Clinical Study Protocol

A Phase 1, blinded, single center study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines, derived from a modified Sabin 2 infectious cDNA clone, in healthy adults previously primed with inactivated polio vaccine (IPV)

Product	- nOPV2 candidate 1 (S2/cre5/S15domV/rec1/hifi3) - nOPV2 candidate 2 (S2/S15domV/CpG40)
Protocol Number	UAM4a
EudraCT Number	2017-000908-21
Clinical Phase	I
Clinical Indication	Oral polio vaccine immunization
Issue Date (Version)	21-Mar-2017

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 1, blinded, single center study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines, derived from a modified Sabin 2 infectious cDNA clone, in healthy adults previously primed with inactivated polio vaccine (IPV).

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 1, blinded, single center study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines, derived from a modified Sabin 2 infectious cDNA clone, in healthy adults previously primed with inactivated polio vaccine (IPV).

Name:	Christopher Gast, Ph.D.
Affiliation:	Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center
Address:	1100 Fairview Ave N., Seattle, WA 98109

'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 Investigator

Title: A Phase 1, blinded, single center study to evaluate the safety and immunogenicity of two novel live attenuated serotype 2 oral poliovirus vaccines, derived from a modified Sabin 2 infectious cDNA clone, in healthy adults previously primed with inactivated polio vaccine (IPV).

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 – UAM4a			
Document	**Issue Date**	**Amendment Type**	**Comments**
Initial Clinical Study Protocol	21-Mar-2017	-	

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PROTOCOL SYNOPSIS

Study Title	**A Phase 1, blinded, single center study to evaluate the safety and immunogenicity attenuated serotype 2 oral poliovirus vaccines, derived from a modified Sabin 2 infectious cDNA clone, in healthy adults previously primed with inactivated polio vaccine (IPV).**		
Product	- nOPV2 candidate 1 (S2/cre5/S15domV/rec1/ hifi3) - nOPV2 candidate 2 (S2/S15domV/CpG40)	**Clinical Phase**	I
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Objectives:

The **primary objectives** of the study are

- to assess safety (serious adverse events [SAEs] and severe* adverse events [AEs]) of nOPV2 candidate 1 and nOPV2 candidate 2;
- to assess viral shedding following nOPV2 candidate 1 and nOPV2 candidate 2 administration in all stool samples.

Secondary objectives are to assess

- safety (any solicited and unsolicited AEs (including SAE and severe AE)), laboratory assessments) of nOPV2 candidate 1 and nOPV2 candidate 2;
- immunogenicity (seroprotection rate, seroconversion rate, median antibody titer (post-vaccination)) of nOPV2 candidate 1 and nOPV2 candidate 2;
- neurovirulence of shed virus (as measured in animal model(s)) in a subset of stool samples of all subjects.

* 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.

Exploratory objectives are

- to assess immunogenicity (geometric mean titer [GMT]) of nOPV2 candidate 1 and nOPV2 candidate 2;
- to assess the genetic stability, including but not limited to the modified regions of shed virus in a subset of stool samples of all subjects;
- to assess viral shedding following nOPV2 candidate 1 or nOPV2 candidate 2 administration in nasopharyngeal swabs of all subjects.

Overview of Study Design:

This will be a single center, blinded study in 30 healthy IPV-only vaccinated adults (age range 18 to 50 years) as follows:

- 15 subjects to receive 1 dose of nOPV2 candidate 1 (Group 1);
- 15 subjects to receive 1 dose of nOPV2 candidate 2 (Group 2).

Subjects who will pass screening but for any reason will drop out before vaccination will be defined as screen-failures and will be replaced.

15 subjects will be evaluated for the 1-dose regimen nOPV2 candidate 1 (Group 1).

15 subjects will be evaluated for the 1-dose regimen nOPV2 candidate 2 (Group 2).

Recruitment will occur as follows:

All subjects will have screening in Week 0, before their vaccination visit (Day 0), in order to collect a pre-vaccination stool sample and baseline lab. To avoid risk of transmission between subjects receiving different candidate oral polio vaccines, the study will be conducted with each candidate vaccine sequentially. After randomization of the first subject the next 14 subjects will be assigned to the same Group of nOPV2 candidate. In Week 1, vaccination will be limited to three subjects with a 48-hour time delay between first and second as well as second and third subject. Absence of any SAE or severe AE report will support continuation of recruitment. Any SAE or severe AE occurring up through 48 hours after vaccination of the last of these 3 subjects will be presented to the DSMB for its consideration. Further recruitment will be halted 24 hours until DSMB has given its advice. Once a positive opinion is given, recruitment of this Group can continue.

Enrollment of the Group with the other nOPV2 candidate will follow the same design and can start as soon as all subjects of the first Group have left the containment unit and the whole facility has been cleaned and decontaminated. Interval between subjects receiving candidate 1 (last subject out) and candidate 2 (first subject in) should be sufficient to avoid any cross-contamination.

The DSMB has established stopping rules for safety prior to study start, detailed in the DSMB charter, which will be continuously assessed.

The study will be conducted at 1 temporary contained facility of the Antwerp University, Belgium, in the proximity of the Antwerp University Hospital emergency department, Belgium. For the purpose of this study a temporary containment unit has been built to avoid environmental contamination with the nOPV2 candidates. During their stay subjects will have individual bedrooms and will share fitness and entertainment rooms, kitchen/dining room and lavatory facilities. After vaccination all subjects of the same Group will remain at this facility until shedding has been deemed insignificant on 3 consecutive samples for every subject of this Group but with a maximum of 28 days after enrollment of the last subject of this Group. If shedding continues on this time point, subjects will be asked to further collect stool samples daily (for testing), on an ambulatory way and will be reminded of the necessity of adherence to the in/exclusion criteria. In addition, an ambulatory stool collecting system will be provided to continue to collect all stools during the shedding period. The assessments performed are summarized per visit in the Time and Events Schedule.

After study completion all subjects will be offered the possibility to receive an additional vaccination of IPV, outside the study, on a voluntary basis.

Study Population:

Healthy adults previously vaccinated with IPV (age range 18 to 50 years)

Inclusion Criteria:

1. Healthy male or female, between 18 and 50 years old, extremes included, having received at least 3 doses of IPV in the past (more than 12 months before the start of the study);
2. In good physical and mental health as determined on the basis of medical history, laboratory screening tests and general physical and psychological examination;
3. 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 vaccine administration;
4. Willing to adhere to the prohibitions and restrictions specified in this protocol;
5. Willing to adhere to the restrictions of containment for duration as specified in the protocol;
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.

