment will be carried out.

The Code of conduct contains an overview of in-/exclusion criteria and hygienic measures to be taken by the subject during the study. Subjects will be explained the content of this document and asked for their agreement during screening. They will receive a copy as a memory aid at home. At each visit and weekly by email or sms subjects will be reminded of the content of this document.

Adverse events and the intake of concomitant medication will be monitored continuously from informed consent until the last study-related activity.

After the predose procedures, (signing of Informed consent and Code of Conduct, reviewing of in-/exclusion criteria, medical history, medication history, demographic data, performing physical examination, pregnancy test, assessing vital signs and blood sample cfr. Time and Events Schedule), the subjects will receive the first dose of study vaccine according to the procedure described in Section 6.7, followed by further assessments as outlined in the Time and Events schedule.

The subjects will be kept under medical supervision for at least 30 min after each vaccination.

Thermometers and a diary card will be distributed to subjects and the use of the diary card will be explained.

Unscheduled visits can be planned for instance:

- to obtain additional information to ensure safety to the subject. Additional blood and urine samples for clinical assessment may be taken at the discretion of the Investigator.

Findings made during unscheduled visits should be reported in the source documents and in the designated sections of the eCRF.

TIME AND EVENTS SCHEDULE – GROUPS 1 AND 3

Assessments					
Visit	**1**	**2**	**3**	**4**	**5**
Time of Visit (days) **Visit window**	**D0**	**D7** **(+/- 2D)**	**D14** **(+/- 3D)**	**D28[a]** **(+/- 2D)**	**D 42** **(+/- 4D)**
Informed consent[b]	X				
In-/exclusion criteria	X				
Medical history/concomitant diseases	X				
Medication history[c]	X				
Demographic data	X				
Physical examination[d]	X[e]	X	X	X	X
Vital signs[f]	X[e,f]	X	X	X	X
Clinical laboratory tests[g]	X[e]	X	X	X	
Pregnancy test[h]	X[e]				X
Serology[i]	X[e]				
Randomization	X				
Administration of vaccine[j]	X				
Serum sample for polio antibodies	X[e]			X	
Stool sample for viral culture/quantitative PCR, stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay[l]	X----------X[l]		X	X[l]	X
Solicited systemic AEs (Diary)[k]	X----------X				
Remote contact for safety follow-up[m]					
Concomitant therapies[n]	X	X	X	X	X
Adverse events[n]	X	X	X	X	X
Reminder Code of Conduct[o]	X	X	X	X	X

a. In case of early termination, the subjects will be asked to return to the clinical site within 14 days after discontinuation for assessments as outlined on Day 28.
b. No study-related assessment is to be carried out before signing of the informed consent form.
c. Including polio vaccination history.
d. Includes weight and height at Day 0. After Day 0, symptom-directed physical examination.
e. Prior to vaccination.
f. Blood pressure and heart rate (supine) and oral body temperature. On Day 0, vital signs will be assessed prior to and 30 min after vaccination.
g. For a list of assessments, please see Appendix 1: Overview of Laboratory assessments.

h. In women of childbearing potential, a urine pregnancy test will be performed.
i. Includes HBsAg, anti-HCV and anti-HIV antibodies, and IgA.
j. The subjects will be kept under medical supervision for at least 30 min after vaccination.
k. Solicited AEs will be collected for Days 0-7.
l. Subjects will be asked to collect the first stool of every day in the provided container and to store this in the provided thermal bag with cooling elements: daily collection on Days 0 to 10 plus collection on Days 14, 21, V4 minus 1 or 2 days and V5 minus 1 or 2 days. At Day 0 the storage procedure will be explained and the collection material provided. Subjects who are still shedding virus in one of the 3 last stool samples will be asked to further collect 3 consecutive stool samples every 3 weeks till end of shedding.
m. Remote contact (text message by sms, WhatsApp or email) on Day 3 and on Day 21 for safety follow-up and reminder stool collection scheme.
n. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity. The subjects can record unsolicited AEs on their diary card.
o. After signing on screening visit, weekly reminder Code of Conduct by email or sms and on each visit to emphasize the need and importance of hygienic measures and respecting the inclusion/exclusion criteria until end of study

TIME AND EVENTS SCHEDULE – GROUPS 2, 4, 5, 6, AND 7

Assessments								
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Time of Visit (days) **Visit window**	**D0**	**D7** **(+/- 2D)**	**D14** **(+/- 3D)**	**D28** **(+/- 2D)**	**D35** **(+/- 2D)**	**D42** **(+/- 3D)**	**D56[a]** **(+/- 2D)**	**D 70** **(+/- 4D)**
Informed consent[b]	X							
In-/exclusion criteria	X							
Medical history/concomitant diseases	X							
Medication history[c]	X							
Demographic data	X							
Physical examination[d]	X[e]	X	X	X[e]	X	X	X	X
Vital signs[f]	X[e,f]	X	X	X[e]	X	X	X	X
Clinical laboratory tests[g]	X[e]	X	X	X[e]	X	X	X	
Pregnancy test[h]	X[e]			X[e]				X
Serology[i]	X[e]							
Randomization	X							
Administration of vaccine[j]	X			X				
Serum sample for polio antibodies	X[e]			X[e]			X	
Stool sample for viral culture/quantitative PCR, stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay[l]	X----------X[l]		X	X[e]-------X[l]		X	X[l]	X
Solicited systemic AEs (Diary)[k]	X----------X			X----------X				
Remote contact for safety follow-up[m]								
Concomitant therapies[n]	X	X	X	X	X	X	X	X
Adverse events[n]	X	X	X	X	X	X	X	X
Reminder Code of Conduct	X	X	X	X	X	X	X	X

a. In case of early termination, the subjects will be asked to return to the clinical site within 14 days after discontinuation for assessments as outlined on Day 56.
b. No study-related assessment is to be carried out before signing of the informed consent form.
c. Including polio vaccination history.
d. Includes weight and height at Day 0. After Day 0, symptom-directed physical examination.
e. Prior to vaccination.

f. Blood pressure and heart rate (supine) and oral body temperature. On Day 0 and Day 28, vital signs will be assessed prior to and 30 min after vaccination.
g. For a list of assessments, please see Appendix 1: Overview of Laboratory assessments.
h. In women of childbearing potential, a urine pregnancy test will be performed.
i. Includes HBsAg, anti-HCV and anti-HIV antibodies, and IgA.
j. The subjects will be kept under medical supervision for at least 30 min after vaccination.
k. Solicited AEs will be collected for Days 0-7 and Days 28-35.
l. Subjects will be asked to collect the first stool of every day in the provided container and to store this in the provided thermal bag with cooling elements: after each vaccine dose, daily collection on Days 0 to 10 plus collection on Days 14, 21,V4 minus 1 or 2 days (= V7 minus 1 or 2 days after 2nd vaccination) and V8 minus 1 or 2 days. At Day 0, the storage procedure will be explained and the collection material provided. Subjects who are still shedding virus in one of the 3 last stool samples will be asked to further collect 3 consecutive stool samples every 3 weeks till end of shedding.
m. Remote contact (text message by sms, WhatsApp or email) on Day 3 and on Days 21, 31 and 49 for safety follow-up and reminder stool collection scheme.
n. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity. The subjects can record unsolicited AEs on their diary card.
o. After signing on screening visit, weekly reminder Code of Conduct by email or sms and on each visit to emphasize the need and importance of hygienic measures and respecting the inclusion/exclusion criteria until end of study.

8.2 IMMUNOGENICITY

8.2.1 *Immunogenicity Variables*

Neutralizing Type 2 Poliovirus Antibody Titers

Blood samples for the determination of neutralizing type 2 poliovirus antibodies will be taken at the time points specified in the Time and Events schedule.

Neutralizing antibodies against type 2 poliovirus will be determined using a sero-neutralization assay.

Detailed descriptions of the collection, handling, transport and processing of the blood samples will be included in the laboratory manual.

Viral Shedding

Stool samples will be collected at the time points outlined in the Time and Events schedule.

As a measure of intestinal immunity, shedding of type 2 poliovirus in the stools will be evaluated using:

- Quantitative PCR (viral identity).
- $CCID_{50}$ determination (titer).

The shedding analyses will initially be conducted using a subset of samples in the 10 days following administration of each dose (e.g. day 0, 3, 7, and 10) and all samples at day 14 and thereafter. The remaining samples will be stored pending evaluation of the initial analyses; PCR and $CCID_{50}$ will be conducted on these stored samples if deemed important for the complete interpretation of the study results.

Shedding results for last stool sample will be provided to the sites in a timely manner in order to inform subjects about the need for further stool sampling every 3 weeks and taking precautions.

