Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Isanaka S, Guindo O, Langendorf C, et al. Efficacy of a low-cost, heat-stable oral rotavirus vaccine in Niger. N Engl J Med 2017;376:1121-30. DOI: 10.1056/NEJMoa1609462

This supplement contains the following items:

1. Original protocol (p.2), final protocol (p.70), summary of changes (p.138)

2. Original statistical analysis plan (p.141), final statistical analysis plan, summary of changes (p.209)

Efficacy and safety of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis in Niger

Study Protocol

Version 6

September 8, 2013

Centre Collaborateur de l'OMS
pour la Recherche en Epidémiologie
et la Réponse aux Maladies Emergentes

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Title	Efficacy and safety of a pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in Niger
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INVESTIGATORS' SIGNATURE PAGE

By my signature below, I hereby confirm that I will conduct the study described in this protocol in compliance with ICH/GCP and the version of such protocol agreed to by the applicable regulatory authorities and approved by all Institutional Review Board and Ethical Committees.

Primary Sponsor, Epicentre
Rebecca Grais

_____ _____
Signature **Date**

Secondary Sponsor, Médecins Sans Frontières-Operational Center Geneva
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TRIAL REGISTRATION DATA

Primary Registry and Trial Identifying Number: To be completed with ClinicalTrials.gov

Date of Registration in Primary Registry: To be completed with ClinicalTrials.gov

Secondary Identifying Numbers: None

Sources of Monetary and Material Support
Médecins Sans Frontières-Operational Center Geneva will provide funding for the trial. Vaccine and placebo are to be provided in-kind by the Serum Institute of India, Limited.

Primary Sponsor
Epicentre takes responsibility for initiating, registering and conducting the trial, and as such, will be involved in the study design; collection, management and analysis, and interpretation of data; and writing of the report. Epicentre takes responsibility for ensuring the trial is properly monitored and results made available.

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Secondary Sponsor
Médecins Sans Frontières- Operational Center Geneva has agreed with the primary sponsor to act as the secondary sponsor and the primary sponsor's legal representative in relation to the trial site and provide funding for the trial. The secondary sponsor will be involved in the study design, interpretation of data and writing of the report.

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Public Title

Efficacy and safety of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis in Niger

Scientific Title

Randomized, double-blind, placebo-controlled phase III trial to assess the efficacy and safety of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis among infants in Niger

Country of Recruitment

Niger

Health Condition(s) or Problem(s) Studied

Severe rotavirus gastroenteritis

Interventions

Active comparator: Live attenuated bovine-human [UK] reassortant rotavirus vaccine manufactured by the Serum Institute of India, Limited (SIIL). The pentavalent vaccine (BRV-PV) contains rotavirus serotypes G1, G2, G3, G4, and G9 (≥5.6 log10 FFU/serotype/dose). The vaccine is in lyophilized form and supplied with 2.5 ml of citrate bicarbonate buffer that is added for reconstitution just before oral administration.

Placebo comparator: Same constituents as the active vaccine but without the viral antigens; manufactured by SIIL.

Key Inclusion and Exclusion Criteria

The study will be performed in infants in Madarounfa, Niger. Healthy male and female infants meeting the following inclusion criteria are eligible for enrollment:

(1) aged 6-8 weeks at the time of inclusion
(2) able to swallow and no history of vomiting within 24 hours
(3) resident in Madarounfa Health District within 15 km of central health facility
(4) intending to remain in the study area for 2 years
(5) parent/guardian providing informed consent

Exclusion criteria will include the following:

(1) unable to swallow or history of vomiting within 24 hours
(2) known history of congenital abdominal disorders, intussusception, or abdominal surgery
(3) receipt of intramuscular, oral, or intravenous corticosteroid treatment within 2 wks
(4) receipt of a blood transfusion or blood products, including immunoglobulins
(5) non-resident in Madarounfa Health District within 15 km of central health facility
(6) not intending to remain in the study area for 2 years
(7) parent/guardian informed consent not provided
(8) any other condition in which, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or the parent/guardian's ability to give informed consent

Study Type

Interventional, individually randomized, double (e.g. investigator and participant) blinded, parallel two-arm, phase III trial to assess the efficacy, safety and immunogenicity of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis when administered concomitantly with EPI vaccines in Niger. Infants will be individually randomized in a 1:1 ratio to receive three doses of the vaccine or placebo administered orally. The initial dose will be at 6-8 weeks of age. Each subsequent dose will be administered after a 4-week intervals (-1 to +4 weeks), with a maximum age at last dose of 24 weeks.

Unique identification numbers will be allocated by SIIL using a computer-generated random number list using permuted blocks of random sizes. Block sizes will not be disclosed to reduce predictability of the random sequence and ensure allocation concealment. Study physicians who oversee randomization will be given a subset of sequentially numbered, sealed, opaque envelopes that will be prepared by SIIL and contain printed randomization numbers and a corresponding group code. The study physician will be instructed to assign the randomization number and group code noted in the next envelope to each eligible infant as (s)he is enrolled.

Vaccine and placebo packages will be labeled with an assigned code and delivered to the study site in otherwise identical presentations. Group assignment will remain concealed from study personnel, investigators and caregivers of participating infants for the whole study period. The Data and Safety Monitoring Board (DSMB) and a sponsor statistician not involved in the rest of the trial will be masked to the group assignment. Investigators conducting the final analysis will remain masked to the group assignment until the end of the analysis.

The study code will be broken only in case of a medical event in which the Medical Coordinator deems the participant cannot be appropriately treated without knowing his/her group assignment. A set of sealed envelopes with group assignment will be securely held at the field site with the Medical Coordinator. Any such case will be fully documented by the Medical Coordinator and written notification will be provided to the sponsor within 48 hours.

Date of First Enrollment
December 2013

Target Sample Size
Assuming a 2% attack rate of severe rotavirus gastroenteritis at 1 year, a 50% true vaccine efficacy and a 20% participant non-assessibility (including withdrawal and loss to follow up), the study will enroll 5138 children to have at least 90% power to detect a 95% confidence interval for vaccine efficacy at 1 year that would be above 0%.

Recruitment Status
Pending: participants are not yet being recruited or enrolled at any site.

Primary Outcome
The primary endpoint is vaccine efficacy of three doses of the SIIL pentavalent rotavirus vaccine vs. placebo against a first episode of severe wild-type rotavirus gastroenteritis at 1 year of age. Gastroenteritis events will be detected through facility- and weekly home-based surveillance and will be defined as the passage of three of more loose or watery stools within a 24 hour period and/or forceful vomiting. Severe gastroenteritis will be defined clinically as an episode of gastroenteritis that needed overnight treatment in hospital and/or rehydration therapy equivalent to the WHO Plan B (oral rehydration therapy) or Plan C (intravenous rehydration therapy) in health facility, or using the 20-point Vesikari scale, where an episode of gastroenteritis with a score of 11 or more is considered severe. In the primary per-protocol analysis, infants who have at least one episode of severe wild-type rotavirus gastroenteritis during the period from 28 days post-Dose 3 until the date the infant reaches 1 year of age will be considered as having achieved the primary outcome. A secondary intention-to-treat (ITT) analysis of the primary endpoint will be conducted including all infants vaccinated with at least one dose of vaccine or placebo and follow up beginning from the time of randomization.