Furthermore, willing to adhere to following restrictions as long as shedding will be observed at the end of the containment period:

7. No intention to travel to the Netherlands and to polio endemic countries (updated list will be made available at the start of the study);
8. No professional handling of food, catering or food production activities;
9. Not having household or professional contact with known immunosuppressed people or people without full polio vaccination (i.e. complete primary infant immunization series), e.g. babysitting;
10. No neonatal nursing activities or other professional contact with children under 6 months old;

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. Ever having received any OPV in the past;
3. Having Crohn's disease or ulcerative colitis or having had major surgery of the gastrointestinal tract involving significant loss or resection of the bowel;
4. A known allergy, hypersensitivity, or intolerance to the study vaccine, or to any of its components or to any antibiotics;
5. Any confirmed or suspected immunosuppressive or immunodeficiency condition (including human immunodeficiency virus [HIV] infection, hepatitis B and C infections or negative for total serum IgA);
6. Chronic administration (i.e., longer than 14 days) of immunosuppressant drugs or other immune-modifying drugs within 6 months prior to the administration of study vaccine or planned use during the study. For instance, for corticosteroids, this means prednisone, or equivalent, $\geq$ 0.5 mg/kg/day (inhaled and topical steroids are allowed whereas intra-articular and epidural injection/administration of steroids are not allowed);
7. 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;
8. Indications of drug abuse or excessive use of alcohol at Day 0;
9. Being pregnant or breastfeeding. Women of childbearing potential will undergo a pregnancy test at Screening (serum) and at Day 0 (urine). Subjects with a positive pregnancy test will be excluded;
10. 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 administration of study vaccine, or planned use during the study period;
11. Administration of any vaccine other than the study vaccine within 28 days prior to the administration of study vaccine and during the entire study period;
12. Administration of polio vaccine within 12 months before the start of the study;
13. Having had a transfusion of any blood product or application of immunoglobulins within the 4 weeks prior to the administration of study vaccine or during the study;
14. 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:

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 ($CCID_{50}$).

The vaccines will be administered orally. One dose of vaccine (0.3 ml) is contained in six drops which are delivered from the dropper supplied with the vaccine.

Study/Treatment Duration:

Maximum study duration is expected to be 6 weeks after vaccine administration, including follow-up contact on D42.

Subjects will be asked to collect stool samples daily until shedding is PCR negative on 3 consecutive samples for each subject of the same Group, which then determines end of containment for this Group. However, maximum duration of containment will be 28 days after enrollment of last subject in the respective Group. If shedding continues longer, subjects will be asked to collect further (on a daily basis) stool samples ambulatory. In addition, an ambulatory stool collecting system will be provided to continue to collect all stools during the shedding period. A subject will be considered to have completed the study in the respective Group, if he or she has completed all study-related procedures 42 days after the administration of the study vaccine and shedding is PCR negative on 3 consecutive stool samples.

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.

Viral shedding:
- Viral shedding positivity rate (as determined using PCR) will be assessed at each stool sample collection time point
- Median 50% cell culture infective dose ($CCID_{50}$; titer) of shed virus after viral extraction from stool samples will be assessed at each stool sample collection time point that is positive for type-2 poliovirus via quantitative PCR
- Time-to-cessation of type-2 viral shedding will be assessed
- A combined index of the prevalence, quantity and duration of shedding, will be assessed using fixed stool sample collection time points

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 Groups 1 and 2;
- the incidence, type, causality and severity of unsolicited adverse events throughout the study period in both groups;
- the incidence, causality and description of deviations from normal safety labs at Day 0, Day 7 and Day 28 for Group 1;
- the incidence, causality and description of deviations from normal safety labs at Day 0, Day 7 and Day 28 for Group 2.

Immunogenicity:
- Median titers of type 2 polio antibodies at Days 0 and 28 in Group 1;
- Median titers of type 2 polio antibodies at Days 0 and 28 in Group 2;

- Seroprotection rate of type 2 polio antibodies at Days 0 and 28 in Group 1;
- Seroprotection rate of type 2 polio antibodies at Days 0 and 28 in Group 2;

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

- Seroconversion rate of type 2 polio antibodies at Day 28 for Group 1;
- Seroconversion rate of type 2 polio antibodies at Day 28 for 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.

Viral shedding:
- Neurovirulence of shed virus (as measured in animal model(s)) in a subset of stool samples of all subjects.

Exploratory
- GMT of type 2 polio antibodies at Days 0 and 28 in Group 1;
- GMT of type 2 polio antibodies at Days 0 and 28 in Group 2;
- assessment of the genetic stability, including but not limited to the modified regions of shed virus in a subset of stool samples of all volunteers;
- Assessment of viral shedding in nasopharyngeal swabs of all subjects;

Statistical Methods:

Sample size

The sample size chosen for this study is not selected to satisfy any specific statistical criteria; rather, 15 subjects per group is considered reasonable and sufficient for a first-in-human contained study of investigational vaccines as agreed with WHO to gain a preliminary assessment of safety, shedding and characteristics of shed virus, in advance of a larger Phase I study intended to be conducted without containment measures upon successful completion of this study. No hypotheses will be tested in this study and all analyses will be descriptive in nature.

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 positivity will be determined at each daily stool collection, and the rate of positivity (percent positive of those samples provided) will be computed. Additionally, the $\log_{10}$ $CCID_{50}$ of shed virus among those positive for shedding via quantitative PCR will be obtained and summarized. Also, 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 a fixed set of stool samples taken from each subject following each vaccine dose.

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 current Medical Dictionary for Regulatory Activities (MedDRA).

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

Separate listings will be created for those subjects who died or experienced a severe or serious AE. Additional 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 overall.

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, including causality, 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 of abnormalities) at each assessment time point and by group, and overall.

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.

TIME AND EVENTS SCHEDULE-GROUPS 1 AND 2

Assessments						**Follow-up contact**
Visit	**Screening**	**1**	**2**	**3**	**4**	**5**
Time of Visit (days) **Visit window**	**Pre-D0[p]**	**D0**	**D7** **(+/- 2D)**	**D14[o]** **(+/- 3D)**	**D28[a,o]** **(+/- 2D)**	**D 42[o]** **(+/- 4D)**
Informed consent[b]	X					
In-/exclusion criteria	X	X				
Medical history/concomitant diseases	X					
Medication history[c]	X					
Demographic data	X					
Physical examination[d]	X	X[e]	X		X	
Psychological examination	X					
Vital signs[f]	X	X[e,f]	X		X	
Clinical laboratory tests[g]	X		X	X	X	
Pregnancy test[h]	X	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, stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay[l]	X[n]	X----------X[l]				
Nasopharyngeal swab		X----------X[q]				
Solicited systemic AEs (Diary)[k]		X----------X				
Concomitant therapies[m]		X	X	X	X	X
Adverse events[m]		X	X	X	X	X

a. In case of early termination, assessments will be done 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 Screening. After Screening, symptom-directed physical examination. At the end of containment an additional physical examination, including vital signs and check of adverse events, will be offered.
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 pregnancy test will be performed, at Screening on serum and at D0 on urine.
i. Includes HBsAg, anti-HCV, HIV antibodies, total serum IgA and HCG.
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: daily collection until end of shedding (confirmation of 3 consecutive negative samples as defined above) of all subjects of the same Group. Stool samples will be stored frozen at the contained facility. The collection material will be provided on a daily basis.
m. 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.
n. At Screening subjects will be asked to collect a pre-vaccination stool sample within 2 days prior to V1.
o. D14, D28 and D42 can be ambulatory in case containment isn't required anymore on these time points.
p. Screening evaluations may be completed up to 14 days before D0.
q. Nasopharyngeal swabs will be taken on D0, D3, D7 and last day of containment.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