Detailed descriptions of the collection, handling, transport and processing of the stool samples will be included in the laboratory manual.

8.2.2 *Immunogenicity Criteria*

For an overview of endpoints, see Section 3.

The following endpoints will be based on neutralizing type 2 poliovirus antibody titers:

- Seroprotection: poliovirus type 2-specific titers ≥1:8;
- Seroconversion: a change from seronegative to seropositive and antibody titers of ≥1:8, and in seropositive subjects, as an antibody titer increase of ≥ 4 fold over baseline titers;
- Median titer and GMT 28 days post-vaccination and 56 days post-vaccination for Groups 2, 4, 5 and 6.

8.3 SAFETY EVALUATIONS

The safety assessment in this study will be based on AEs, clinical laboratory tests, vital signs, and physical examination, as described in the following sections.

8.3.1 *Adverse Events*

Adverse events will be monitored continuously from informed consent until the last study-related activity. At regular intervals during the study, subjects will be asked non-leading questions to determine the occurrence of any AEs. All AEs reported spontaneously during the course of the study will be recorded as well.

For detailed definitions and reporting procedures of AEs, see Section 11.

Solicited AEs will be recorded for 7 days following each vaccine dose using a Diary Card.

8.3.2 *Clinical Laboratory Tests*

Blood samples of about 15 mL will be collected at the time points indicated in the Time and Events schedule.

Biochemistry and hematology testing will be performed on these samples, as well as serology testing (HBsAg, anti-HCV, anti-HIV antibodies, and total serum IgA) on the sample from Day 0. In female subjects of childbearing potential, also urine β-human chorionic gonadotropin (hCG) will be tested at Day 0 and at last visit in each group and at Day 28 in Groups 2, 4, 5, 6 and 7.

Standard laboratory tests as outlined in Appendix 1 will be performed by the local laboratory.

The Investigator must review the laboratory report, document this review, and record any change occurring during the study he/she considers to be clinically relevant in the source documents and in the AE section of the eCRF. Laboratory values outside the normal range will be flagged and their clinical relevance and causality will be assessed by the Investigator. A copy of all laboratory reports must be filed in the subject's medical records.

Samples that remain after protocol-specific assessments have been performed may be used for further exploratory work on polio. No human DNA or RNA analysis will be performed.

8.3.3 *Vital Signs*

Vital sign parameters will be assessed after 5 minutes in supine position at the time points indicated in the Time and Events schedule.

The vital sign parameters that will be assessed are supine systolic and diastolic blood pressure (SBP and DBP, respectively), pulse rate and oral body temperature.

Blood pressure and heart rate will be measured using a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values will be registered on a built-in recorder so that measurements are observer-independent.

Any abnormal vital sign values occurring during the study that are considered to be clinically relevant by the Investigator should be recorded in the source documents and the AE section of the eCRF.

Fever is defined as oral body temperature ≥37.5°C.

8.3.4 *Physical Examination*

Physical examination will be performed at the time points indicated in the Time and Events schedule.

Physical examination at Day 0 will include height and weight. To obtain the actual body weight, subjects must be weighed lightly clothed. The height should be measured barefoot.

Any change in physical examination occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the source documents and the AE section of the eCRF.

8.4 EXPLORATORY EVALUATIONS

8.4.1 *Neurovirulence*

As noted above, a pre-specified subset of the stool samples collected for viral extraction (as outlined in the Time and Events schedule) will be analyzed by PCR and cell culture infectivity (CCID50). Of these, a further subset will be assessed for neurovirulence and genetic sequence.

For the neurovirulence testing of virus derived from stool samples of clinical trial participants vaccinated with oral polio vaccines, a modified transgenic mouse neurovirulence test (mTgmNVT) may be applied. The TgmNVT that will be used is adapted from the WHO SOP (Neurovirulence Testing of Oral Polio Vaccine Using TgPVR21 Transgenic Mice) used for vaccine release.

In the mTgmNVT, blinded, cell culture-amplified virus material (from stool) is titrated and then diluted to a fixed target dose/inoculum selected to detect reversion of the Sabin-2 strain. The inoculum is intraspinally injected into transgenic mice susceptible to poliovirus. Control virus is tested concurrently in the TgmNVT to assess the validity of a given test.

The inoculated mice are observed daily for the presence of clinical signs of poliovirus infection (weakness, paresis, and paralysis). After the observation period of 14 days, the in-vivo phase is ended. Mice are euthanized and a clinical end score (paralyzed or non-paralyzed) is assigned to each mouse. Results are reported as percent paralysis observed per sample. Samples that induce paralysis above a critical threshold may be further tested in a multi-dose format of the TgmNVT for further characterization.

NV data from this study may be combined with data from prior studies to further optimize this assay and also to draw comparisons between shed virus from Sabin-2 and the novel vaccine candidates.

8.4.2 *Sequencing*

Viral sequencing methods (e.g. deep sequencing) will be performed on selected stool samples taken at one or more of the time points specified in the Time and Events schedule to explore the heterogeneity of shed virus. Sequence information on shed virus may be compared with the results of neurovirulence testing, if available.

8.4.3 *Further exploratory work*.

Serum or stool samples that remain after protocol-specific assessments have been performed may be used for further exploration of safety lab parameters, and/or exploratory work on polio for maximum 10 years after study closure. No human DNA or RNA analysis will be performed.

8.5 APPROPRIATENESS OF MEASUREMENTS

The assessments which will be made in this study are either standard or are scientifically justified.

Each biological assay contains a reference material used as a control, to ensure comparability between these study vaccine arms and the historical control arm. Additional re-test of some samples may be conducted to ascertain the presence of temporally-related variability in assay results.

9. STUDY TERMINATION/COMPLETION

9.1 STUDY COMPLETION

A subject 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 or stable or until the event is otherwise explained.

Also, if type 2 virus shedding is detected by PCR on one of the last 3 scheduled stool samples the study duration for this individual will be extended. As soon as these results are known (anticipated approximately 3 weeks after the last such sample provided for evaluation) the subject will be asked to further collect 3 consecutive stool samples (with a maximum of one sample per day) after the last per-protocol sample, and to repeat this until shedding is PCR-negative for type 2 poliovirus on 3 consecutive stool samples.

If the last stool sample is missing, subject will be asked to provide a new sample as soon as possible in order to determine end of study for this person or the need for further stool sample collection according to the collection cadence until shedding is PCR negative on 3 consecutive stool samples.

9.2 REMOVAL OF SUBJECTS FROM STUDY OR INVESTIGATIONAL PRODUCT

9.2.1 *Removal from Study*

Subjects have the right to withdraw from the study at any time for any reason, including personal reasons. A subject can withdraw without giving a reason. The Investigator should however try to find out why a subject withdraws from the study and document the reason for withdrawal in the source documents and on the eCRF.

Subjects **may** be withdrawn from the study in the event of:

- a severe AE or SAE;
- difficulties in obtaining blood or other samples;
- failure of the subject to comply with the protocol requirements or to cooperate with the Investigator.

Subjects **must** be withdrawn from the study in the event of:

- withdrawal of consent;
- for safety reasons, it being in the best interest of the subject that he/she be withdrawn, in the Investigator's opinion.

In the event of a subject being withdrawn from the study, the monitor and Sponsor should be informed: in case of withdrawal due to an SAE (for details on AE reporting see Section 11), the Sponsor should be notified within 24 hours; in case of withdrawal for other reasons, the Sponsor should be notified within 2 days from the event.

If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned.

Subjects who are withdrawn from the study prior to completion of the scheduled study procedures for any reason (AE, withdrawal of consent, etc.) should be invited to complete the assessments as much as possible: as long as the subject consents, all relevant assessments of the day on which the subject withdrew from the study should be completed, at least those related to safety. In case of an AE, the appropriate follow-up will be done.

Subjects who are withdrawn from the study will not be replaced.

9.2.2 *Removal from Investigational Product*

Removal from investigational product administration concerns subjects who do not receive a complete vaccination schedule as planned per protocol. A subject withdrawn from further investigational product administration need not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information pertaining to premature discontinuation of the investigational product will be documented in the eCRF. The Investigator will document whether the decision to discontinue further vaccination was made by the subject or the Investigator, and which of the following possible reasons was responsible for withdrawal:

- SAE;
- AE;
- other (specify).

10. STATISTICAL METHODS

10.1 STATISTICAL ANALYSIS

All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalized before database lock. For Groups 1 through 4, comparative analyses to the historical control companion study (UAM1) will be specified in the SAP, and the statistical methods and definitions described in this protocol and accompanying SAP supersede those of the UAM1 study.