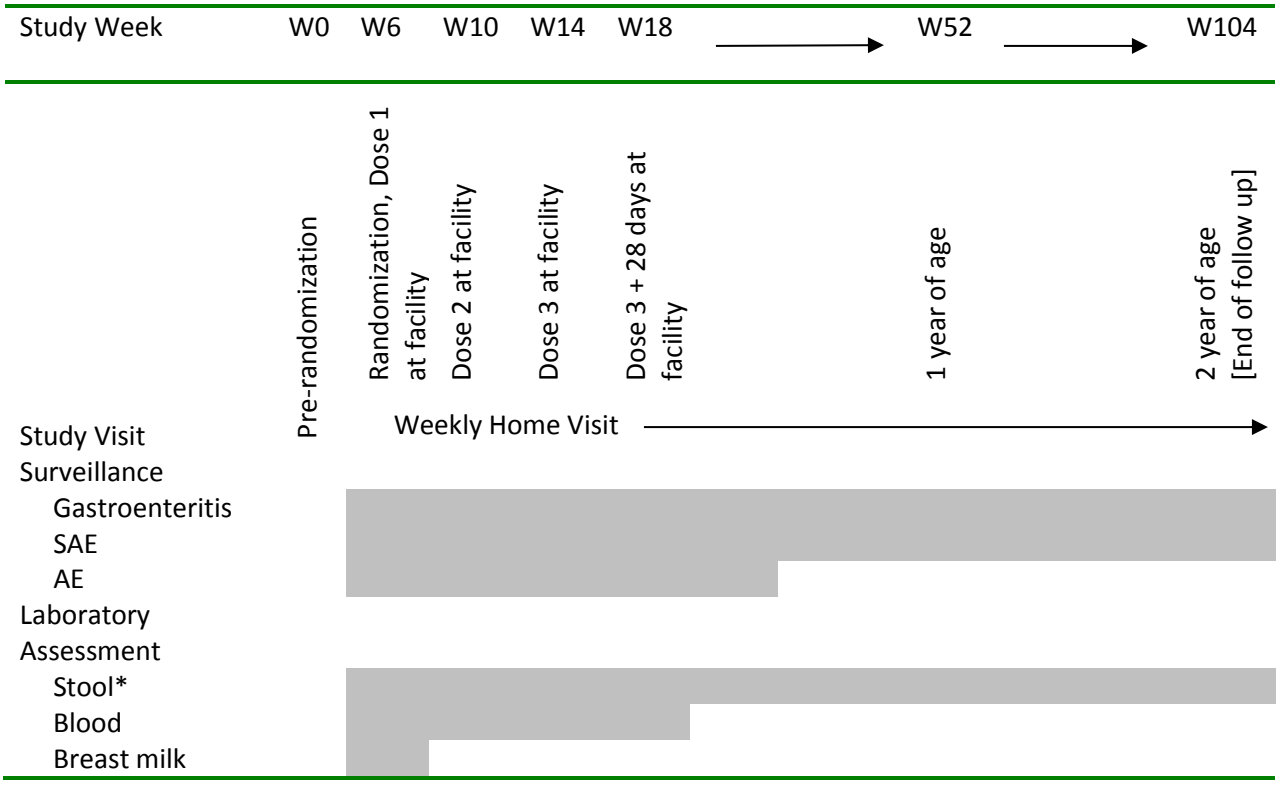
Key Secondary Outcomes
Secondary efficacy endpoints include: vaccine efficacy against severe rotavirus gastroenteritis during the second year of life and for the combined period for the first from 28 days post-Dose 3 until 2 years of age; rotavirus gastroenteritis of any severity; rotavirus gastroenteritis with a Vesikari score of ≥ 17; rotavirus gastroenteritis by serotype; gastroenteritis of any cause; longitudinal prevalence of rotavirus gastroenteritis; hospitalization due to rotavirus gastroenteritis; and hospitalization for any reason. The rates of all secondary efficacy endpoints will be compared by study intervention as in the per-protocol and ITT analyses of the primary efficacy endpoint.

Secondary safety endpoints include: the risk of adverse events from the time of the first dose to 28 days post-Dose 3 and the risk of serious adverse events from the time of the first dose until 2 years of age. Adverse events will include fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in Standard Operating Procedures), diarrhea, vomiting, decreased appetite, decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia). Serious adverse

events will be defined as any new health-related problem that results in disability, incapacity or death; necessitates hospitalization or is life-threatening, and according to the Brighton Collaboration for adverse events following immunization for oral vaccines, will also include generalized convulsive seizure, hypotonic-hyporesponsive episodes, intussusception, and persistent crying. The rates of all adverse and serious adverse events will be compared by study intervention in the ITT population, i.e. all infants who received at least one dose of the study vaccine or placebo, and include follow up from the time of enrollment until 28 days post-dose 3 (adverse events) or the end of follow up (serious adverse events).

The secondary immunogenicity endpoints will be assessed in a sub-sample of participants and include sero-conversion, defined as at least a three-fold serum titre rise of anti-rotavirus IgA, and differences in geometric mean titres from the first dose to 28 days (± 7 days) after the third dose of vaccine or placebo. Immune factors in breast milk at the time of immunization have been hypothesized to contribute to lower immunogenicity of live oral rotavirus vaccines in developing countries, therefore secondary immunogenicity analyses will assess concentrations of maternal anti-rotavirus IgA antibodies in breast milk in order to compare immunogenicity by level of maternal anti-rotavirus IgA concentration. Statistical differences in the risk of sero-conversion by level of maternal IgA concentration in breast milk will be assessed using the chi-square test. As the study vaccine will be administered concomitantly with the oral polio vaccine as part of the standard EPI, interference of the study vaccine with anti-poliovirus antibody serum titres will be assessed in the immunogenicity sub-sample. Differences in the concentration of anti-polio antibodies by study intervention will be compared using the chi-square test.

Table A. Schema of overall study design



* Stool collected at facility or home one time per episode of loose or watery stools and/or forceful vomiting within 7 days onset.

ABBREVIATIONS

AE	Adverse Event
BRV-PV	Bovine-human reassortant rotavirus vaccine-pentavalent
CERMES	Centre de Recherche Médicale et Sanitaire
CRF	Case Report Form
DHS	Demographic and Health Survey
DSMB	Data and Safety Monitoring Board
EPI	Expanded Programme on Immunization
FORSANI	Forum Santé Niger
GAVI	Global Alliance for Vaccines and Immunization
GEMS	Global Enteric Multicenter Study
HIV	Human Immunodeficiency Virus
GCP	Good Clinical Practice
IMCI	Integrated Management of Childhood Illness
ITT	Intention To Treat
MEM	Minimum Essential Medium
MSF	Médecins Sans Frontières
NGO	Non-governmental organization
ORS	Oral rehydration salts
PATH	Program for Appropriate Technology in Health
PI	Principal Investigator
ROC	Receiver Operating Characteristic
SAE	Serious adverse event
SIIL	Serum Institute of India, Limited
SOP	Standard Operating Procedure
UN	United Nations
UNICEF	United Nations Children's Fund
WHO	World Health Organization

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BACKGROUND AND RATIONALE

Epidemiology of childhood diarrhea. Acute diarrhea remains one of the major causes of morbidity and mortality among children, accounting for 11% of child deaths worldwide, more than malaria (7%) and far greater than HIV (2%) (1). Diarrhea-specific mortality has decreased in recent decades, but worldwide it is estimated to still account for 800,000 deaths in children < 5 years of age each year (2) and there has been little progress in reducing the incidence of diarrheal illness (3). There remains an estimated 2.5 billion episodes of childhood diarrhea per year (4). Children < 2 years of age are thought to experience an average of three to five episodes of diarrhea per year, with the highest rates (six to eight episodes per year) among infants 6-11 months old (5).