List of Abbreviations

AE	Adverse event
bpm	Beats per minute
$CCID_{50}$	50% cell culture infective dose
CI	Confidence interval
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
GMT	Geometric mean titer
GPEI	Global Polio Eradication Initiative
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
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
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
PCR	Polymerase Chain Reaction
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
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

SPONSOR	University of Antwerp
SPONSOR REPRESENTATIVE:	
Principal investigator:	Pierre Van Damme, MD, PhD University of Antwerp Centre for the Evaluation of Vaccination Vaccine & Infectious Disease Institute Campus Drie Eiken Universiteitsplein 1 2610 Antwerpen (Wilrijk) - Belgium
Clinical Project Manager:	Leen Suykens University of Antwerp Centre for the Evaluation of Vaccination Vaccine & Infectious Disease Institute
Investigator:	Ilse De Coster, MD University of Antwerp Centre for the Evaluation of Vaccination Vaccine & Infectious Disease Institute
Clinical Center	Centre for the Evaluation of Vaccination (CEV), Vaccine &Infectious Disease Institute, University of Antwerp Campus Drie Eiken, Universiteitsplein1, 2610 Antwerpen (Wilrijk), Belgium
MONITORING	Assign Clinical Research GmbH Hainburger Strasse 33, 1030 Vienna
Project Manager:	Lenka Brandejsová
BIOSTATISTICS	Vaccine and Infectious Disease Division, Fred Hutchison Cancer Research Center 1100 Fairview Ave N., Seattle, WA 98109
Biostatistician:	Christopher Gast, Ph.D.

VIRAL ANALYSES	Centers for Disease Control and Prevention Polio and Picornavirus Laboratory Branch 1600 Clifton Road, NE MS-G17, Room 6053 Atlanta, GA 30333 USA Contact: William Weldon, Ph.D. Team Lead, Population Immunity Laboratory Ph – 404-639-5485
	Viroclinics Biosciences B.V. Rotterdam Science Tower Marconistraat 16 3029 AK Rotterdam The Netherlands
CLINICAL LAB	AML, Emiel Vloorsstraat 9, 2020 Antwerpen
MANUFACTURING	Bio Farma JL.Pasteur No.28, Pasteur, Sukajadi, Kota Bandung, Jawa Barat 40161, Indonesia
	Intravacc Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands
	ABF Pharmaceutical Services GmbH Gastgebgasse 5-13 1230Vienna, Austria Phone +43 1 890 12 00 Fax +43 1 890 12 00 – 11
	CSM Europe sa Watson &Crick Hill Rue Granbonpré 11 1435 Mont-Saint-Guibert, Belgium
PHARMACOVIGILANCE	Assign Data Management and Biostatistics GmbH, Stadlweg 23, 6020 Innsbruck

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 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 where wild-type poliovirus transmission still occurs - Nigeria, Afghanistan and Pakistan. In 2016, 37 cases of paralytic poliomyelitis due to wild poliovirus were reported globally, compared to 74 in 2015. All the cases were caused by wild poliovirus type 1.

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. However, in many developing countries, a reduced immune response to polioviruses type 1 and 3 has been observed with tOPV. The bivalent oral polio vaccine (bOPV), which does not contain type 2, is more effective against the two remaining wild poliovirus types. It has been documented that immune-mediated responses tend to increase in proportion to the relative valency of the vaccine with bivalent vaccines offering protection equivalent or non-inferior to monovalent preparations. 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, 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 type 2 viruses. 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, in improved response to any future use of a monovalent type 2 polio vaccine in the case of an outbreak, in a reduction of

transmission of a reintroduced type 2 virus; and in 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.

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 SAGE recommendations. Following this OPV2 cessation, stockpiles of mOPV2 are being maintained for 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.

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.
This first-in-human (FIH) phase 1 study is designed to evaluate in contained conditions the safety, shedding and genetic stability of both nOPV2 vaccines in IPV-primed adults before testing in a larger adult and adolescent (> 16 y of age) population, and then in young children and infants. The primary objectives of the subsequent Phase 1 and 2 studies will include the general safety, the shedding and genetic stability of the two candidate vaccines, primarily based on comparison with historical data obtained in the Phase 4 studies of Sabin mOPV2 noted above.
The novel vaccine is expected to be eventually be licensed based on 3 criteria: non-inferior safety to the currently licensed mOPV2 of the Sabin strain, non-inferior immunity, less reversion to virulence and less shedding in stool. As all comparisons are to be made to the licensed vaccine, a placebo arm was not included in the Phase IV studies or in this study as it does not add value for licensure.

This Phase 1 study will include IPV-only vaccinated adults to be vaccinated with the study vaccines and followed in contained conditions to obtain safety, shedding and genetic stability data relevant to the decision to advance to future studies with testing in un-

contained conditions in adult (OPV-primed), adolescent (IPV-primed) and children who have not been exposed to OPV2. The study is designed to first obtain safety data from an adult population which is IPV primed, without prior exposure to OPV2, before moving into larger groups of OPV-primed as well as IPV-primed individuals.

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 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).

Because the study will be conducted after global withdrawal of Sabin mOPV2, this FIH study will take place in containment to avoid environmental contamination with the nOPV2 candidates.

During their stay subjects will have individual bedrooms and will share fitness and entertainment rooms, kitchen and dining room and lavatory facilities. All waste water and stools will be collected in tanks and be decontaminated in a standardized way before further processing in ordinary sewage.
To avoid risk of transmission between subjects receiving different candidate vaccines, the study will be conducted with each candidate vaccine sequentially.
After vaccination of the first Group all subjects will remain at the facility until shedding has been deemed insignificant on 3 consecutive samples for every subject of this Group, but with a maximum of 28 days after enrollment of the last subject of this Group. If shedding continues on this time point, subjects will be asked to further collect stool samples daily (for testing), on an ambulatory way and will be reminded of the necessity of adherence to the in/exclusion criteria. In addition, an ambulatory stool collecting system will be provided to continue to collect all stools during the shedding period. A mobile chemical toilet will be provided which enables the subjects to collect all stools in a disposable box. This box will be discarded in a container for hazardous medical waste which can be collected and destroyed by the appropriate waste company. Enrollment of the Group with the second nOPV2 candidate will follow the same design and can start as soon as all subjects of the first Group have left the containment unit and all facilities have been cleaned and decontaminated. The contained unit will be decontaminated after use by each cohort of volunteers by application of Chlorine Dioxide Gas, according to standardized procedures. Biological indicators (consisting of spores) will be used to validate the process (Appendix 4).