The following populations will be considered for analysis:

- Intention-to Treat (ITT) population, defined as all subjects who are randomized according to randomized treatment assignment.
- Total Vaccinated population (TVP), defined as all subjects who are in the ITT population and who received at least one dose of study vaccine. Drop out from ITT to TVP will be described.
- Per-Protocol (PP) population, consisting of all eligible study participants who are in the TVP and who receive all of the immunizations scheduled for the group to which they are allocated and excludes those subjects who meet any of the criteria outlined in Section 5.3. All deviations and violations occurring in the study will be reviewed prior to database lock and classified as either minor or major. The PP population requires adherence to all vaccinations (including timing) for those from Groups 1, 3, 5 and 6, but subjects will not be removed from the population for missing outcome assessments; these will be handled case by case as missing data. In case of missing second vaccination of a subject in a 2-dose Group (Groups 2 and 4) but adherence to the protocol until that timepoint, all previous collected data will still be analysed in the PP population. A subject matching items under Section 5.3 will be removed from the PP analysis.

The TVP will be used for primary safety population analysis and the PP population for primary immunogenicity analysis; all immunogenicity analyses (primary and secondary) will be repeated in the TVP.

10.1.1 *Comparative Analyses*

The study UAM1, conducted in a pre-OPV2 cessation period (2015-2016), was specifically designed to provide active control comparator data for both safety and immunogenicity for the OPV-vaccinated adults given the novel vaccine candidates in this study. For all endpoints, summaries of data from this study will be presented alongside corresponding summaries from UAM1. Summaries of demographics, baseline characteristics, and immunogenicity data from this study will be prepared by site and overall, to assess comparability of sites. Comparative analyses will be limited to comparison of each vaccine to the UAM1 Sabin 2 control.

10.1.2 *Initial Characteristics Data of the Subject Sample*

Descriptive statistics will be provided per group and by site for demographic (e.g., age, height, weight, body mass index race, gender) and other initial subject characteristics

(clinical laboratory values, physical examination, medical and surgical history, concomitant diseases, concomitant medications). Descriptive statistics include mean, standard deviation (SD), median, maximum, minimum, and range for continuous variables and the number and percentage in each group for categorical variables.

Unless specified otherwise in the SAP, statistical tests and confidence intervals will be computed using a two-sided 5% significance level.

Prior and concomitant medications will be coded using the current WHO Drug Dictionary. For comparison with the UAM1 study UAM1 data will be recoded if necessary.

10.1.3 *Immunogenicity Data*

For an overview of primary, secondary and exploratory endpoints, see Section 3.

Neutralizing Type 2 Poliovirus Antibody Titers

At each post-vaccination time point where neutralizing antibody titers are obtained:

- Seroprotection rate with 95% CIs will be tabulated by group and site.
- Seroconversion rate with 95% CIs will be tabulated for post-vaccination time points by group and site
- Median of $\log_2$ antibody titers will be computed along with 95% Cis by group and site
- GMT with accompanying 95% CIs will be computed by group and site
- Plots of the reverse cumulative distribution of antibody titers will be generated by group and site

The primary immunogenicity endpoint, seroprotection after a single dose of each vaccine candidate, will be formally compared to the corresponding endpoint from UAM1 via a non-inferiority test of each of the novel candidates to the UAM1 Sabin 2 control, each using $\alpha = 0.025$ and a non-inferiority margin of 10%.

Secondary endpoints will be compared to corresponding endpoints from UAM1 in the same manner, using $\alpha = 0.025$ and margin = 10% for binary endpoints (seroconversion), and 2/3 $\log_2$ for median antibody titer and GMT ratios. GMTS will be estimated with likelihood-based methods to accommodate censoring at assay ULOQ and LLOQ, and ANCOVA methods will be used to address imbalances in baseline immunity.

Non-inferiority comparisons will be conducted by computing the difference in endpoint between each candidate vaccine and the UAM1 Sabin 2 control (novel minus comparator), with corresponding two-sided $\alpha = 0.05$ confidence intervals, with inference drawn from comparison of the lower confidence limit to the non-inferiority margin. Score-based confidence intervals will be used for binary comparisons, asymptotic normal-based methods will be used for GMTs, and bootstrap methods will be used for median titers and other continuous endpoints.

Immunogenicity comparisons will be based on the combined data from each site in this study compared to the single-site data from the historical control study, UAM1. In the event that between-site comparisons within this study indicate that significant differences in outcomes (two-sided tests for differences at $\alpha= 0.05$) exist between sites, additional

comparisons for all immunogenicity endpoints will be added to compare vaccine candidates using only data at the site in common between both studies. Comparisons of binary endpoints between sites in this study will be based on 95% two-sided score intervals for the difference; comparison of continuous endpoints will be based in two-sided 95% bootstrap-based confidence intervals for the difference in medians (median titer), or normal-based methods (for GMTs). Differences between sites will be indicated by a nominal value (percent/median/mean difference of zero) excluded from the confidence interval coverage region.

Viral Shedding

For each group (excluding Group 7), site, and time point, viral shedding positivity and concentration will be summarized. For each subject, a viral shedding index will be calculated as the average of $\log_{10}$-transformed values of viral concentration in stool samples as determined using quantitative PCR (viral identity) and $CCID_{50}$ (titer) from select stool samples taken following each vaccine dose, and this index will be summarized by group. The time to cessation of shedding, defined as the time interval between administration of vaccine and the last day of shedding positivity prior to cessation, will be assessed with Kaplan-Meier methods to describe and compare the duration of shedding.

Descriptive analysis and plots of the reverse cumulative distribution of the viral shedding index will be generated.

Comparative analyses to the Phase IV historical control arm will involve comparison of the binary shedding positivity endpoint using summary statistics, as well as 95% score-based confidence intervals for the difference in proportions at each time point.

The $CCID_{50}$ will be $\log_{10}$ transformed, described with summary statistics, and compared to historical control using two-sided randomization tests and bootstrap 95% confidence intervals for the difference in median.

Time-to-shedding cessation will be summarized using quantiles and corresponding 95% confidence intervals, and tests between candidate vaccines and the historical control will be conducted with two-sided log-rank tests using a Type I error rate of 5%.

10.1.4 *Safety Data*

For an overview of primary and secondary endpoints, see Section 3.

Safety parameters will be tabulated and analyzed descriptively. Comparisons to the historical control will be based on descriptive statistics, using rates for adverse events and laboratory deviations, and continuous summaries for safety laboratory values.

Adverse Events

Analyses described below will be performed for solicited and unsolicited AEs as well as for SAEs, severe AEs.

The original terms used in the designated sections of the eCRFs by Investigators to identify AEs will be fully described and coded according to the current Medical

Dictionary for Regulatory Activities (MedDRA).For comparison with the M1 study M1 data will be recoded if necessary. All AEs will be summarized by type, seriousness, severity, causality, by group, and overall.

Separate tables and listings will be created for those subjects who died, discontinued the study vaccine due to an AE, or experienced a severe or serious AE. Summaries, listings, and narratives may be provided, as appropriate.

Clinical Laboratory Tests

Each continuous biochemistry and hematology laboratory test will be evaluated by means of descriptive statistics (i.e., number of subjects, mean, SD, median, minimum, and maximum) on the actual values, at each assessment time point and by group. Changes from baseline will also be summarized by assessment time point and by group.

Clinical laboratory test values will be evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (toxicity grades) or in accordance with the normal ranges of the clinical laboratory (below, within, or above normal range) for parameters for which no toxicity grades are defined.

While all safety lab values, including abnormalities, will be summarized, for the specific safety lab parameters listed below, the rate of elevated values will be presented at the subject level overall, by timepoint, by group, and by abnormality grade, and accompanied by a two-sided 90% confidence interval for the risk difference of each vaccine candidate vs. the placebo group.

Safety lab values of OPV-vaccinated subjects will be compared with those of the historical control UAM1 study where possible (e.g. ALT/AST), and in addition, all safety lab values (ALT, AST, CK, GGT, bilirubin and albumin) of OPV-vaccinated subjects and of IPV-vaccinated subjects (Groups 5 and 6) will be compared to the placebo group (Group 7).

Parameters: CPK, ALT, AST, GGT, bilirubin and albumin plus routine other lab. Eventual further testing to include CK subtypes, electrophoresis, auto-antibodies (e.g. ANA, AMA, LKM-1, SLA/LP) and other viral pathogens can be done if any parameter is found to be consistently abnormal.

A listing of subjects with any clinical laboratory test result outside the reference ranges will be provided.

Vital Signs

Pulse rate, SBP, DBP and body temperature will be evaluated by means of descriptive statistics (actual values and changes from baseline and frequency tabulations at each assessment time point and by group).