Acute diarrhea in children is often caused by a diverse group of infectious agents, including viruses, bacteria, and parasites. Transmission may occur through fecal-oral routes, respiratory secretions, or fomites (inanimate objects such as kitchen utensils). The recently completed Global Enteric Multicenter Study (GEMS) provides new evidence regarding the incidence, etiology and clinical outcome of moderate-to-severe diarrhea in seven sites throughout sub-Saharan Africa and south Asia (6). Using a comprehensive panel of microbiological assays to identify the etiology of moderate-to-severe diarrhea, a substantial proportion of diarrheal disease was attributed to 4 pathogens: rotavirus, *cryptosporidium*, and heat stable toxin producing *enterotoxigenic e.coli*, and *shigella*. In the first two years of life, the attributable incidence of moderate-to-severe diarrhea was dominated by rotavirus: incidence in infancy (7 episodes per 100 child-years) was more than double that of any other pathogen.

Current strategies for the management of childhood diarrhea. Acute diarrhea is rapidly dehydrating and can be life-threatening unless fluid therapy is initiated. In 1978, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) adopted oral rehydration salts (ORS) solution as the primary tool to fight dehydration. For more than 25 years, ORS was the only drug recommended by WHO and UNICEF for the prevention and treatment of dehydration. Rehydration therapy, however, does not decrease stool output, nor the duration or incidence of diarrhea, and ORS uptake is low: it is estimated that only one-third of children with diarrhea in developing countries currently receive ORS for treatment of their illness(4).

In 2004, WHO and UNICEF released revised recommendations for the management of diarrhea that also included therapeutic zinc supplementation for 10 to 14 days and incorporated zinc in the WHO Essentials Medicines List making it possible for zinc to be stocked in UNICEF warehouses (7). Despite the extensive body of scientific evidence supporting zinc for diarrhea management (8), many developing countries have been slow to adopt zinc as an explicit component of their diarrhea control programs, and zinc tablets appropriate for young children remain largely unavailable (4).

To accelerate progress towards reducing childhood diarrhea, calls for continued scaling up of proven interventions continue (9). In 2009, UNICEF and WHO issued the report, 'Diarrhea: why

are children still dying and what can be done,' and called for the large-scale implementation of 7 interventions for comprehensive diarrhea control in developing countries. The plan includes a treatment package: fluid replacement (with low-osmolarity ORS and continued breastfeeding or feeding) and zinc treatment to decrease diarrhea severity and duration; and a prevention package: rotavirus and measles vaccination, promotion of early and exclusive breast-feeding and vitamin A supplementation, promotion of hand washing with soap, improved water supply and quality including household water treatment and safe storage of household water, and community wide sanitation (4).

Epidemiology of rotavirus and public health response. Rotavirus is the leading cause of severe gastroenteritis in children and is responsible for an estimated 450,000 deaths per year in children < 5 years of age, with most of the deaths occurring in developing countries (10). In sub-Saharan Africa, the proportion of hospitalizations for diarrhea associated with rotavirus ranges from 29% to 52% (11), with a global estimate of 34% in a meta-analysis of recent studies published between 2006 and 2008 (12).

Rotavirus genotypes are based on the two structural proteins of the virus outer capsid, VP7 (G glycoprotein) and VP4 (P protein). Four genotypes (G1P[8], G2P[4], G3P[8], G4P[8]) were historically recognized to be the most frequent, representing 88% of all strains worldwide in a meta-analysis from 2005, with the single G1P[8] genotype responsible for over 70% of infection in North America, Europe and Australia (13). More recently, G9P[8] has emerged worldwide and other rare genotypes, such as G12 and G6, are also emerging in Asia and Africa (14-16). Overall, recent studies have shown a wider variety of rotavirus genotypes circulating in developing countries compared to industrialized countries (11, 17).

Two rotavirus vaccines are currently available and prequalified by the WHO. Rotarix® (GlaxoSmithKline) is a live, attenuated vaccine derived from the human 89-12 strain which belongs to G1P[8] type, while Rotateq® (Merck) is a pentavalent human-bovine reassortant strain containing the G1, G2, G3, G4 and P[8] proteins. These vaccines have been shown to be safe and efficacious in high and middle-income countries (18, 19), where the impact of their introduction in routine vaccination programs on rotavirus-related hospital admissions and deaths has been demonstrated (20-24). Recent vaccine trials in Africa and Asia have shown substantially lower vaccine efficacy in low-income countries than in high or middle-income countries, with efficacies ranging from 50% to 64% (25-27). Despite these reduced efficacies, WHO has extended their recommendation for rotavirus vaccine introduction to all countries based on the higher predicted number of deaths averted in low-income countries (28).

Most of the difference in vaccine efficacy between high and low-income countries may be due to lower immunogenicity of oral live vaccines in developing countries (29, 30). Enteropathy and malnutrition are thought to be major factors responsible for the reduced immune response to oral vaccines (31, 32). Whether the greater variety of locally circulating genotypes and lower correlation with vaccine genotypes also contribute to the reduced efficacy remains a question for debate. Pooled data from randomized controlled trials show that the G1P[8]-containing Rotarix® vaccine offers some protection against non-G1 and non-P[8] strain (33), but vaccine

effectiveness studies in countries that have seen the emergence of the fully heterotypic G2P[4] genotype after introduction of Rotarix® have shown contradictory results on heterotypic protection (34, 35).

Countries that have introduced rotavirus vaccines into their immunization programs have seen an improvement in child health. Recent studies show the swift and significant impact of rotavirus vaccines following introduction in national immunization programs. In Mexico, diarrheal deaths in children < 5 years of age decreased by 46% during 2007-2009 (36). In Australia, Belgium, El Salvador and the United States, hospitalizations and clinic visits for rotavirus-related diarrhea in children < 5 years of age declined by 60-94% between 2007 and 2010 (22). This reduction of severe diarrhea underscores the potential for rotavirus vaccines to save children's lives. Since 2011, Sudan, Ghana, Rwanda, Moldova, Yemen, Malawi, Armenia and Tanzania have introduced rotavirus vaccines into their national immunization programs. At present, 22 other countries in sub-Saharan Africa are expected to introduce rotavirus vaccines over the next several years (37).

Ongoing support from the Global Alliance for Vaccines and Immunization (GAVI) allows eligible countries to purchase vaccine at reduced cost, but the sustainability of time-limited vaccine subsidies remains a concern. Further, introducing current presentations of rotavirus vaccine into existing immunization programs may substantially disrupt the vaccine supply and cold chains. The added volume of new vaccines could displace other Expanded Programme on Immunization (EPI) vaccines from storage and transport space, overwhelm transport and storage at lower levels of the supply chain, and reduce the availability of all EPI vaccines at health centers where they are delivered. This scenario was born out during the 2006 to 2007 RotaTeq® and Rotarix® introductions in 7 Latin American countries. RotaTeq® and Rotarix® were too large for many of the existing supply chains, surpassing refrigerator capacities of many health centers and forcing health care workers to carry extra thermoses and cold boxes (38). Because no contingency plans were in place, these unexpected consequences resulted in the expiration of large stocks of vaccines. While this experience compelled manufacturers to re-design their vaccine packaging, it also underscores the possibility for new vaccines to not fit smoothly into supply chains, failing to reach their target populations and preventing other vaccines from reaching clinics.

Rotavirus in Niger. Since 2009, Epicentre has led a large-scale surveillance effort to gather data on the epidemiology of rotavirus in urban and rural Niger (39). From December 2009 to March 2012, 10,597 children aged 0-59 months presenting to health facilities in Niamey and Maradi with watery diarrhea and signs of dehydration were enrolled. Stool specimens were systematically collected at presentation, with a rapid test performed on-site to determine the presence of rotavirus and genotyping performed on a subsample of rotavirus-positive specimens to determine genotype distribution and evolution during the study period. Surveillance identified 30.4% (95% CI: 29.6-31.3) of diarrhea to be rotavirus-positive, with 80% of all rotavirus cases found among children < 1 year of age and 96% of all cases found among children < 18 months. A higher proportion of rotavirus was found among cases in rural health centers than urban hospitals (32.4% versus 23.3%). Severe rotavirus-positive diarrhea represented 1.2% of cases among children < 5 years of age and 3.0% among children < 1 year of

age. Cases were seen year-round, with a consistent peak in the dry and cool season (October to December) and a natural switch from G2P[4] to G12P[8] genotype predominance observed during the study period. With 30% of children aged 0-59 months with diarrhea and dehydration positive for rotavirus, these results confirm the high burden of rotavirus in Niger, particularly in children < 18 months of age.