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 IPV in the past;
- They are willing to adhere to the restrictions of containment for duration as specified in the protocol;
- 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 are willing to adhere to the following restrictions as long as shedding will be observed at the end of containment:
    - No intention to travel to the Netherlands and to polio endemic countries;
    - No professional handling of food, catering or food production activities;
    - No household or professional contact with known immunosuppressed people or people without full polio vaccination (i.e. complete primary infant immunization series), e.g. babysitting;
    - No neonatal nursing activities or other professional contact with children under 6 months old.

To minimize the risk of potential transmission to study staff, study staff will be checked for history of OPV or IPV vaccination (in collaboration with the occupational health department of the Antwerp University) and will be trained on protective measures by the Infectious Diseases Department of the Antwerp University Hospital (e.g. one layer

clothing, gloves and shoes (heightened personal protective equipment)) to be taken each time they will enter or leave the containment unit. The same measures will apply to all additional "temporary" personnel that will enter the facility. Study staff will be offered a booster IPV vaccination at least one month before the start of the study and will be checked for not having been vaccinated with OPV during the last 4 weeks before study start, by the occupational health department. The same measures will apply to all staff that will enter the facility and has close contact with the subjects (e.g. concierge, cleaning personnel).

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. 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.

1.3.2 *Potential Benefits*

Subjects who previously completed childhood polio vaccination schedule and received a dose of nOPV2 in this study may have a boost in immunity for poliovirus type 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

2.1 PRIMARY OBJECTIVE

Primary objectives of the study are to assess:

- safety (serious adverse events [SAEs] and severe[†] adverse events [AEs]) of nOPV2 candidate 1 and nOPV2 candidate 2;
- viral shedding following administration of nOPV2 candidate 1 and nOPV2 candidate 2 in all stool samples.

2.2 SECONDARY OBJECTIVES

Secondary objectives are to assess:

- safety (any solicited and unsolicited AEs, laboratory assessments) of nOPV2 candidate 1 and nOPV2 candidate 2;
- immunogenicity (seroprotection rate, seroconversion rate, median antibody titer (post-vaccination)) of nOPV2 candidate 1 and nOPV2 candidate 2;
- neurovirulence of shed virus (as measured in animal model(s)) in a subset of stool samples of all subjects.

2.3 EXPLORATORY OBJECTIVES

Exploratory objectives are to assess

- immunogenicity (geometric mean titer [GMT]) of nOPV2 candidate 1 and nOPV2 candidate 2;
- genetic stability, including but not limited to the modified regions of shed virus in a subset of stool samples of all subjects;
- assessment of viral shedding following nOPV2 candidate 1 or nOPV2 candidate 2 administration in nasopharyngeal swabs of all subjects.

[†] 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.

3. STUDY ENDPOINTS

3.1 PRIMARY ENDPOINTS

The following endpoints will be evaluated by group and overall:

Safety:

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

Viral shedding:

- Viral shedding positivity rate (as determined using PCR) will be assessed at each stool sample collection time point
- Median 50% cell culture infective dose ($CCID_{50}$; titer) of shed virus after viral extraction from stool samples will be assessed at each stool sample collection time point that is positive for type 2 poliovirus via quantitative PCR
- Time-to-cessation of type-2 viral shedding will be assessed
- A combined index of the prevalence, quantity and duration of shedding, will be assessed using fixed stool sample collection time points from each subject.

3.2 SECONDARY ENDPOINTS

The following safety and immunogenicity endpoints will be evaluated by group and overall.

Safety:

- Incidence, type, causality and severity of solicited AEs for Days 0-7 in Groups 1 and 2;
- Incidence, type, causality and severity of unsolicited AEs throughout the study period in both groups;
- Incidence, causality and description of deviations from normal safety labs at Day 0, Day 7, and Day 28 for Group 1;
- Incidence, causality and description of deviations from normal safety labs at Day 0, Day 7, and Day 28 for Group 2;

Immunogenicity:

- Median titers of type 2 polio antibodies at Days 0 and 28 in Group 1;

- Median titers of type 2 polio antibodies at Days 0 and 28 in Group 2;

- Seroprotection rate of type 2 polio antibodies at Days 0 and 28 in Group 1;
- Seroprotection rate of type 2 polio antibodies at Days 0 and 28 in Group 2;

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

- Seroconversion rate of type 2 polio antibodies at Day 28 for Group 1;
- Seroconversion rate of type 2 polio antibodies at Day 28 for 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.

Viral shedding:

- Neurovirulence of shed virus (as measured in animal model(s)) in a subset of stool samples of all subjects.

3.3 EXPLORATORY ENDPOINTS:

- GMT of type 2 polio antibodies at Days 0 and 28 in Group 1;
- GMT of type 2 polio antibodies at Days 0 and 28 in Group 2;
- Assessment of the genetic stability, including but not limited to the modified regions of shed virus in a subset of stool samples of all volunteers.
- Assessment of nasopharyngeal viral shedding in swabs of all subjects.

4. STUDY DESIGN

4.1 OVERVIEW OF STUDY DESIGN

This will be a single center, blinded study in 30 healthy IPV-only vaccinated adults (age range 18 to 50 years), as follows:

- 15 subjects to receive 1 dose of nOPV2 candidate 1 (Group 1);
- 15 subjects to receive 1 dose of nOPV2 candidate 2 (Group 2).

Subjects who will pass screening but for any reason will drop out before vaccination will be defined as screen-failures and will be replaced.

15 subjects will be evaluated for the 1-dose regimen nOPV2 candidate 1 (Group 1).

15 subjects will be evaluated for the 1-dose regimen nOPV2 candidate 2 (Group 2).

Recruitment will occur as follows:

All subjects will have screening in Week 0, before their vaccination visit (Day 0), in order to collect a pre-vaccination stool sample and baseline lab. To avoid risk of transmission between subjects receiving different candidate vaccines, the study will be conducted with each candidate vaccine sequentially. After randomization of the first subject the next 14 subjects will be assigned to the same Group of nOPV2 candidate. In Week 1, vaccination will be limited to three subjects with a 48-hour time delay between first and second as well as second and third subject. Absence of any SAE or severe AE report will support continuation of recruitment. Any SAE or severe AE occurring up through 48 hours after vaccination of the last of these 3 subjects will be presented to the DSMB for its consideration. Further recruitment will be halted 24 hours until the DSMB has given its advice. Once a positive opinion is given, recruitment of this Group can continue.