The percentage of subjects with values beyond clinically important limits (as defined in Appendix 2) will be summarized.

Physical Examination

Abnormal findings in physical examination will be listed.

10.1.5 *Exploratory*

If any stool samples assessed via PCR and $CCID_{50}$ yield a qualifying sample for the deep sequencing and neurovirulence assays (qualification based on the assay input cutoff(s)

provided by the contracted CRO), descriptive analysis of viral sequence heterogeneity will be conducted, as well as descriptive and comparative analysis of neurovirulence assay results to results obtained from samples collected in the Phase IV historical control study.

10.1.6 *Missing Data*

In spite of best efforts to collect complete data for all study subjects, some data will be missing at the end of the trial. The reasons for missing data will be ascertained and appropriate statistical methods will be used to accommodate these absences in the analyses of trial data that minimize potential biases and maximize efficiency conditional on the causes for data being missing. Data values that are identified by quality control procedures to be spurious will not be used in final analyses of trial data.

10.2 DETERMINATION OF SAMPLE SIZE

Groups 1 through 4:

Sample size is chosen here to mirror that of the historical control study, UAM1. The serotype 2 Day 28 seroprotection rate among OPV-vaccinated individuals is expected to be 95% for subjects receiving a single dose of either investigational vaccine or the type 2 Sabin OPV control vaccine (historical control). 77 evaluable subjects per vaccine group are required to achieve 80% power for a test of non-inferiority to the historical control with a type I error rate of $\alpha = 0.025$ and a non-inferiority margin of 10%. 45 subjects are required to have 90% probability of observing at least one occurrence of an adverse event, when the true adverse event rate is 5%. Allowing for a 10% non-evaluability/dropout rate requires 50 subjects for the safety evaluations. For each vaccine candidate, both 1- and 2-dose groups may be used to assess the Day 28 immunogenicity endpoint, providing 100 subjects for the primary immunogenicity comparison. Therefore, 50 subjects will be allocated to each dose group, to also collect adequate safety data for each group, as defined above.

Groups 5 through 7:

Due to observed transient and asymptomatic elevated values of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) in the first-in-human phase 1 study, UAM4a, conducted in IPV-vaccinated adults in a contained environment, CK, ALT, AST, GGT, bilirubin and albumin have been designated for further evaluation in this study. Data from OPV-primed individuals in study UAM1 suggest the background rate of spurious elevations with the safe mOPV2 vaccine may be approximately 6%. If the true rate with either candidate vaccine is 4 times the expected rate, 44 subjects per group (each vaccine, plus control) would be required to have 80% power to detect a significant increase in the rate (lower one-sided 95% confidence bound for risk difference > 0) relative to the placebo group. If, upon review of interim safety data, the DSMB agrees that a) despite all reasonable efforts, recruitment has stagnated, and b) sufficient data have accrued to satisfy safety objectives relevant to these groups, group sizes can be truncated.

11. ADVERSE EVENT REPORTING

11.1 DEFINITIONS

Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including clinical laboratory test abnormalities.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose meets any of the following conditions:

- results in death;
- is life-threatening, i.e., the subject was at risk of death at the time of the event (e.g., ventricular fibrillation and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing inpatient hospitalization:
  Hospitalization refers to an overnight admission into hospital for the purpose of investigating and/or treating the AE. Hospitalization for an elective procedure, or routinely scheduled treatment for a pre-existing condition that has not worsened, is not an SAE.
- results in persistent or significant disability/incapacity, i.e., causing substantial disruption of the subject's ability to conduct normal life;
- is a congenital anomaly/birth defect;
- is medically important: Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations such as important medical events (IMEs) that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of drug dependency or drug abuse.

Solicited Adverse event

A selected sign or symptom ('adverse event') occurring in the hours and days following a vaccination, to be collected by the subject for 8 consecutive days (D0-D7), using a pre-defined checklist in a diary card.

The following adverse events are included in the diary checklist:

- Headache
- Fatigue
- Myalgia
- Arthralgia
- Paresthesia
- Anesthesia
- Paralysis
- Nausea
- Vomiting
- Diarrhea
- Abdominal pain

Other solicited reaction

Body temperature for fever (oral), to be collected by the subject for 8 consecutive days (D0-D7) after vaccination. Fever is defined as temperature of 37.5°C or higher.

11.2 INTENSITY OF ADVERSE EVENTS

Severity of solicited AEs will be scored as indicated on the Diary Card.

Each unsolicited AE must be rated on a 3-point scale of increasing intensity as outlined below:

Grade 1:

Mild; an AE that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.

Grade 2:

Moderate; an AE that is sufficiently discomforting to interfere with normal everyday activities.

Grade 3:

Severe; an AE that prevents normal everyday activities. Such an AE would, for example, prevent attendance at work and would necessitate the administration of corrective therapy.

Fever will be assessed using the NIH CTCAE grading scale:

Grade 1: 37.5°C to 38.0°C

Grade 2: 38.1°C to 39.0°C

Grade 3: >39.0°C

Lab results will be assessed using the NIH CTCAE version 4.03 (toxicity grades). For grade 1 values the investigator will use his clinical judgement to determine these values as clinically significant. Lab values above grade 1 will automatically be deemed clinically significant, and recorded as adverse events.

11.3 CAUSALITY ASSESSMENT

The following categories will be used by the Investigator to describe the causality assessment:

Unrelated – there is not a reasonable possibility that the study vaccine caused the AE.

Unlikely – suggests that only a remote connection exists between the study vaccine and the event. Other conditions, including concurrent illness, progression or expression of the disease state or reaction to concomitant medication, appear to explain the AE.

Possible – suggests that the association of the AE with the study vaccine is unknown, however the event is not reasonably supported by other conditions.

Probable – suggests that a reasonable temporal sequence of the AE with vaccine administration exists and, in the Investigator's clinical judgment, it is likely that a causal relationship exists between the vaccine administration and the AE, and other conditions (concurrent illness, progression or expression of the disease state, or concomitant medication reactions) do not appear to explain the AE.

11.4 ACTION TAKEN REGARDING THE STUDY VACCINE

The action taken towards the study vaccine must be described as follows:

- Permanently discontinued;
- Stopped temporarily;
- No action taken;
- Not applicable.

11.5 OUTCOME

The outcome of each AE must be rated as follows:

- Recovered/resolved;
- Recovered with sequelae/resolved with sequelae;
- recovering/resolving;
- not recovered;
- Fatal;
- Unknown.

11.6 RECORDING OF ADVERSE EVENTS

All (S)AEs occurring during the clinical investigation must be documented in the source documents and on the AE forms of the eCRF. The Investigator will inquire about the occurrence of AEs/SAEs at every visit/contact during the study.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the (S)AE to the study vaccine in the source documents and on the eCRF. All measures required for (S)AE management must be recorded in the source documents and reported according to Sponsor's instructions.

All AEs occurring at any time during the study will be followed by the Investigator until satisfactory resolution (e.g., value back to baseline value) or stabilization or until last visit. If necessary, in order to obtain additional information to ensure safety to the subject, additional blood and urine samples may be taken at the discretion of the Investigator. Certain long-term AEs related to therapy cannot be followed until resolution within the setting of this study. In these cases follow-up will be the responsibility of the treating physician.

11.7 REPORTING OF SERIOUS ADVERSE EVENTS TO THE SPONSOR AND ASSIGN DATA MANAGEMENT AND BIOSTATISTICS GMBH

All SAEs independent of the circumstances or suspected cause must be reported on a Serious Adverse Event Form by the Investigator to the Sponsor and to Assign Safety Desk within 24 h of their knowledge of the event by fax (+43 512 281 514 77).

The SAE form should include a clearly written narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

Follow-up and outcomes should be reported for all subjects who experience an SAE.

It is critical that the information provided on the Serious Adverse Event Form matches the information recorded in the source documents and on the eCRF for the same event.

Copies of additional laboratory tests, consultation reports, postmortem reports, hospital case reports, autopsy reports, and other documents should be sent when requested and applicable. Follow-up reports relative to the subject's subsequent course must be submitted to the Sponsor and to Assign Safety Desk until the event has subsided or, in case of permanent impairment, until the condition stabilizes.

11.8 PREGNANCY

All initial reports of pregnancy in subjects must be reported to the Sponsor by the Investigator within 24 h of his/her knowledge of the event using a Pregnancy Report Form. Any subject who becomes pregnant during the study must be withdrawn from further vaccination (cfr. Section 9) but will continue in the study for safety follow-up.

The Investigator will contact the subject at the expected time of delivery for follow-up. Abnormal pregnancy outcomes (e.g., spontaneous or induced abortion, stillbirth, neonatal

death, congenital abnormality, birth defect) are considered SAEs and must be reported using the Serious Adverse Event Form.