The Ministry of Health of Niger has been formally approved for a GAVI-subsidized introduction of rotavirus vaccine, but has chosen to delay introduction until at least 2015 due to concerns about vaccine presentation, storage and cold chain requirements among others.

Serum Institute of India, Limited rotavirus vaccine. The currently in-development rotavirus vaccine (live attenuated bovine-human [UK] reassortant rotavirus vaccine) manufactured by the Serum Institute of India, Limited (SIIL) holds great promise for immunization programs in countries like Niger. The pentavalent vaccine (BRV-PV) contains rotavirus serotypes G1, G2, G3, G4 and G9 ($\geq$5.6 $\log_{10}$ FFU/serotype/dose) and is delivered in lyophilized form supplied with 2.5 ml of citrate bicarbonate buffer that is added for reconstitution just before oral administration. The proposed schedule includes a three-dose series of oral vaccine administered , concomitantly with EPI vaccines. The initial dose is given at 6-8 weeks of age, with each subsequent dose given following a 4-week interval.

Compared to the 2 WHO prequalified vaccines, the SIIL formulation introduces important advantages for immunization programs (Table 1). First, BRV-PV is expected to be more affordable than available vaccines, with the potential for dramatic cost savings after 2015 when GAVI subsidies can expire. Second, BRV-PV offers the unique potential to be delivered out of cold chain. BRV-PV was tested and found to be stable at 37°C for 1 year and 40°C for 6 months; if delivered out of cold chain, this formulation could introduce significant logistical advantages for national programs in sub-Saharan Africa where cold chain capacity is limited.

Pre-clinical evaluation of BRV vaccine. Because of the promising safety and immunogenicity profile observed in a phase I clinical trial, SIIL conducted a two-center, double-blind, randomized, placebo-controlled phase II clinical trial with three doses of the pentavalent rotavirus vaccine in Pune, India. Sixty healthy infants received three administrations containing Log_{10} 5.6-5.8 FFU/dose or placebo at 8-10 wks, 12-14 wks & 16-18 wks of age, with at least 4 weeks interval between each dose. Safety parameters included recording of solicited symptoms in the 14-day follow-up period after each dose, monitoring and recording of adverse events (AEs) and serious adverse events (SAEs) in the post-dose 28 day follow-up period, and changes in laboratory parameters including hematology, biochemistry, changes noted during physical examination and vital signs assessment. In total, 132 AEs were reported during the study, and 26 (86.7%) participants in the vaccine arm reported at least one AE during the study compared to 21 (67.7%) participants in placebo arm. Most reported adverse events were mild in intensity (97.4% in vaccine group and 94.6% in placebo group), and all events recovered without any sequelae before the study completion. No SAEs were reported during the entire period of the study. Overall, as the type, frequency, and severity of adverse events observed in vaccine-

treated participants appeared similar to those of placebo-treated participants, the SIIL rotavirus vaccine at 3 doses (Log_{10} 5.6-5.8 FFU/dose) was found to be safe and tolerable in infants.

Table 1. Summary of rotavirus vaccines

	Rotarix® (GlaxoSmithKline)	**Rotateq® (Merck)**	**BRV-PV (SIIL)**
Origin	Human monovalent	Bovine pentavalent	Bovine pentavalent
Genotype(s)	G1, P[8]	G1, G2, G3, G4, P[8], G6P[7]	G1, G2, G3, G4, G9
Vaccine course	2 doses – oral	3 doses – oral	3 doses – oral
Schedule	With DTP1 / 2	With DTP1 / 2 / 3	With DTP1 / 2 / 3
Age restrictions	First dose at 6-15 wk of age; Max age for last dose at 32 wk	First dose at 6-15 wk of age; Max age for last dose at 32 wk	First dose at 6-8 wk of age; Max age for last dose at 24 wk
Intussusception risk	None observed	None observed	None observed
Presentation	Lyophilized and reconstituted; or liquid	Liquid, single dose pouch	Lyophilized and reconstituted
Volume per dose	259.8 cm^3/1 dose box	798 cm^3/10 dose box	202.5 cm^3/1 dose box
Storage	2-8^oC; diluent at room temp	2-8^oC	< 25 ^oC to central facility and out of cold chain to distribution; diluent at room temp
Price (USD)	$2.5 / dose (GAVI)	$5 / dose (GAVI)	< $ 2 / dose (expected)
WHO pre-qualification	2007	2008	

Sero-conversion rates at 28 days post-Dose 3 in the phase II trial were 60.0% in the vaccine arm and 7.7% in the placebo arm, indicating that the vaccine is highly immunogenic as compared to placebo. The net sero-conversion rate of 52.3% [60.0% (BRV vaccine) – 7.7% (placebo)] observed in this study is similar to that observed with Rotarix® and Rotateq® vaccines in India and other developing countries (40-49). At baseline, 23.3% of participants in the vaccine arm and 38.5% of participants in the placebo arm had an IgA concentration of ≥20.00 units/ml, indicating an early exposure to natural rotavirus infection. These results were not unexpected, as similarly high initial sero-conversion rates (in the absence of prior vaccination) have been observed in South Asia in past surveillance and previous rotavirus vaccine studies (40, 41, 50). Nevertheless, the good immunogenic response seen in initially sero-positive infants (57.1%) implies that the vaccine can successfully colonize the infant gut, induce a robust immune response and significantly increase initial antibody levels, even in the presence of pre-existing IgA antibodies.

In summary, preliminary study has demonstrated that, compared to placebo, the SIIL rotavirus vaccine has a similar reactogenicity profile and is immunogenic in an environment where a substantial proportion of infants are initially sero-positive. These encouraging results justify conducting a phase III clinical trial to evaluate the protective efficacy of the SIIL rotavirus vaccine with dose Log_{10} 5.6-5.8 FFU/serotype/dose.

Permission for a phase III clinical trial was granted by the Drug Controller General of India in May 2013. This will be a double-blind, placebo-controlled study to assess efficacy of the SIIL pentavalent vaccine in prevention of severe rotavirus gastroenteritis. The multi-centric study will be conducted in 6 sites across India in collaboration with the Program for Appropriate Technology in Health (PATH) and was approved by the Western Institutional Review Board, USA. The study is expected to start in September 2013; 7500 children will be enrolled and followed until September 2016.

Study rationale. Sub-Saharan Africa carries the largest burden of rotavirus-related mortality, but immunization against rotavirus presents unique challenges. Current supply of the 2 WHO prequalified vaccines is constrained (51), and in many African settings, national immunization programs are challenged by supply shortages and a lack of trained health workers. Unreliable transportation systems and storage facilities also make it difficult to preserve vaccines that require refrigeration. If rotavirus vaccine is to be brought to the infants that need it most through national immunization programs in the region, new vaccines that address these challenges are urgently needed. The BRV-PV vaccine is a relatively low-cost and heat-stable formulation whose introduction into national immunization programs may help minimize the burden on already-strained national programs throughout sub-Saharan Africa.