Enrollment of the Group with the other nOPV2 candidate will follow the same design and can start as soon as all subjects of the first Group have left the containment unit and all facilities have been cleaned and decontaminated. Interval between subjects receiving candidate 1 (last subject out) and candidate 2 (first subject in) should be sufficient to avoid any cross-contamination.

The DSMB has established stopping rules for safety prior to study start, detailed in the DSMB charter, which will be continuously assessed.

The study will be conducted at 1 temporary contained facility of the Antwerp University, Belgium, in the proximity of the Antwerp University Hospital emergency department, Belgium. For the purpose of this study a temporary containment unit has been built to avoid environmental contamination with the nOPV2 candidates. During their stay subjects will have individual bedrooms and will share fitness and entertainment rooms, kitchen and dining room and lavatory facilities. After vaccination all subjects of the same Group will remain at this facility until shedding has been deemed insignificant on 3 consecutive samples for every subject of this Group but with a maximum of 28 days after enrollment of last subject of this Group. If shedding continues on this time point, subjects will be asked to further collect stool samples daily (for testing), on an ambulatory way and will be reminded of the necessity of adherence to the in/exclusion criteria. In

addition, an ambulatory stool collecting system will be provided to continue to collect all stools during the shedding period.

After study completion all subjects will be offered the possibility to receive an additional vaccination of IPV, outside the study, on a voluntary basis.

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

5. SELECTION OF STUDY POPULATION

5.1 INCLUSION CRITERIA

1. Healthy male or female, between 18 and 50 years old, extremes included, having received at least 3 doses of IPV in the past (more than 12 months before the start of the study);
2. In good physical and mental health as determined on the basis of medical history, laboratory screening tests and general physical and psychological examination;
3. 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 vaccine administration;
4. Willing to adhere to the prohibitions and restrictions specified in this protocol;
5. Willing to adhere to the restrictions of containment for duration as specified in the protocol;
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.

Furthermore, willing to adhere to following restrictions as long as shedding will be observed at the end of the containment period:

7. No intention to travel to the Netherlands and to polio endemic countries (updated list will be made available at the start of the study);
8. No professional handling of food, catering or food production activities;
9. Not having household or professional contact with known immunosuppressed people or people without full polio vaccination (i.e. complete primary infant immunization series), e.g. babysitting;
10. No neonatal nursing activities or other professional contact with children under 6 months old;

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. Ever having received any OPV in the past;
3. Having Crohn's disease or ulcerative colitis or having had major surgery of the gastrointestinal tract involving significant loss or resection of the bowel;
4. A known allergy, hypersensitivity, or intolerance to the study vaccine, or to any of its components or to any antibiotics;
5. Any confirmed or suspected immunosuppressive or immunodeficiency condition (including human immunodeficiency virus [HIV] infection, hepatitis B and hepatitis C infection or negative for total serum IgA);
6. Chronic administration (i.e., longer than 14 days) of immunosuppressant drugs or other immune-modifying drugs within 6 months prior to the administration of study

vaccine 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);

7. 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;
8. Indications of drug abuse or excessive use of alcohol at Day 0;
9. Being pregnant or breastfeeding. Women of childbearing potential will undergo a pregnancy test at Screening (serum) and Day 0 (urine). Subjects with a positive pregnancy test will be excluded;
10. 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 administration of study vaccine, or planned use during the study period;
11. Administration of any vaccine other than the study vaccine within 28 days prior to the administration of study vaccine and during the entire study period;
12. Administration of polio vaccine within 12 months before the start of the study;
13. Having had a transfusion of any blood product or application of immunoglobulins within the 4 weeks prior to the administration of study vaccine or during the study;
14. 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 or an employee or the Investigator.

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 could significantly affect the subject's immune status.
- administration of any vaccine other than the study vaccine within 28 days of receipt of study vaccine and during the entire study period. Subjects receiving any other vaccine prior to the day 28 visit will be removed from the per-protocol population.

These subjects will be included in the Total Vaccinated population (see section 10.1) and will continue in the study for safety follow-up.

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.

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 administration of the study vaccine.

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 site in vials filled in 1.1 ml aliquots, sufficient for 3 doses per vial, and presented as an aqueous 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 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

Not applicable.

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.

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 subjects will receive one of the nOPV2 candidates in a blinded manner.

To avoid risk of transmission between subjects receiving different candidate vaccines, the study will be conducted with each candidate vaccine sequentially. After randomization of the first subject of Group 1 the next 14 subjects will all be enrolled in the same Group and receive the same nOPV2 candidate and the next 15 subjects will be enrolled in the other Group and receive the corresponding nOPV2 candidate. Prior to the start of the study Assign will provide the site with 2 randomization envelopes for the first subject. By randomly choosing 1 of the envelopes first subject will be dedicated to a certain nOPV2 candidate and this will determine the allocation of the next 14 subjects to the same Group.

Study staff will be blinded in the same way and will be blinded for individual shedding results of the participants of a Group until end of containment of this Group. At that time point study staff will be notified by CDC of any remaining shedding to be able to provide these subjects with adequate instructions to take home.

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.

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 administration of the study vaccine, 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.

Subjects will be informed about the importance of the use of contraception during the study and condoms and counseling will be readily available to the participants during the period of containment.

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.

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, 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 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 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, with consent from the participant.

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 2

Assessments						**Follow-up contact**
Visit	**Screening**	**1**	**2**	**3**	**4**	**5**
Time of Visit (days) **Visit window**	**Pre-D0[p]**	**D0**	**D7** **(+/- 2D)**	**D14[o]** **(+/- 3D)**	**D28[a,o]** **(+/- 2D)**	**D 42[o]** **(+/- 4D)**
Informed consent[b]	X					
In-/exclusion criteria	X	X				
Medical history/concomitant diseases	X					
Medication history[c]	X					
Demographic data	X					
Physical examination[d]	X	X[e]	X		X	
Psychological examination	X					
Vital signs[f]	X	X[e,f]	X		X	
Clinical laboratory tests[g]	X		X	X	X	
Pregnancy test[h]	X	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, stool sample for potential poliovirus sequencing, stool sample for potential neurovirulence assay[l]	X[n]	X----------X[l]				
Nasopharyngeal swab		X----------X[q]				
Solicited systemic AEs (Diary)[k]		X----------X				
Concomitant therapies[m]		X	X	X	X	X
Adverse events[m]		X	X	X	X	X

a. In case of early termination, assessments will be done 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 Screening. After Screening, symptom-directed physical examination. At the end of containment an additional physical examination, including vital signs and check of adverse events, will be offered.
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 pregnancy test will be performed, at Screening on serum and at D0 on urine.
i. Includes HBsAg, anti-HCV, HIV antibodies, total serum IgA and HCG.
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: daily collection until end of shedding (confirmation of 3 consecutive negative samples as defined above) of all subjects of the same Group. Stool samples will be stored frozen at the contained facility. The collection material will be provided on a daily basis.
m. 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.
n. At Screening subjects will be asked to collect a pre-vaccination stool sample within 2 days prior to V1.
o. D14, D28 and D42 can be ambulatory in case containment isn't required anymore on these time points.
p. Screening evaluations may be completed up to 14 days before Day 0.
q. Nasopharyngeal swabs will be taken on D0, D3, D7 and last day of containment.