11.9 REPORTING OF SERIOUS ADVERSE EVENTS TO COMPETENT AUTHORITIES/ETHICS COMMITTEES

Assign Data Management and Biostatistics GmbH assumes responsibility for appropriate reporting of AEs to the regulatory authorities. Assign Data Management and Biostatistics GmbH will also report to the Investigator all SAEs that are unlisted (unexpected) and associated with the use of the vaccine. The Investigator (or the sponsor via Assign Data Management and Biostatistics GmbH where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

Adverse events reporting, including suspected unexpected serious adverse reactions (SUSARs), will be carried out in accordance with applicable local regulations.

After termination of the clinical study (determined as LSLV), any unexpected safety issue that changes the risks benefit analysis and is likely to have an impact on the subjects who have participated in the study, together with proposed actions, will be reported by the Sponsor/ Assign Data Management and Biostatistics GmbH to the competent authority(ies) concerned as soon as possible.

11.10 DATA MONITORING COMMITTEE

A Data Safety Monitoring Board (DSMB) will monitor the safety aspects of this trial.

The composition and functioning of the Board is documented elsewhere.

12. ETHICAL ASPECTS

12.1 STUDY-SPECIFIC DESIGN CONSIDERATIONS

Potential subjects will be fully informed of the nature of the study and of the risks and requirements of the study before any study-related assessment will be carried out. During the study, subjects will be given any new information that may affect their decision to continue participation. They will be informed that their participation in the study is voluntary and that they may withdraw from the study at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and who provide their consent voluntarily will be enrolled in the study.

12.2 REGULATORY ETHICS COMPLIANCE

12.2.1 *Investigator Responsibilities*

The Investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the study, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB/IEC, or the regulatory authority(ies).

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles originating from the Declaration of Helsinki (1964 and revisions), and that the clinical study data are credible.

12.2.2 *Independent Ethics Committee or Institutional Review Board (IEC/IRB)*

An IRB/IEC should safeguard the rights, safety, and well-being of all study subjects. Special attention should be paid to studies that may include vulnerable subjects.

Before the start of the study, the Investigator (or Sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- final protocol and, if applicable, amendments;
- Investigator's Brochure
- Sponsor-approved ICF (and any updates or any other written materials to be provided to the subjects);
- Sponsor-approved subject recruiting materials;
- Information on the placebo

- information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable;
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by the IEC/IRB);
- information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects;
- any other documents that the IEC/IRB may require to fulfill its obligation (including insurance certificate).

This study will be undertaken only after the IEC/IRB has given full written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials, and any other written information to be provided to the subjects, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study, the Investigator (or Sponsor where required) will send the following documents and updates to the IEC/IRB for its review and approval, where appropriate:

- protocol amendments;
- revision(s) to the ICF and any other written materials to be provided to the subjects;
- new or revised subject recruiting materials approved by the Sponsor;
- revisions to compensation for study-related injuries or payment to subjects for participation in the study;
- summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually);
- reports of AEs that are serious, unlisted, and associated with the investigational medicinal product (IMP) (SUSARs);
- new information that may adversely affect the safety of the subjects or the conduct of the study;
- deviations from or changes to the protocol to eliminate immediate hazards to the subjects;
- report of death of any subjects under the Investigator's care;
- notification if a new Investigator is responsible for the study at the clinical site;
- Development Safety Update Report, Short-Term Study Specific Safety Summary and Line Listings, where applicable;
- any other requirements of the IEC/IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study subjects. If a deviation from or a change to the protocol was implemented to

eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IEC/IRB as soon as possible.

The Investigator (or Sponsor where required) will notify the IEC/IRB about the study completion within 90 days after the end of the study (defined as LSLV).

12.2.3 *Informed Consent*

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and the reviewing IEC/IRB. The informed consent should be in accordance with the principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before enrollment in the study, the Investigator or an authorized member of the clinical staff must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may refuse to participate or withdraw consent to participate at any time, without penalty or loss of benefits to which the subject was entitled. Finally, they will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access, and agrees to allow his or her study physician to re-contact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The ICF will include a paragraph whereby the participants allow/or not the use of their biological samples for additional polio related research, if needed.

The language about the study used in the oral and written information, including the ICF, should be non-technical and practical and should be understandable to the subject. The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained consent, a copy of the ICF must be given to the subject.

If a subject is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the ICF after the oral consent of the subject is obtained, if permitted by local law.

12.2.4 *Privacy of Personal Data*

The collection and processing of personal data from subjects enrolled in the study will be limited to those data that are necessary to investigate the safety and immunogenicity of the IMP used in the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data need to agree to keep the identity of the study subjects confidential.

The informed consent obtained from the subjects includes explicit consent for the processing of personal data and for the Investigator to allow direct access to subjects' original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

13. ADMINISTRATIVE REQUIREMENTS

13.1 PROTOCOL AMENDMENTS/NOTIFICATIONS

Neither the Investigator nor the Sponsor will modify this protocol without a formal amendment (except modifications that do not alter the benefit/risk-see next paragraph). All protocol amendments must be issued by the Sponsor and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IEC/IRB approval nor when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazard to the subjects, in which case an amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IEC/IRB must be provided to the Sponsor or its designee.

When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

13.2 SUBJECT IDENTIFICATION AND ENROLLMENT LOGS

The Investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure subject confidentiality, no copies will be made. All reports and communications related to the study will identify subjects by initials and/or assigned number only.

13.3 SOURCE DOCUMENTATION

At a minimum, source documentation, prepared by the site, must be available for the following to confirm data collected in the eCRF: subject identification, eligibility, and study identification; study discussion and date of informed consent, dates of visits, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow-up of AEs, concomitant medication, study vaccine receipt/dispensing/return records, study vaccine administration and 30 minutes post-vaccination observation information, laboratory printouts, date of study completion, and reason for early discontinuation of study vaccine or withdrawal from the study, if applicable.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded on the eCRF are consistent with the original source data.

It is recommended that the author of an entry in the source documents be identifiable.

At a minimum, the type and level of detail of source data available for a study subject should be consistent with that commonly recorded at the clinical site as a basis for standard medical care. Specific details required as source data for the study will be reviewed with the Investigator before the study and will be described in the monitoring guidelines (or other equivalent document). The nature and location of all source documents will be identified in the Source Document Identification Form. Data that will be recorded directly into the eCRF are specified in the Source Document Identification Form.

13.4 CASE REPORT FORM COMPLETION

Electronic Data Capture (EDC) will be used for this study. The study data will be transcribed by study personnel from the source documents onto an eCRF, and transmitted in a secure manner to the Sponsor. The electronic file will be considered to be the eCRF.

All eCRF entries, corrections, and alterations must be made by the Investigator or other authorized study-site personnel.

Worksheets may be used for the capture of some data to facilitate completion of the eCRF. Any such worksheet will become part of the subject's source documentation. Such worksheet should not resemble an eCRF. All data related to the study must be recorded on the eCRFs prepared by the Sponsor. Data must be entered into the eCRFs in English. Designated site personnel must complete the eCRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

The Investigator must verify that all data entries on the eCRFs are accurate and correct.

13.5 MONITORING

The monitoring of the study will be done under the responsibility of the Sponsor by Assign Data Management and Biostatistics GmbH.

The monitor will perform on-site monitoring visits as frequently as necessary. The monitor will record the dates of the visits in a study site visit log that will be kept at the clinical site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the eCRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the Sponsor and clinical staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the clinical staff.

Direct access to source documentation (medical records) must be allowed at all times for the purpose of verifying that the data recorded in the eCRF are consistent with the original source data. Findings from this review of eCRFs and source documents will be discussed with the clinical staff. During on-site monitoring visits (notified and agreed upfront with the clinical staff), the relevant clinical staff will be available, the source documentation will be accessible, and a suitable environment for review of study-related documents will be provided. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

13.6 DATA MANAGEMENT

Data management of the study will be performed under the responsibility of the Sponsor by Assign Data Management and Biostatistics GmbH.

After the monitor has reviewed the data entered into the eCRFs for completeness and accuracy and the data are released by the Investigator, data will be uploaded into the clinical database to perform cleaning activities. Computerized data cleaning checks will be used in addition to manual review, including listings review, to check for discrepancies and to ensure consistency and completeness of the data.

If necessary, queries will be generated in the EDC tool. The Investigator or an authorized member of the clinical staff must adjust the eCRF (if applicable) and complete the query. If corrections to an eCRF are needed after the initial entry into the eCRF, this can be done in 3 different ways: 1- site personnel can make corrections in the EDC tool at their own initiative or as a response to an auto query (generated by the EDC tool), 2- the site manager can generate a query (field data correction form [DCF]) for resolution by the clinical staff, and 3- the clinical data manager can generate a query for resolution by the clinical staff.