The WHO Expert Committee on Biological Standardization has recommended that the efficacy of new rotavirus vaccines be demonstrated in diverse geographical regions including developing countries before widespread implementation (52). The Ministry of Health of Niger, Médecins Sans Frontières (MSF) – Operational Center Geneva and Epicentre along with other partners have formed a research consortium to bring additional evidence to inform public health decision making on the potential value of the BRV-PV vaccine in an African setting. The goal of the present study is to collect additional data on the efficacy profile of BRV-PV vaccine in a randomized controlled setting, while gaining further experience with vaccine-related adverse events. This will be conducted through the performance of a phase III trial in Niger conducted in compliance with the version of the protocol agreed to by the applicable regulatory authorities and Good Clinical Practice (GCP).

Evidence supporting the efficacy and safety of this formulation in an African setting would support the pre-qualification and increased global access to the BRV-PV vaccine. If shown to be efficacious and pre-qualified, the government of Niger would benefit from a low cost vaccine adapted to the logistical and supply demands of the national immunization program.

STUDY OBJECTIVES AND ENDPOINTS

Primary objective:

(1) To estimate the efficacy of three doses of the SIIL pentavalent rotavirus vaccine vs. placebo against severe rotavirus gastroenteritis at 1 year of age.

Secondary objectives:

(2) To estimate the efficacy of three doses of the SIIL pentavalent rotavirus vaccine vs. placebo against severe rotavirus gastroenteritis at 2 years of age.

(3) To estimate the safety of three doses of the SIIL pentavalent rotavirus vaccine in terms of adverse and serious adverse events from the time of the first dose until 28 days post-Dose 3 (adverse events) or 2 years of age (serious adverse events).

(4) To estimate the immunogenicity of three doses of the SIIL pentavalent rotavirus vaccine in terms of anti-rotavirus IgA sero-conversion in a sub-sample of participants.

(5) To assess the difference in immunogenicity by level of breast milk concentration of rotavirus-specific IgA in a sub-sample of participants.

(6) To assess the difference of anti-poliovirus antibody titres at 28 days post-Dose 3 by study group in a sub-sample of participants.

Primary endpoint. The primary endpoint is vaccine efficacy against a first episode of severe wild-type rotavirus gastroenteritis from 28 days after the final dose until 1 year of age. Severe gastroenteritis will be defined clinically as an episode of gastroenteritis that needed overnight treatment in hospital and/or rehydration therapy equivalent to the WHO Plan B (oral rehydration therapy) or Plan C (intravenous rehydration therapy) in health facility, or using the 20-point Vesikari scale (53), where an episode of gastroenteritis with a score of 11 or more is considered severe.

Secondary endpoints. Secondary efficacy endpoints include vaccine efficacy against a first episode of severe wild-type rotavirus gastroenteritis during the second year of life and for the combined period from 28 days after the final dose until 2 years of age; rotavirus gastroenteritis of any severity; rotavirus gastroenteritis with a Vesikari score of ≥ 17; rotavirus gastroenteritis by serotype; gastroenteritis of any cause; longitudinal prevalence of rotavirus gastroenteritis; hospitalization due to rotavirus gastroenteritis; and hospitalization for any reason.

Safety endpoints include the risk of adverse events from the time of the first dose to 28 days post-Dose 3 and the risk of serious adverse events from the time of the first dose until 2 years of age. Adverse events will include fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in Standard Operating Procedures), diarrhea, vomiting, decreased appetite,

decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia). Serious adverse events will be defined as any new health-related problem that results in disability, incapacity or death; necessitates hospitalization; or is life-threatening, and according to the Brighton Collaboration for adverse events following immunization for oral vaccines will also include generalized convulsive seizure, hypotonic-hyporesponsive episodes, intussusception, and persistent crying (54, 55).

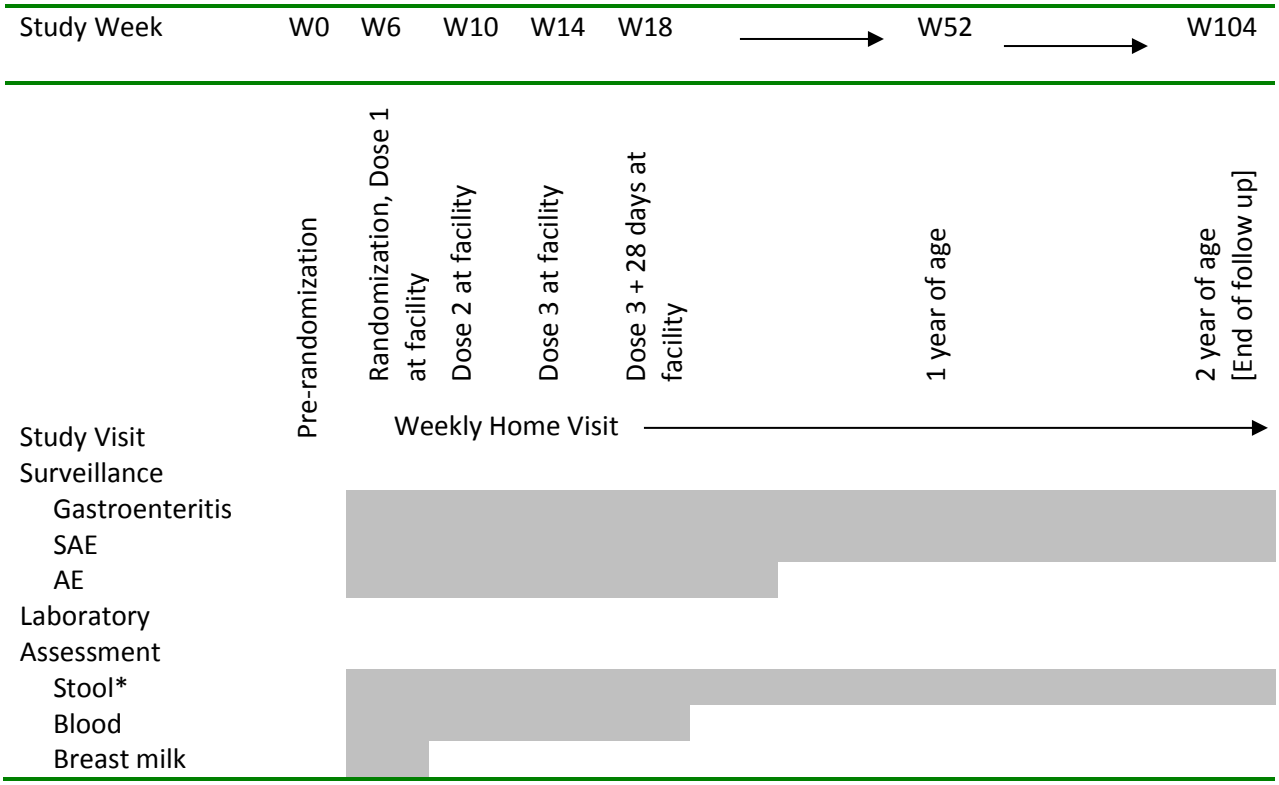
Immunogenicity endpoints will be assessed in a sub-sample of participants. The principal endpoint will include three-dose sero-conversion, defined as at least a three-fold serum titre rise for anti-rotavirus IgA from the first dose to 28 days post-Dose 3. Sero-conversion following Dose 1 and Dose 2, as well as geometric mean titres following all doses, will be considered secondary immunogenicity endpoints. Immunogenicity analyses will consider concentrations of maternal anti-rotavirus IgA antibodies in breast milk in order to compare immunogenicity by level of maternal anti-rotavirus IgA concentration, as well as anti-poliovirus antibody serum titres to assess interference of the study vaccine with anti-polio immunogenicity.