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).

Blinded shedding results will be provided to the site in a timely manner in order to inform subjects about the need for further stool sampling and remaining in containment conditions.

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

Phenotypic neurovirulence of shed virus on a subset of stool samples (last sample with sufficient virus to be submitted to the assay) from each subject will be evaluated using a modified version of the WHO transgenic mice assay (TgPVR mice) (Appendix 3).

Testing of additional stool samples for phenotypic neurovirulence may be conducted on an exploratory basis.

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 at Day 0 and Day 28 post-vaccination.

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 vaccine dose administration 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 screening sample. In female subjects of childbearing potential, also β-human chorionic gonadotropin (hCG) will be tested at Screening on serum and at D0 on urine in each Group.

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 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 *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.

8.4.2 *Nasopharyngeal shedding and contamination*

Nasopharyngeal swabs will be taken at the time points outlined in the Time and Events schedule.

Shedding of polio virus type 2 will be evaluated on these swabs by using PCR.

Detailed descriptions of the collection, handling and processing of the nasopharyngeal swabs will be included in the laboratory manual.

8.4.3 *Further exploratory work*.

Serum or stool samples that remain after protocol-specific assessments have been performed may be used for further 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.

9. STUDY TERMINATION/COMPLETION

9.1 STUDY COMPLETION

A subject will be considered to have completed the study in the respective Group if he or she has completed all study related procedures 42 days after administration of the study vaccine and 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. To lower the likelihood of drop out during the study, a sound screening of the participating volunteers will be needed where a lot of attention shall be paid on this issue, because of the contained dimension of this study.

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

- a severe AE or SAE;
- 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. In any case the subject will be informed about the measures to be followed at home to minimize the risk of vaccine virus transmission.

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 any case the subject will be informed about the measures to be followed at home to minimize the risk of vaccine virus transmission.

In case of an AE, the appropriate follow-up will be done.

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

10. STATISTICAL METHODS

10.1 STATISTICAL ANALYSIS

All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalized before first subject in (FSI).

The following populations will be considered for analysis:

- Intention-to Treat (ITT) population, defined as all subjects who successfully complete the screening visit and are invited to participate in the study.
- Total Vaccinated population (TVP), defined as all subjects who are in the ITT population and who received 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 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 TVP will be used for primary safety analysis and the PP population for immunogenicity analysis; all immunogenicity analyses (primary and secondary) will be repeated in the TVP.

10.1.1 *Initial Characteristics Data of the Subject Sample*

Descriptive statistics will be provided per group and overall 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 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.

10.1.2 *Immunogenicity Data*

For an overview of primary, secondary and exploratory endpoints, see Section 3. All immunogenicity results will be descriptive in nature; no statistical hypotheses will be tested.

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

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

Viral Shedding

For each group and time point, viral shedding positivity and concentration (among positive samples) 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 the 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, 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.

The $CCID_{50}$ will be log_{10} transformed and described with summary statistics along with bootstrap 95% confidence intervals for the median.

Time-to-shedding cessation will be summarized using quantiles and corresponding 95% confidence intervals.

Neurovirulence of the shed virus will be assessed. See Appendix 3 for a description of modifications to the WHO TgMNVT neurovirulence assay.

10.1.3 *Safety Data*

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

Safety parameters will be tabulated and analyzed descriptively, using continuous or categorical summaries, as appropriate.

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). 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. Additional 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, including causality, 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.

10.1.4 *Exploratory*

Descriptive analysis of viral sequence heterogeneity will be conducted.

10.1.5 *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. For example, interpolation may be used in the estimation of viral shedding area-under-the-curve if necessitated by missing stool samples. Details will be provided in the Statistical Analysis Plan. 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

The sample size chosen for this study is not selected to satisfy any specific statistical criteria; rather, 15 subjects per group is considered reasonable and sufficient for a first-in-human contained study of investigational vaccines to gain a preliminary assessment of safety, shedding and characteristics of shed virus, in advance of a larger Phase 1 study to be conducted (without containment measures) upon successful completion of this study. No hypotheses will be tested in this study and all analyses will be descriptive in nature.

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

A 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 depency 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°C

Grade 2: 38,1°C to 39°C

Grade 3: >39°C

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, who agree to abide by the containment requirement, 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 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 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 Clinical Research GmbH.

During the period of containment of the subjects remote monitoring will be performed to avoid exposure of the monitors. For this remote monitoring source documents (only identifiable by subject number) will be scanned by the study staff. After each cohort has left the facility and the facility has been decontaminated, monitors can enter the site for monitor completion and source verification.

The monitor will record the dates of the remote monitoring visits in a study site visit log that will be kept at the other clinical site of the University. The first post-initiation monitoring 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 scanned 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.

At the end of containment and after decontamination of the site direct access to source documentation (medical records) must be allowed 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 the 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.

14. BIBLIOGRAPHY

1. Global Polio Eradication Initiative website: http://www.polioeradication.org/.Accessed 24 January 2017.
2. Sutter RW, John TJ, Jain H et al. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. The Lancet. 2010; 376:1682–1688.
3. Aylward B, Tangermann R. The global polio eradication initiative: lessons learned and prospects for success. Vaccine.2011; 29 (Suppl.4): D80-85.
4. Platt LR, Estívariz CF, Sutter RW. Vaccine-associated paralytic poliomyelitis: a review of the epidemiology and estimation of the global burden. J. Infect. Dis. 2014;210(Suppl. 1): S380–S389.
5. Bandyopadhyay AS, Garon J, Seib K, Orenstein WA. Polio vaccination: past, present and future. Future Microbiol.2015;10:791-808
6. SAGE Polio Working Group Report. Proposed policy for a switch from tOPV to bOPV globally. April 10, 2012.
7. WHO, Meeting of the Strategic Advisory Group of Experts on immunization, November 2013 – conclusions and recommendations.
8. WHO, Meeting of the Strategic Advisory Group of Experts on immunization, April 2015 – conclusions and recommendations.
9. Dragunsky E. et al., Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study. Bulletin of the World Health Organization 2003, 81 (4).
10. Macadam AJ, Ferguson G, Stone DM et al., Rational design of genetically stable, live attenuated poliovirus vaccines of all three serotypes: relevance to poliomyelitis eradication. J. Virol. 80(17), 8653–8663 (2006).
11. Laurin AS, Jones JO, Andino R. Rationalizing the development of live attenuated virus vaccines. Nat. Biotechnol. 28(6), 573–579 (2010).
12. Burns CC, Shaw J, Campagnoli R et al. Modulation of poliovirus replicative fitness in HeLa cells by deoptimization of synonymous codon usage in the capsid region. J. Virol.80(7), 3259–3272 (2006).