The clinical database will be locked as soon as it is considered clean. Only authorized and well-documented updates to the study data are possible after database lock. The locked database is used in the final statistical analysis for study reporting. Measures will be undertaken to protect subject data handed over by the Investigator to the data management department and during inspections against disclosure to unauthorized third parties. Subject confidentiality will be maintained at all times.

13.7 DATA QUALITY ASSURANCE

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor or designate.

The Sponsor or his designee will review the eCRF system for accuracy and completeness during (on-site) monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. After upload of the data into the clinical study database, their accuracy verified using appropriate validation programs.

In accordance with Good Clinical Research Practice Guidelines and Recommendations, the Sponsor will be entitled to audit the facilities used in the clinical and laboratory parts of the study, as well as to access all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

13.8 ON-SITE AUDITS

Representatives of the Sponsor's clinical quality assurance department or any other qualified auditor appointed by the Sponsor may visit the clinical site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eCRFs. Subject privacy must, however, be respected. The Investigator and clinical staff are to be present and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or its designees.

Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the

Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

13.9 STUDY TERMINATION

The Sponsor has the right to terminate the study at any time. In case of an early termination of the study for any reason or temporary halt by the Sponsor, the IEC/IRB and the regulatory authority should be notified within 15 calendar days and should be provided with a detailed written explanation of the reasons for the termination/halt.

An end-of-study declaration will be submitted to the regulatory authorities and IEC/IRB after the complete study has ended. This notification will be submitted within 90 days after the end of the study.

13.10 RECORD RETENTION

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eCRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 25 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 25 years have elapsed since the formal discontinuation of clinical development of the IMP. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

13.11 USE OF INFORMATION AND PUBLICATION

All information, including but not limited to, information regarding the study vaccine or the Sponsor’s operations (e.g., patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the Sponsor to the Investigator and not previously published, and any data generated as a result of this study are considered confidential and remain the sole property of the Sponsor. The Investigator agrees to maintain this information in confidence, to use this information

only to accomplish this study, and not to use it for other purposes without the Sponsor's prior written consent.

The Investigator understands that the information generated in this clinical study will be used by the Sponsor in connection with the continued development of the study vaccine, and thus may be disclosed as required to other clinical Investigators or regulatory agencies. To permit information derived from the clinical studies to be used, the Investigator is obliged to provide the Sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated under the responsibility of the Sponsor and will contain eCRF data from all clinical sites that participated in the study. In the Clinical Study Report clinical narratives will be written for the following events (for example):

- all deaths (irrespective of vaccine relationship);
- all other SAEs after vaccination;
- all discontinuations of the study vaccine due to AEs (irrespective of vaccine relationship);
- at the discretion of the team and after statistical analysis of the data, certain discontinuations not related to AEs or treatment failure, i.e., related to lost to follow-up or withdrawal of consent (irrespective of treatment group);
- any events of special interest explicitly requested by the regulatory agencies.

The coordinating Investigator will sign off the final version of the Clinical Study Report. A summary of this final version will be provided to the Investigators, the applicable regulatory authorities, and the IECs/IRBs, if required by the applicable regulatory requirements, within 1 year after the end of the study (LSLV).

The Sponsor shall have the right to publish study data and information without approval from the Investigator. If an Investigator wishes to publish information from the study, a copy of the manuscript must be provided to the Sponsor for review at least 30 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations, or other materials. If requested by the Sponsor in writing, the Investigator will withhold such publication for up to an additional 30 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the Sponsor will review these issues with the Investigator. The Sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicenter study designs and substudy approaches, results may need to be published in a given sequence (e.g., substudies should generally not be published before the primary endpoints of a study have been published). Similarly, Investigators will recognize the integrity of a multicenter study by not publishing data derived from an individual clinical site until the combined results from the completed study have been published in full, within 12 months after conclusion, abandonment, or termination of the study at all clinical sites, or the Sponsor confirms there will be no multicenter study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the

study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

13.12 REGISTRATION OF CLINICAL STUDIES AND DISCLOSURE OF RESULTS

The Sponsor will register the existence and disclose the results of clinical studies as required by law.

13.13 INVESTIGATOR INDEMNITY

The Sponsor holds and will maintain an adequate insurance policy covering damages arising out of University of Antwerp sponsored clinical research studies.

The Sponsor will indemnify the Investigator and hold him/her harmless for claims related to damages arising from the investigation, provided that the study vaccine was administered under the Investigator's or deputy's supervision and in strict accordance with accepted medical practice and the study protocol.

The Investigator must notify the Sponsor immediately upon notice of any claims or lawsuits.

13.14 CONFIDENTIALITY

All study documents are provided by the Sponsor to the Investigator and appointed clinical staff in confidence. None of this material may be disclosed to any party not directly involved in the study without the Sponsor's written permission.

The Investigator must assure that subjects' rights to privacy and the confidentiality of their medical information will be maintained in accordance with all applicable laws and regulations. Data of subjects will only be forwarded in a coded way by subject number without full names. The Investigator will keep a separate list with at least the initials, the subjects' study numbers, names, addresses, and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from the Sponsor.

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APPENDIX 1: OVERVIEW OF LABORATORY ASSESSMENTS

Serology[a]	**Hematology**	**Chemistry**
Human immunodeficiency virus	Hemoglobin	Total bilirubin
Hepatitis B surface antigen	Hematocrit	Direct bilirubin[b]
Hepatitis B core antibody	Red blood cells (RBC)	Glucose
Hepatitis C virus	White blood cells (WBC) with differential	Blood urea nitrogen (or urea)
Total serum IgA	Lymphocytes	Creatinine
	Monocytes	Calcium
	Neutrophils	Phosphate, inorganic
	Eosinophils	Potassium
	Basophils	Sodium
	Platelets	Alanine amino transferase
		Aspartate amino transferase
		Gamma-glutamyl transferase
		Creatine phosphokinase
		Albumin
		C-reactive protein
Other Assessments	**Coagulation**	
Urine pregnancy test	Prothrombin time	
	Activated partial thromboplastin time	
	Fibrinogen	

[a] Day 0 only

[b] Assay if total bilirubin is above normal range.

APPENDIX 2: NORMAL RANGES FOR VITAL SIGNS

NORMAL RANGES FOR VITAL SIGNS

Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Pulse rate (bpm)	Oral temperature (°C)
$90 \leq SBP \leq 150$	$45 \leq DBP \leq 90$	$40 \leq HR \leq 100$	$35.0 \leq t° < 37.5$

These normal ranges are applicable in supine position (after 5 minutes).

APPENDIX 3: SYNOPSIS PROTOCOL UAM1

Study Title	**A Phase 4 study to evaluate the safety and immunogenicity of monovalent oral polio vaccine type 2 in healthy OPV-vaccinated adults**		
Product	SABIN monovalent Oral Polio Vaccine type 2 (mOPV2)	**Clinical Phase**	IIV
Protocol Number	UAM1	**Indication**	Oral polio vaccine immunization
Eudract Number 2015-003325-33			

Sponsor	University of Antwerp
Sponsor Representative	Prof Dr Pierre Van Damme, PhD
Clinical Center(s)	Centre for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute, University of Antwerp Campus Drie Eiken, Universiteitsplein 1, 2610 Antwerpen (Wilrijk), Belgium

Objectives:

The primary objectives of this study are to assess the safety (serious adverse events [SAEs] and severe[††] adverse events [AEs]) and immunogenicity (seroprotection rate) of SABIN mOPV2 in healthy OPV-vaccinated adults.

Secondary objectives are

- assess the safety (any solicited and unsolicited AEs, laboratory assessments) of SABIN mOPV2 in healthy OPV-vaccinated adults;
- immunogenicity (seroconversion rate, median antibody titer) of SABIN mOPV2 in healthy OPV-vaccinated adults.

Exploratory objectives are to

- assess immunogenicity (geometric mean titer [GMT] of SABIN mOPV2);
- investigate viral shedding following mOPV2 administration in a subset of stool samples;
- exploratory objectives may also include assessment of the genetic sequence heterogeneity and potential for neurovirulence (as measured in animal model(s)) of shed virus in a subset of stool samples;

Overview of Study Design:

This will be a single center, open, randomized study in 100 healthy OPV-vaccinated adults (age range 18 to 50 years), as follows:

- 50 OPV-vaccinated adults to receive 1 dose of mOPV2 (Group 1);
- 50 OPV-vaccinated adults to receive 2 doses of mOPV2 (Group 2).