METHODOLOGY

Study design. We propose a double-blind, placebo-controlled randomized phase III trial with two parallel groups in Madarounfa, Niger to assess the efficacy, safety and immunogenicity of BRV-PV, a pentavalent rotavirus vaccine manufactured by SIIL. The primary aim of the study is to assess the efficacy of three doses of pentavalent rotavirus vaccine vs. placebo against severe wild-type rotavirus gastroenteritis at 1 year of age when administered concomitantly with EPI vaccines. Infants aged approximately 6-8 weeks will be individually randomized in a 1:1 ratio using permuted blocks of random sizes to receive three doses BRV-PV or placebo and followed until 2 years of age. Episodes of gastroenteritis, adverse events and serious adverse events will be identified through facility- and home-based surveillance until 2 years of age (28 days post-Dose 3 for adverse events). Immunogenicity will be assessed in a sub-sample of study participants 28 days following each dose.

Placebo, instead of an active comparator, will be used in this trial as no other licensed rotavirus vaccine is yet available in Niger and data from an African setting are needed to inform WHO prequalification of BRV-PV (see Choice of Placebo Comparator below).

Table 2. Schema of overall study design



* Stool collected at facility or home one time per episode of loose or watery stools and/or forceful vomiting within 7 days of onset.

Study setting. Niger is one of the poorest countries in the world, ranking 186 of 187 in 2011 on the Human Development Index(56). Fertility is high, estimated by the 2006 Demographic and Health Survey (DHS) at 7·1 children per woman(57). While rates of child and neonatal mortality in Niger have been among the highest in the world, significant progress has been made with investments in maternal, child and newborn program and policy. Recent data suggests child mortality in Niger declined 43% between 1998 and 2009, from 226 to 128 deaths per 1000 live births(58). Progress in reducing neonatal mortality however has been slower, with the same data showing high levels of neonatal mortality and no significant reduction in neonatal mortality between 1998 and 2009 (39 vs. 33 neonatal deaths per 1000 live births).

The health system in Niger is a pyramidal system in line with the 1985 Lusaka agreements, based on health structures with increasing levels of service capacity: health posts (cases de santé) provide basic care and preventive services and are most often staffed by community health workers who are helped by community representatives. Health centers (centres de santé intégrés) are staffed by nurses and ensure the provision of all services not requiring hospitalization. Complications are referred to the district hospital and to the regional hospital from the district hospital. Integrated Management of Childhood Illness (IMCI) algorithms provide the basis for the organization of care and referral system for children. In this organization, severity signs are assessed at the level of health centers and only severe cases are referred to the district hospital, with the exception of severe dehydration, which should be treated immediately at the health center.

The current organization of the health system is based on several international initiatives launched in 1995-1996. In Niger, this period was marked by the creation of Health Districts for decentralization of care, the gradual introduction of IMCI in all districts, and the implementation of cost recovery systems following the Bamako Initiative. Despite these efforts, the most recent final report from the national DHS in Niger in 2006 showed that only 17% of caretakers of children < 5 years of age had sought advice or treatment in case of diarrhea in their child (57). With the aim of reducing child mortality in line with the UN Millennium Development Goals, free care for children under 5 years of age was introduced in April 2007.

The study will take place in the region of Maradi, in south-central Niger along the Nigerian border. The region of Maradi is comprised of seven Health Districts with a total estimated population in 2009 of about 3 million inhabitants. The proposed site for this study is the Madarounfa Health District, a rural area of 4700 km^2 largely representative of the Sahel region of Niger and sub-Saharan Africa (59). In 2009, the average number of public health structures per 100 km^2 was 1.5 and the public health system coverage, defined as the proportion of the population with access to any health structure within a distance of less than 5 km, was 83% (60).

In collaboration with the Ministry of Health, MSF has been supporting pediatric care in the Madarounfa Health District of Maradi since 2001. Since 2009, project activities have been transferred to local control and implemented through a Nigerien non-governmental organization, Forum Santé Niger (FORSANI) in collaboration with the Ministry of Health. FORSANI provides care and treatment to over 30,000 children in the Madarounfa Health District

each year. Epicentre, the epidemiologic and research organization affiliated with MSF has been present in Niger since 2009. In close partnership with the Ministry of Health, MSF, the CERMES and other NGOs working in the area, Epicentre develops and conducts research aimed at responding to the medical and operational objectives of local and regional public health actors. A team of medical professionals, epidemiologists, biologists and data management specialists work in Maradi and Niamey.

Study intervention (vaccine and placebo). The study vaccine, BRV-PV, is a pentavalent rotavirus vaccine containing rotavirus serotypes G1, G2, G3, G4, and G9 manufactured by SIIL. Each dose of vaccine contains an estimated potency of ≥ 5.6 $\log_{10}$ infectious units / serotype per dose; dose selection was based on demonstrated immunogenicity in phase II trials. Placebo, also manufactured by SIIL, will contain the same constituents as the active vaccine but without the viral antigens; both are lyophilized and will be reconstituted with 2.5 ml of liquid citrate bicarbonate buffer before administration. The initial dose of study vaccine or placebo will be administered orally at a health facility by a study physician at approximately 6-8 weeks of age. The second and third doses of study vaccine or placebo will each be administered following a 4 week internal (-1 to +4 weeks), with a maximum age at last dose of 24 weeks.

Study vaccine and placebo will be administered concomitantly with EPI vaccines, however, administration of the study intervention will not be delayed if EPI vaccines are unavailable at the time of study dosing. Infants who have already received EPI vaccines at the time of study dosing can still receive the study intervention.

Table 2. Characteristics of study interventions

Study Vaccine	Live attenuated Bovine-Human (UK) reassortant pentavalent rotavirus vaccine containing ≥ $\log_{10}$5.6 FFU/dose of each serotype G1, G2, G3, G4 and G9
Placebo	Placebo, consisting of the lyophilized MEM culture medium used to grow the vaccine, excipients and stabilizers
Diluent	Buffered Diluent (containing 25.6 mg/mL of sodium bicarbonate and 9.6 mg/mL citric acid per liter)
Manufacturer	Serum Institute of India Limited, India

Preparation and administration. The process for preparation and administration of vaccine and placebo will be fully detailed in the study Standard Operating Procedures (SOP). In brief, the vaccine and placebo are dispensed as single dose and are for one time use only. The reconstituted vaccine and placebo, as well as the vial containing buffered diluent, should be inspected visually for any foreign particulate matter and/or abnormal physical appearance prior to administration. In the event either is observed, the vaccine or placebo will be discarded.

BRV-PV and placebo are for oral use only and should under no circumstances be injected. Neither vaccine nor placebo should be mixed with other medicinal products. Procedures for reconstitution are as follows:

- Remove plastic caps from the vials containing buffered diluent and lyophilized powder.
- Fix transfer adapter to 5 ml disposable syringe (without needle).
- Connect syringe/adapter to the vial containing buffered diluent.
- Pull the plunger back and aspirate 2.5 ml of buffered diluent from vial into the syringe.
- Remove the assembly from buffer diluent vial and connect it with the vaccine or placebo vial containing lyophilized powder.
- Inject the entire contents of the syringe into the vial.
- With syringe still attached shake the vial and examine for complete suspension of the powder. The reconstituted vaccine or placebo will appear as a clear, pinkish solution.
- Pull the plunger back and aspirate reconstituted solution from vial into the syringe.
- Remove the syringe from the transfer adapter.

Study vaccine and placebo must be administered within one hour of reconstitution.

Packaging and labeling. Each single oral dose of study vaccine and placebo will be approximately 2.5 ml in volume. Packaging for both study vaccine and placebo will contain 1 vial of lyophilized vaccine/placebo, 1 vial of citrate bicarbonate buffer, 1 adapter and 1 syringe (without needle) of 5ml capacity for vaccine reconstitution. Only the specific buffer diluent provided must be used for reconstitution.