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		
HCG	Lymphocytes	Creatinine
	Monocytes	Calcium
	Neutrophils	Phosphate, inorganic
	Eosinophils	Potassium
	Basophils	Sodium
	Platelets	Alanine amino transferase
		Aspartate amino transferase
		C-reactive protein
Other Assessments	**Coagulation**	
Urine pregnancy test	Prothrombin time	
	Activated partial thromboplastin time	
	Fibrinogen	

[a] Screening 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: MODIFIED WHO TGPVR21 TRANSGENIC MICE NEUROVIRULENCE ASSAY

There currently exists no generally accepted method for assessing the neurovirulence of poliovirus strains excreted in stool. For the purpose of assessing the degree of neurovirulence of shed virus from this study, this approach modifies the existing WHO neurovirulence assay to satisfy this requirement, making modifications wherever necessary and keeping most of the original assay's definitions and methods in place. The document which will serve as the basis for the modified version is The WHO SOP for Neurovirulence Testing of Oral Polio Vaccine Using TgPVR21 Transgenic Mice, Version 6 (2012).

This transgenic mouse test for all three poliovirus serotypes is part of the WHO recommendations for the production and control of OPV. It is based on clinical observations of transgenic mice carrying the human poliovirus receptor (TgPVR21 mice) which are inoculated intra-spinally with either a test vaccine or a standardized WHO reference vaccine at varying concentrations. The mice are scored according to precise definitions, as "normal" or "paralyzed", and the dose level is related to the frequency (probability) of paralysis through a statistical model.

The WHO assay requires two doses (expressed in units of $\log_{10}$ $CCID_{50}$/5µl) for each serotype, which are produced according to a recommended method in the document *WHO Recommendations to Assure the Quality, Safety, and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral).* Doses are required to be produced within a precision of ±0.3 $\log_{10}$ $CCID_{50}$/5µl or better (95% confidence limits on the mean).

The WHO assay requires a specific strain of mice, and a specific training program to be followed to qualify a laboratory to conduct the test. The assay also describes methods to randomize both male and female mice to cages and dose levels.

Statistical analysis stems from fitting a logistic regression model to the binary paralyzed/not paralyzed data. Vaccine batches/lots are passed or failed according to specific criteria on the log odds ratio. The relative neurovirulence of the test vaccine vs. the WHO reference must lie within specified boundaries in order for the vaccine to "pass". These limits, established from historical data, are established such that a test vaccine which is equivalent to the reference will have a probability of 0.95 of passing, and a probability of 0.01 of failing.

The WHO assay will be modified to provide a characterization of the neurovirulence of the excreted strain obtained from stool samples on a given day. The viral population will be expanded from the clinical sample via cell culture with a fixed number of passages. One or more dose levels of the shed virus will be administered to TgPVR21 mice,

alongside the WHO reference material (the latter to be used as a quality-control measure, as opposed to a direct comparator). Each subject from the study would then provide data that describes the probability of paralysis at the dose level(s) tested. In practice a single estimate (or group of *estimates*, in the event of multiple dose levels) will be estimated from the combined data via a statistical model that accounts for sampling variation and between-subject variability. Key endpoints may then be extracted from this model, such as the probability of paralysis at a given dose level, and/or the dose level at which 50% paralysis is expected to occur. Full method details will be described in the statistical analysis plan.

For more details on the WHO SOP Neurovirulence Testing of Oral Polio Vaccine Using TgPVR21 Transgenic Mice, Version 6 (2012) is available at:
http://www.who.int/biologicals/vaccines/TgmNVT_SOPv6_Final_09112012.pdf

APPENDIX 4: THE BENEFITS OF CHLORINE DIOXIDE GAS

decon logic	UZA Preliminar technical information on CD decontamination	Date: 24/01/2017 Reference: 009001001

The benefits of Chlorine Dioxide gas

decon logic	UZA Preliminar technical information on CD decontamination	Date: 24/01/2017 Reference: 009001001

1. Distribution and efficacy

Gaseous methods (chlorine dioxide, ethylene oxide, and formaldehyde) are the most effective for decontamination due to their natural diffusion within the space being decontaminated. Gasses will reach all surfaces including inside cracks and crevices to ensure complete decontamination throughout the facility. Chlorine dioxide gas has the added benefit of being smaller than all microorganisms with a molecule size of 0.124 nm ($1.24x10^{-7}$ mm) so no organism can hide from CD gas. No organism can survive either, as CD gas is a sterilant capable of eliminating all viruses, bacteria, fungi, and spores. Additionally, effectiveness has been shown against beta lactams, pinworm eggs, and more. There is no post-decon residue either, so all equipment (including sensitive electronics) can be left inside the facility during a decontamination. Chlorine dioxide gas can be accurately measured in real time from multiple points within the area being decontaminated, guaranteeing that the correct dosage needed for an effective decontamination has been met before the decontamination is deemed complete and aeration is started.

2. Flexibility, Repeatability and Cycle Development

The process flexibility and repeatability of CD gas allows for simple setup of a decontamination as very little affects the cycle parameters used. With the integrated (and highly accurate) concentration monitor controlling the cycle, the proper dosage is able to be met each and every time. CD gas injection rates are constant and not variable depending on room sizes or shapes. There are no special studies, such as airflow or temperature mapping that need to be performed on areas being decontaminated for the first time. Initial relative humidity levels within the space do not affect the cycle parameters, as condensation and the dew point are not factors towards the CD gas cycle. The load pattern or density of the load within the space do not affect the CD gas cycle either, the CD gas cycle performs equally whether the space is empty or filled with components. Combining these factors, chlorine dioxide gas does not need a long prep time in order for an effective cycle to be configured.

3. Decontamination verification

Biological Indicators (BIs), most commonly inoculated with 10^6 bacterial spores and referred to as spore strips, are used to challenge the efficacy of a decontamination/sterilization cycle as they are considered among the hardest organisms to kill. Sterilization, often referred to as the 6-log reduction of organisms occurs when there is a million-fold reduction in living organisms which is why these spore strips generally consist of approximately 1 million bacterial spores.