A total of 100 OPV-vaccinated subjects will be evaluated for the 1-dose mOPV2 regimen and 50 subjects will be evaluated for the 2-dose regimen.

Allocation of subjects to Group 1 or 2 will occur in a randomized way.

[††] List of severe AEs as mentioned in the diary cards: fever > 39°C, headache, fatigue, myalgia, arthralgia , paresthesia, anesthesia, paralysis, or gastrointestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain) that prevent normal activity or any other severe AE that prevents normal activity;

The DSMB will establish stopping rules for safety which will be continuously assessed.

Study Population:
Healthy OPV-vaccinated adults (age range 18 to 50 years)

Eligibility Criteria:

Inclusion Criteria:

1. Healthy male or female, between 18 and 50 years old, extremes included;
2. Received at least 3 doses of OPV in the past (more than 12 months before start of the study);
3. In good physical and mental health as determined on the basis of medical history and general physical examination performed at Day 0;
4. Female subjects of childbearing potential must agree to the use of an effective method of birth control throughout the study and up to 3 months after last vaccine dose;
5. Willing to adhere to the prohibitions and restrictions specified in this protocol (see Section 5.5);
6. Informed Consent Form (ICF) signed voluntarily by the subject before any study-related procedure is performed, indicating that the subject understands the purpose of and procedures required for the study and is willing to participate in the study.

Exclusion Criteria:

1. A condition that, in the opinion of the Investigator, could compromise the well-being of the subject or course of the study, or prevent the subject from meeting or performing any study requirements;
2. Having Crohn's disease or ulcerative colitis or having had major surgery of the gastrointestinal tract involving significant loss or resection of the bowel;
3. A known allergy, hypersensitivity, or intolerance to the study vaccine, or to any of its components, or to any antibiotics;
4. Any confirmed or suspected immunosuppressive or immunodeficiency condition (including human immunodeficiency virus [HIV] infection);
5. Will have household or professional contact with known immunosuppressed people or people without full polio vaccination (i.e. complete priming) within 28 days after vaccination;
6. Neonatal nurses or others having professional contact with children under 6 months old within 28 days after vaccination;
7. Chronic administration (i.e., longer than 14 days) of immunosuppressant drugs or other immune-modifying drugs within 6 months prior to the first vaccine dose or planned use during the study. For instance, for corticosteroids, this means prednisone, or equivalent, ≥ 0.5 mg/kg/day (inhaled and topical steroids are allowed, whereas intra-articular and epidural injection/administration of steroids are not allowed);
8. Presence of contraindications to administration of the study vaccine on Day 0: acute severe febrile illness deemed by the Investigator to be a contraindication for vaccination or persistent diarrhea or vomiting;
9. Indications of drug abuse or excessive use of alcohol at Day 0;
10. Being pregnant or breastfeeding. Women of childbearing potential will undergo a urine pregnancy test at Day 0. Subjects with a positive pregnancy test will be excluded;
11. Participation in another clinical study within 28 days prior to entry in this study or receipt of any investigational product (drug or vaccine) other than the study vaccine within 28 days prior to the first administration of study vaccine, or planned use during the study period;
12. Planned administration of any vaccine other than the study vaccine within 28 days of the first dose of study vaccine and during the entire study period.
13. Administration of polio vaccine within 12 months before the start of the study.
14. Having had a transfusion of any blood product or application of immunoglobulins within the 4 weeks prior to the first administration of study vaccine or during the study.
15. Subject is an employee of the Investigator or study site, with direct involvement in the proposed study or other studies under the direction of that Investigator or study site, or is a family member of an employee or the Investigator.

Test Product, Dose, Mode of Administration:

Polio Sabin™ Mono Two (oral) is a licensed, monovalent, live attenuated poliomyelitis virus vaccine of the Sabin strain Type 2 (P 712, Ch, 2ab), propagated in MRC5 human diploid cells. Each two-drop dose (0.1 mL) contains not less than $10^{5.0}$ $CCID_{50}$ of Type 2. Magnesium chloride is used as a stabilizer. Polio Sabin™ Mono Two (Oral) contains trace amounts of neomycin sulphate and polymyxin B sulphate.

One dose of vaccine (0.1 ml) is contained in two drops which are delivered from the polyethylene dropper supplied with vaccine.

Study Duration: 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.

Criteria for Evaluation:

Primary

The following endpoints will be evaluated by group and overall:

- ***Safety***: incidence, type and causality of SAEs and severe AEs throughout the study period.
- ***Immunogenicity***: seroprotection rate of type 2 polio antibodies at Day 28, following a single dose of mOPV2 (combined Groups 1 and 2).
  Seroprotection is defined as type 2-specific antibody titers ≥1:8.

Secondary

The following endpoints will be evaluated by group and overall:

Safety:

- the incidence, type, causality and severity of solicited adverse events for days 0-7 in both groups combined and days 28-35 in Group 2;
- the incidence, type, causality and severity of unsolicited adverse events throughout the study period;
- Incidence, causality and description of deviations from normal safety labs at Day 0 (both groups), Day 7 (both groups), Day 28 (both groups), Day 35 (Group 2), and Day 56 (Group 2).

Immunogenicity:

- Median titers of type 2 polio antibodies at Day 28 in Groups 1 and 2 combined;
- Seroprotection rate and median titers of type 2 polio antibodies at Day 56 in Group 2;
- Seroconversion rate of type 2 polio antibodies at Day 28 for both groups combined and at Day 56 in Group 2.
  Seroconversion is defined as a change from seronegative to seropositive and antibody titers of ≥1:8, and in seropositive subjects, as an antibody titer increase of ≥ 4 fold over baseline titers.

Exploratory

- GMT of type 2 polio antibodies at Day 28 in both groups combined and at Day 56 in Group 2;
- Descriptive analysis of viral shedding as determined using quantitative PCR (viral identity) and 50% cell culture infective dose ($CCID_{50}$; titer) after viral extraction from stool samples taken at one or more time points in a subset of stool samples;
- Exploratory endpoints may also include assessment of the genetic sequence heterogeneity and potential for neurovirulence (as measured in an animal model) of shed virus in a subset of stool samples;

Statistical Methods:

Sample size

Serotype 2 Day 28 seroprotection rates in this population are expected to be 95% for subjects receiving either a single dose of type 2 Sabin OPV or a future comparator vaccine. 77 evaluable subjects per group are required to achieve 80% power for a test of non-inferiority to this historical control with a type I error rate of alpha = 0.025 and a non-inferiority margin of 10%. 45 subjects are required to detect a nonzero adverse event rate, when the true adverse event rate is 5% with 90% power, and a type I error rate of alpha = 0.05. Allowing for a 10% non-evaluability/dropout rate requires 50 subjects for the safety comparisons. Both dose groups may be used to assess the Day 28 immunogenicity endpoint, so 50 subjects will be allocated to each dose group, yielding 100 total subjects for this study.

Immunogenicity

Neutralizing Type 2 Poliovirus Antibody Titers

At each post-vaccination time point where neutralizing antibody titers are obtained:

- Seroconversion rate and seroprotection rate with 95% CIs will be tabulated.
- Median $\log_2$ antibody titers with accompanying 95% confidence intervals (CIs) will be computed.
- GMT with accompanying 95% CIs will be computed.
- Plots of the reverse cumulative distribution of antibody titers will be generated.

Viral Shedding

- For each subject, a viral shedding index will be calculated as the average of $\log_{10}$-transformed values of viral concentration in stool samples as determined using quantitative PCR (viral identity) and $CCID_{50}$ (titer) from select stool samples taken following each vaccine dose, and this index will be summarized by group
- Descriptive analysis and plots of the reverse cumulative distribution of the viral shedding index will be generated.

Safety

Safety parameters will be tabulated and analyzed descriptively.

Adverse Events

Analyses described below will be performed for solicited and unsolicited AEs as well as for SAEs and severe AEs. The original terms used in the designated sections of the eCRFs by Investigators to identify AEs will be fully described and coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

All AEs will be summarized by group, occurrence in relation to vaccination, and overall.

Separate tables and listings will be created for those subjects who died, discontinued the study vaccine due to an AE, or experienced a severe or serious AE. Summaries, listings, and narratives may be provided, as appropriate.

Clinical Laboratory Tests

Each continuous biochemistry and hematology laboratory test will be evaluated by means of descriptive statistics (i.e., number of subjects, mean, SD, median, minimum, and maximum) on the actual values, at each assessment time point and by group. Changes from baseline will also be summarized by assessment time point and by group.

Clinical laboratory test values will be evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (toxicity grades) or in accordance with the normal ranges of the clinical laboratory (below, within, or above normal range) for parameters for which no toxicity grades are defined.

A listing of subjects with any clinical laboratory test result outside the reference ranges will be provided.