The vials used for administration of the study drugs will be labeled according to the local regulations and requirements of the study protocol. Label text will be approved by the sponsor prior to label printing. All labels will contain the following minimum information**:**

- Name of Sponsor/Manufacturer
- Imprint “For Clinical Trial Use Only”
- Imprint “For Oral use only”
- Blind code to identify content

Finally, a vaccine vial monitor will be affixed to each vial to allow a visual indication of whether the product has been kept at the recommended temperature.

Storage. The study vaccine and placebo will be stored below 25°C up to the central facility. Changes in temperature outside the allowed range before arrival at the central facility-level will be immediately reported and any lots experiencing such out of range changes will be brought to the attention of the sponsor for determination of appropriate action.

After arrival at the central facility-level, study vaccine and placebo will be stored out of cold chain until administration. Ambient temperatures will be recorded on a daily basis at the Epicentre Maradi Weather Station to describe the conditions observed out of cold chain during the study period. Procedures for proper storage from the manufacturer (SIIL) to Niamey, the central facility level (Maradi) and to administration at the rural health facility (Madarounfa) will be fully detailed in the study SOPs.

Study supply and accountability. All study vaccine and placebo will be provided by SIIL. The sponsor will acknowledge receipt of the vaccine and placebo indicating shipment content and condition. The sponsor will maintain an inventory record of study product received and account for all study products used in the study using appropriate accountability records.

Temperature will be monitored during shipment, and the sponsor will check and maintain temperature records on file. The sponsor will inform SIIL immediately of any shipment that is out of range. The lot number of used study products will be recorded at the time of administration for accountability and used vials destroyed on site. All unused or partially used study products and packaging will be returned to SIIL or destroyed on site. The process for disposing of unused or partially used materials will be fully detailed in the study SOPs.

Choice of placebo comparator. Two vaccines are currently WHO prequalified, however, several issues arise with respect to their use in the evaluation of BRV-PV. A placebo-control, instead of an active comparator, has been selected for this trial given the following issues and ethical considerations (61, 62):

- It is known that the current oral vaccines have lower efficacy in low-resource settings than in high-resource settings. To inform eventual WHO pre-qualification and increased access to rotavirus vaccine, it will be important to demonstrate the absolute efficacy of new formulations in low-resource settings. A placebo-controlled design is needed to directly demonstrate absolute efficacy; an active-comparator design would determine relative efficacy and be less informative in the process towards WHO pre-qualification.

- There is no efficacy data on any licensed rotavirus vaccine in Niger, and a placebo-controlled trial remains defensible and appropriate where there is no “proven” intervention, as per the Declaration of Helsinki (Appendix D).

- Rotavirus vaccine is not part of EPI in Niger. As rotavirus vaccination is not a standard of care, ethical considerations permit the use of a placebo, instead of an active comparator (61). Although children in the placebo group will not potentially benefit from vaccination during the study period, all children will benefit from prompt, free and appropriate primary care, minimizing the risks and enhancing benefits to participants. If shown to be safe and efficacious and pre-qualified, the vaccine should be made available to all children in Niger through the national immunization program.

- A non-inferiority trial using an active comparator would require a prohibitively large sample size and involve the disproportionate use of time and resources. It would be practically infeasible to conduct such a trial, and the resulting impact of such an investment has been subject to debate and criticism due to the potential delay in bringing rotavirus vaccines to children who stand to benefit most.

- There is no rotavirus vaccine currently registered in Niger by the National Regulatory Authority, and current supply of the 2 WHO prequalified vaccines is constrained (51), suggesting no practical active alternative to placebo in this setting.

Randomization and blinding procedures. Infants will be individually randomized in a 1:1 ratio to receive three doses of the vaccine or placebo. The initial dose will be orally administered concomitantly with EPI vaccines at approximately 6-8 weeks of age. The second and third doses of study vaccine or placebo will each be administered following a 4 week interval (-1 to + 4 weeks), with a maximum age at last dose of 24 weeks.

A list of unique identification numbers from 1 to 5138 will be allocated to vaccine or placebo by SIIL using a computer-generated random number list using permuted blocks of random sizes. Block sizes will not be disclosed to reduce predictability of the random sequence and ensure allocation concealment. Study physicians who will oversee randomization will be given a subset of sequentially numbered, sealed, opaque envelopes that will be prepared by SIIL and contain printed randomization numbers and a corresponding group code. The study physician will be instructed to assign the randomization number and group code noted in the next envelope to each eligible infant as (s)he is enrolled. Adherence to the randomization list will be periodically verified by the Study Monitor. The randomization list will remain with SIIL for the duration of the study; randomization will therefore be conducted without any influence of sponsors or field personnel.

Vaccine and placebo packages will be labeled with the assigned code and delivered to the study site in otherwise identical presentations. Vaccine and placebo presentation and packaging will be indistinguishable to insure that caregivers and investigators can not identify to which group the infant has been assigned. Group assignment will remain concealed from study team, investigators and parents of participating infants for the whole study period. The Data and Safety Monitoring Board (DSMB) and sponsor statistician will be masked to the group assignment. The DSMB will remain masked unless otherwise deemed necessary by the DSMB members. Investigators conducting the final analysis will remain masked to the group assignment until the end of the analysis.

The study code will be broken only in case of a medical event in which the Medical Coordinator deems the participant cannot be appropriately treated without knowing his/her group assignment. A set of sealed envelopes with group assignment will be held at the field site with the Medical Coordinator. All code breaks will be fully documented and reported to the sponsor within 48 hours, and the date, time and reason for unblinding will be noted. Codes will not be freely available to the sponsors or other study personnel until after the completion of the trial and final data review.

Unblinding will not be sufficient reason for individual discontinuation from the study.

Target population. The study will be performed in infants in Madarounfa, Niger. Healthy male and female infants meeting the following inclusion criteria are eligible for enrollment:

(1) aged 6-8 weeks at the time of inclusion
(2) able to swallow and no history of vomiting within 24 hours
(3) resident in Madarounfa Health District within 15 km of central health facility
(4) intending to remain in the study area for 2 years
(5) parent/guardian providing informed consent

There will be no restriction based on breast feeding around the time of vaccination, receipt of routine pediatric vaccinations, prematurity, low birth weight or HIV status (63). Inability to swallow and history of vomiting within the last 24 hours are the only conditions based on the infant's immediate clinical status to delay oral administration of the study vaccine or placebo.

Exclusion criteria will include the following:

(1) unable to swallow or history of vomiting within 24 hours
(2) known history of congenital abdominal disorders, intussusception, or abdominal surgery
(3) receipt of intramuscular, oral, or intravenous corticosteroid treatment within 2 wks
(4) receipt or planned administration during the study period of a blood transfusion or blood products, including immunoglobulins
(5) non-resident in Madarounfa Health District within 15 km of central health facility
(6) not intending to remain in the study area for 2 years
(7) parent/guardian informed consent not provided
(8) any other condition in which, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or the parent/guardian's ability to give informed consent*.

*While determining eligibility, study physicians will be asked to use good clinical judgment in considering a participant's overall fitness for inclusion. Some participants may not be appropriate for the study even if they meet all inclusion criteria. For instance, medical, occupational or other conditions of the caregiver may make routine home visits and evaluation difficult or make the child a poor candidate for retention.

Discontinuation criteria. The following criteria will be checked at each Weekly Home Visit and if any becomes applicable the participant will be required to discontinue vaccination and/or follow up:

(1) Serious adverse event related to the study intervention
(2) Use of any investigational or non-registered vaccine other than the study vaccine during the study period
(3) Administration of immunosuppressant for > 14 days during the study period
(4) Administration of any blood products during the study period
(5) Parent/guardian consent withdrawal
(6) Permanently migrated or moved from the study area
(7) Loss to follow up

(8) Opinion of the study sponsor that it is not within the subject's best interest to continue vaccination or follow up

The Medical Coordinator will discuss any potential discontinuation with the PI and sponsor. The sponsor will have final authority to discontinue vaccination and/or follow up of any participant according to the above criteria.