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To challenge the decontamination/sterilization cycle, BIs are placed in hard to reach areas

throughout the space being decontaminated; rooms, isolators, biological safety cabinets, etc. Once the decontamination has been completed, the BIs are dropped into growth media using aseptic technique. If the biological indicator does not produce growth within the media tube after the incubation period (typically 36 hours), the decontamination/sterilization cycle is deemed to be successful.

The BIs used for CD decontamination, consist of 10^6 ***Geobacillus stearothermophilus*** spores inoculated on a paper substrate and wrapped in tyvek. The tyvek creates a barrier to prevent the spores from escaping, and prevents other organisms from entering post-decontamination allowing the BIs to be handled and transported easily. The chlorine dioxide gas molecule is small enough however, to permeate the tyvek and kill the spores inside.

As BIs should always be used to challenge decontamination/sterilization cycles, they must be placed in challenging areas. It is recommended to use BIs with a population of 10^6 spores to test the decontamination cycles, even for "challenge" locations.

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4. Material compatibility

Oxidizers are ranked by their tendency to lose electrons, or oxidize, known as the oxidation potential. CD has an oxidation potential of 0.95 volts (V). Oxidation potential is also viewed as a chemical's corrosion potential, as chemicals with higher oxidation are more corrosive than those with lower oxidation potentials. CD's oxidation potential is lower than that of ozone, peracetic acid, sodium hypochlorite (bleach), and hydrogen peroxide as shown below.

Table 1. Material compatibility of sterilizing agents

Sterilant	Mode of Action	Oxidation Potential (V)
Ozone	Oxidation	2.07
Peracetic Acid	Oxidation	1.81
Hydrogen Peroxide	Oxidation	1.78
Sodium Hypochlorite (Bleach)	Oxidation	1.49
Chlorine Dioxide	Oxidation	0.95
Formaldehyde	Alkylation	N/A

CD gas has also been used for the decontamination of equipment and electronics from within laboratories, offices, and production areas without affecting their operation.

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5. Safety

Table 2. Safety classification of sterilizing agents

Sterilant	Classification as a Carcinogen	8-hr safety level (TWA)	Odor treshold
Chlorine dioxide	No	0,1ppm	0,1ppm
Formaldehyde	Yes (IARC)	0,75ppm	0,8-1ppm
Hydrogen peroxide	Confirmed animal carcinogen with unknown relevance to humans (ACGIH)	1ppm	None
Ozone	No	0,1ppm	0,1ppm

CD is non-flammable and although it can be explosive at concentrations over 10% (100,000 ppm), no commercial or industrial decontamination / sterilization application approaches that level. Our use concentration is 360 ppm for most applications. One benefit of CD gas is that it has a low odor threshold of 0.1 ppm, equal to its 8-hr exposure safety level. CD's smell is distinguishable from, but similar to the smell of chlorine. This similarity to the smell of chlorine is beneficial as chlorine's odor is widely known and recognized so there is no learning curve for personnel in recognizing when there is CD present. The difference in smell between chlorine and CD is slight, with chlorine having a slightly sharper smell than CD. While ones nose is not meant to be used as a primary means of odor detection due to the variance in sensitivity to smell that personnel have, it provides an extra layer of personal protection for those working nearby a decontamination. Moreover, small

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portable chlorine dioxide gas detectors (0-1ppm) can be provided to ensure safety to surroundings.

Chlorine dioxide gas aerates much faster than hydrogen peroxide vapor as hydrogen peroxide condenses upon surfaces and takes time (usually 8+ hours) to fully aerate to below OSHA's 8-hr safety threshold for exposure while CD typically aerates in under an hour. If required, aeration through activated carbon can be provided.

6. CD decontamination process

Similar to all other decontamination methods, CD gas decontamination is a multi-step process. The 5 steps of the CD gas process are:

1. Precondition: Raising of relative humidity (RH) levels between 60-75%.
2. Condition: Hold time once relative humidity set point has been reached.
3. Charge: Generation and delivery of chlorine dioxide gas.
4. Exposure: Hold time once gas concentration set point has been reached. The exposure step is controlled through an "area under the curve" accrual of gas concentration until a target dosage has been met.
5. Aeration: Removal of chlorine dioxide gas.

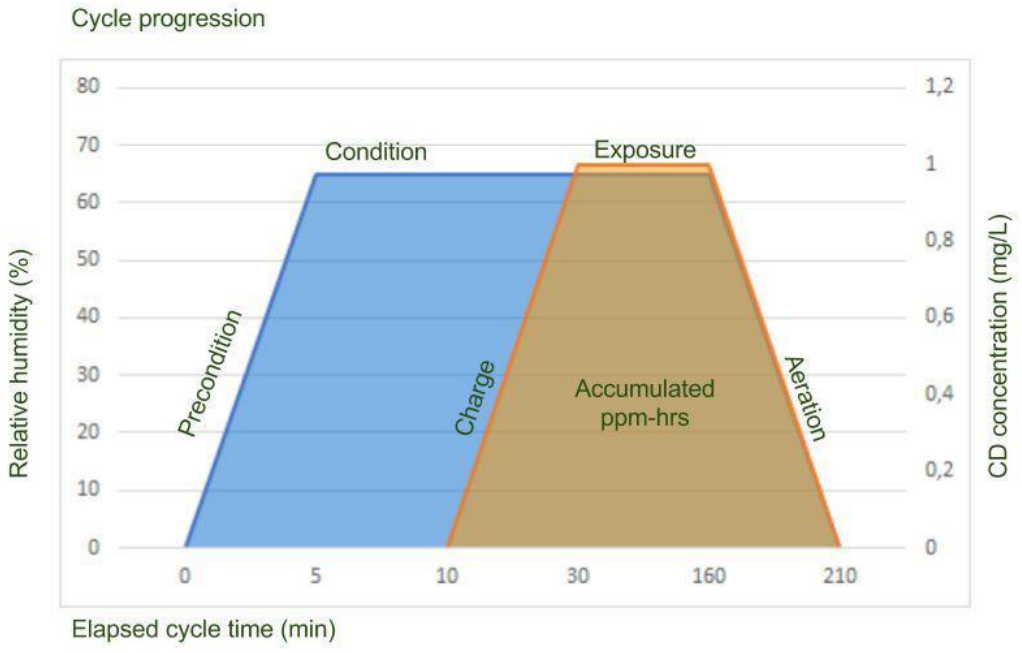

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Figure 1. CD decontamination cycle progression

7. Summary

CD decontamination is a mature technology suited for sterilisation of (parts) of buildings, fulfilling the 3 conditions for a perfect decontamination:

1. Broad biocidal activity (incl. against Polio);
2. Homogeneous distribution and high penetration;
3. Ensurance of sufficient exposure through accurate analytics.

Taking into account the required safety measures, CD decontamination cycles are straightforward and extremely effective. Decon-O-Logic will be pleased to be your decontamination partner.