Vital Signs

Pulse rate, SBP, DBP and body temperature will be evaluated by means of descriptive statistics (actual values and changes from baseline) and frequency tabulations at each assessment time point and by group.

The percentage of subjects with values beyond clinically important limits will be summarized.

Physical Examination

Abnormal findings in physical examination will be listed.

Data Safety Monitoring Board:

A Data Safety Monitoring Board (DSMB) will monitor the benefit-risk and data integrity of this trial.

The composition and functioning of the DSMB is documented elsewhere.

TIME AND EVENTS SCHEDULE – GROUP 1

Assessments	**Group 1**				**Follow-up contact**
Visit	**1**	**2**	**3**	**4**	**5**
Time of Visit (days) **Visit Window**	**D 0**	**D 7 (+/- 2D)**	**D 14 (+/- 3D)**	**D 28[a] (+/- 2D)**	**D 42 (+/- 4D)**
Informed consent[b]	X				
In-/exclusion criteria	X				
Medical history/concomitant diseases	X				
Medication history[c]	X				
Demographic data	X				
Physical examination[d]	X[e]	X		X	
Vital signs[f]	X[e,f]	X		X	
Clinical laboratory tests[g]	X[e]	X		X	
Pregnancy test[h]	X				
Serology[i]	X				
Randomization	X				
Administration of vaccine[j]	X				
Serum sample for polio antibodies	X[e]			X	
Stool sample for viral culture/quantitative PCR[l], stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay	X----------X[l]		X		
Solicited systemic AEs (Diary)[k]	X----------X				
Remote contact for safety follow-up[m]	X----------X[m]				X
Concomitant therapies[n]	X	X	X	X	X
Adverse events[n]	X	X	X	X	X

a. In case of early termination the subjects will be asked to return to the clinical site within 14 days after discontinuation for assessments as outlined on Day 28.
b. No study-related assessment is to be carried out before signing of the informed consent form.
c. Including polio vaccination history.
d. Includes weight and height at Day 0. After Day 0, symptom-directed physical examination.
e. Prior to vaccination.
f. Blood pressure and heart rate (supine) and oral body temperature. On Day 0, vital signs will be assessed prior to and 30 min after vaccination.
g. For a list of assessments, please see Appendix 1: Overview of Laboratory assessments.
h. In women of childbearing potential, a urine pregnancy test will be performed.
i. Includes HBsAg, anti-HCV, and HIV antibodies.
j. The subjects will be kept under medical supervision for at least 30 min after vaccination.
k. Solicited AEs will be collected for Days 0-7.
l. Subjects will be asked to collect the first stool of every day in the provided recipient and bring the samples with them at the next scheduled visit. Daily collection on Days 0 to 10 plus collection on Days 14, 21 and V4 minus 1 or 2 days. Stool samples need to be stored by the subject at 4-8°C for maximum 7 days , before brought to the vaccine center. At Day 0 the storage procedure will be explained and the collection material provided.
m. Daily remote contact (text message by sms, WhatsApp or email) from Day 0 to Day 10 and also on Day 21and 42.

n. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity. The subjects can record unsolicited AEs on their diary card.

TIME AND EVENTS SCHEDULE – GROUP 2

Assessments	Group 2							Follow-up contact
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Time of Visit (days) **Visit Window**	**D 0**	**D 7 (+/- 2D)**	**D 14 (+/- 3D)**	**D 28 (+/- 2D)**	**D 35 (+/- 2D)**	**D 42 (+/- 3D)**	**D 56[a] (+/- 2D)**	**D 70 (+/- 4D)**
Informed consent[b]	X							
In-/exclusion criteria	X							
Medical history/concomitant diseases	X							
Medication history[c]	X							
Demographic data	X							
Physical examination[d]	X[e]	X		X[e]	X		X	
Vital signs[f]	X[e,f]	X		X[e]	X		X	
Clinical laboratory tests[g]	X[e]	X		X[e]	X		X	
Pregnancy test[h]	X			X[e]				
Serology[i]	X							
Randomization	X							
Administration of vaccine[j]	X			X				
Serum sample for polio antibodies	X[e]			X[e]			X	
Stool sample for viral culture/quantitative PCR[l], stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay	X----------X[l]		X	X[e]----------X[l]		X		
Solicited systemic AEs (Diary)[k]	X----------X			X----------X				
Remote contact for safety follow-up[m]	X----------X[m]			X----------X[m]				X
Concomitant therapies[n]	X	X	X	X	X	X	X	X
Adverse events[n]	X	X	X	X	X	X	X	X

a. In case of early termination the subjects will be asked to return to the clinical site within 14 days after discontinuation for assessments as outlined on Day 56.
b. No study-related assessment is to be carried out before signing of the informed consent form.
c. Including polio vaccination history.
d. Includes weight and height at Day 0. After Day 0, symptom-directed physical examination.
e. Prior to vaccination.
f. Blood pressure and heart rate (supine) and oral body temperature. On Day 0 and Day 28 (Group 2 only), vital signs will be assessed prior to and 30 min after vaccination.
g. For a list of assessments, please see Appendix 1: Overview of Laboratory assessments.
h. In women of childbearing potential, a urine pregnancy test will be performed.
i. Includes HBsAg, anti-HCV, and HIV antibodies.
j. The subjects will be kept under medical supervision for at least 30 min after vaccination.
k. Solicited AEs will be collected for Days 0-7 and Days 28-35.

p. Subjects will be asked to collect the first stool of every day in the provided recipient and bring the samples with them at the next scheduled visit. After each vaccine dose, daily collection on Days 0 to 10 plus collection on Days 14, 21 and V4 minus 1 or 2 days (=V7 minus 1 or 2 days after 2nd vaccination). Stool samples need to be stored by the subject at 4-8°C for maximum 7 days), before brought to the vaccine center. At Day 0 the storage procedure will be explained and the collection material provided.
q. Daily remote contact (text message by sms, WhatsApp or email) from Day 0 to Day 10 and also on Day 21 after the first vaccine dose. Thereafter, daily remote contact from Day 28 to Day 38 and also on Day 49 and Day 70.
r. Adverse events and intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity. The subjects can record unsolicited AEs on their diary card.

APPENDIX 4: SAMPLING OF HOUSEHOLD CONTACTS

This addendum is applicable to household contacts of long-term shedders in this phase 2 study with nOPV2 vaccine candidates. (Long-term shedders are defined as subjects in this phase 2 study with at least one PCR-positive stool sample after Day 42 following last study vaccination). A subject 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).

If type 2 virus shedding is detected by PCR on one of the last 3 scheduled stool samples, the study duration for this individual will be extended. As soon as these results are known (anticipated 3 weeks after the last such sample provided for evaluation) the subject will be asked to further collect 3 consecutive stool samples (with a maximum of one sample per day) after the last per-protocol sample, and to repeat this until shedding is PCR-negative for type 2 poliovirus on 3 consecutive stool samples.

As an additional risk assessment measure, household contact monitoring will be offered in case of extended shedding (PCR-positivity of at least one of the follow-up stool samples after D42 following last study vaccination).

Procedure:

In case of extended shedding subjects will be asked to contact their household members with the request that at least one of them volunteers to collect 3 consecutive stool samples and to repeat this until the long-term shedder reaches end of shedding (PCR negative on 3 consecutive stool samples).

Informed consent forms will be distributed by the long-term shedders to their household contacts, sampling material will be made available once the household contact has signed the ICF, and appointments will be made with the study team;

Households are defined as contacts of the original study participant living under the same roof for a minimum period of one day post vaccination and being older than 18 years of age.

If a stool sample of a household member is PCR-positive, risk mitigation measures, such as the use of enhanced hygiene techniques and/or chemical toilets will be offered, and the procedure may be expanded to other household contacts subject to their consent.

Viral shedding assessment:

Collected stools will be analyzed by methods similar to the clinical study UAM4. In brief, stool samples will be shipped in batches to the C.D.C. (U.S.) for analysis by PCR. The method employed allows the identification and discrimination of each candidate from the other and from Sabin OPV2. If the samples are determined to be positive for either candidate vaccine virus, the virus titer may be measured in cell culture as a 50%

infectious dose ($CCID_{50}$). For samples with sufficient titer (e.g. >4.00 log $CCID_{50}$ per gram of stool) or if otherwise assessed to be important based on public health considerations, sequencing may be conducted in order to examine the preservation of the attenuating features of the nOPV2 candidate, particularly the retention of the modified, attenuated domain V region. Assessment of the neurovirulence of culture-amplified shed virus using a transgenic mouse assay is not anticipated; however, such testing would be considered in the event of unexpected clinical or sequencing observations.