In the event of a participant's discontinuation in the study, an Early Termination Visit will be performed by the Medical Coordinator. The Early Termination Visit will include a review of address and contact details; physical examination; update of relevant medical history and intervention; review of vaccination history; and documentation of reason(s) for termination. In the event of a Serious Adverse Event leading to discontinuation, the child will be followed until resolution of the event and/or the end of the study (whichever is earlier). A participant's parents/guardian can withdraw consent for participation in the study at any time without prejudice.

After discontinuation, follow up for all endpoints will cease, and any participant discontinued from the study will not be replaced. Data collected up to the point of last contact will be included in the analysis. Subjects who are discontinued due to migration or lost to follow up but later present and are willing to continue participation will continue to be followed until 2 years of age, and all available data will be included in the analysis.

Study sites. Study activities will take place in the Madarounfa Health District, Maradi, Niger. Health facilities in the Madarounfa Health District, including 1 hospital, 8 health centers and 25 health posts in the 8 rural health zones of Gabi, Safo, Madarounfa, Dan Issa, Tofa, Serkin Yama, Djirataoua, and Moulé will be included as Surveillance Sites, where a study physician or nurse will be present to assess symptoms of gastroenteritis and adverse events. Of the 34 Surveillance Sites, a limited number of facilities will be designated as Enrolment and Dosing Sites. Enrolment and Dosing Sites will be staffed by study physicians trained to seek informed consent and equipped with appropriate emergency medical treatment in the event of any acute serious adverse event post-immunization. The number and location of Enrolment and Dosing Sites will be made to insure reasonable access by the study population and in consideration of available human resources.

Additional study activities will be take place at the participants' home (see Recruitment and Follow up for further detail).

Recruitment. Rotavirus in Niger is known to circulate year-round in the Maradi region (with a consistent peak in October to November), therefore enrolment will occur year-round. Eligible children will be continuously identified during an 18-month enrollment period using community-based recruitment monitors selected from each village by village representatives, often being a women's group leader or midwife. These individuals selected as recruitment monitors are intended to be well regarded and trusted members of the community with broad knowledge of community events, such as births, deaths and migration. Beginning two months

before study initiation, the recruitment monitor will begin to record all pregnancies in his/her village. The registry will be routinely updated, and notice of the time and place of any live birth will be immediately transferred to study staff as they occur during the enrollment period.

Within 48 hours of a live birth, a study nurse will visit mothers at home for a **Pre-randomization Visit**, at which time the nurse will provide the household with information about the study. An informational sheet with details of the study will be provided and discussed with caregivers and heads of households. The Pre-randomization Visit will allow caregivers adequate time to consider their participation in the trial and prepare any questions they may have, thereby reducing the number of defaulters once randomization has occurred. At this time, informed consent will be sought specifically to allow recording of maternal mid-upper arm circumference (MUAC), as well as infant birth weight and gestational age at birth, using standard field procedures. Informed consent to participate in the full trial will be sought at the time of randomization. Infants < 6 wks of age who migrate into the study area will be identified by recruitment monitors and similarly visited at home by a study nurse for a Pre-randomization Visit and confirmation of the child's age. At all Pre-randomization Visits, the study nurse will confirm receipt of routine vaccinations scheduled for administration at birth [e.g. Bacillus Calmette-Guérin and oral poliovirus vaccine] through review of the infant's vaccination card, and as needed, invite the caregiver to have the child vaccinated at home or the nearest health facility.

At 6-8 weeks of age, the recruitment monitors will ask interested caregivers to present with their child to the nearest Enrollment and Dosing Site for the **Randomization Visit**. At this time, a study physician will confirm eligibility with an assessment of age, residence and medical history and will seek informed consent from the parent/guardian for the child's participation in the full study. Issues of confidentiality will be underscored. No financial or non-financial incentives will be provided to study staff for enrolment.

If during the eligibility evaluation a child is found to be unable to swallow or to have history of vomiting within 24 hours but is otherwise eligible for study inclusion, the study physician can delay randomization. The caregiver and infant will be asked to return to the Enrollment and Dosing Site 24 hours after symptoms resolve but before 8 weeks of age for randomization and administration of Dose 1. If a child is found to be entirely ineligible for inclusion, the child will be recorded as an exclusion on an Eligibility Evaluation Case Report Form (CRF), with the reason for exclusion noted.

Once informed consent for participation in the full trial is provided by the parent/guardian during the Randomization Visit, the child will be enrolled in the study and a randomization number assigned. Study procedures, to be detailed in the study SOPs, are as follows:

- Administration of study intervention (Dose 1): The first study dose will be administered at the health facility according to the study's randomization procedures. A Dosing CRF will be completed to record information on the study product received, date and time. If a child vomits any of the study product immediately after administration, a new dose will be

administered and the event will be recorded on the appropriate CRF. A repeat dose will not be administered if the child drools or spits up any of the study product. Study staff will remain with the infant for a minimum of 30 minutes to record any acute serious adverse event post-immunization. Any reactions that occur during this time will be recorded on the standardized Dosing CRF, and appropriate emergency medical treatment will be readily available in case of a rare reaction following administration of a study product. Breastfeeding 30 minutes before or after administration of the study product will be documented on the Dosing CRF.

- Administration of EPI vaccines: During the Randomization Visit, the study physician will verify previous receipt of any EPI vaccine through review of the infant's vaccination card. As needed, the study physician will concomitantly administer any required routine EPI immunization and update the child's vaccination records (see Standard Care below for schedule of EPI vaccines to be provided). Note: Supply of EPI vaccines will be supported by the study sponsor, but if any EPI vaccine is not available at the time of study dosing, administration of the study intervention will not be delayed. Children who have received EPI vaccines prior to randomization remain eligible to receive the study intervention at this time.

- Collection of background information: A detailed background questionnaire will be administered to the caregiver, including information on maternal age, nutritional status (e.g. weight, height and MUAC) and reproductive history (e.g. parity, age of first delivery); household size and socio-demographic characteristics; infant's clinical and breast feeding history; and infant anthropometry.

- Collection of biological specimens (sub-sample of participants and caregivers): Blood and breast milk specimens will be collected for testing of anti-rotavirus IgA concentration from a sub-sample of infants and breastfeeding mothers, respectively (see Laboratory Assessment below).

Note: If a child is found to have gastroenteritis at the time of randomization, randomization and administration of Dose 1 will not be delayed but a stool sample will be collected for detection of rotavirus antigen. HIV status will not be systematically assessed by study staff during the Randomization Visit.

After all procedures of the Randomization Visit have been completed, the caregiver will receive a study identification card, which should be shown at the time of presentation to any health facility and will include study contact information in case of emergency. At this time, the study physician will remind the participant's caregiver of the next scheduled home visit.

The recruitment period will extend over 18 months. We anticipate recruiting 65 infants per week in 8 rural zones of the Madarounfa Health District [e.g. Gabi, Safo, Madarounfa, Dan Issa, Tofa, Serkin Yama, Djirataoua, and Moulé], where there were 9021 live births in 2013(59).

Dosing Visits. A three-dose series of study vaccine or placebo will be orally administered to infants with the initial dose given at time of the Randomization Visit (6-8 weeks of age) and each

subsequent administration given after a 4-week interval (-1 to +4 weeks) . Maximum age at last dose is 24 weeks. All doses of study vaccine or placebo will be administered at a designated Enrollment and Dosing Site by a trained study physician. If a child vomits any of the study product immediately after administration, a new dose will be administered and the event will be recorded on the appropriate Dosing CRF. A repeat dose will not be administered if the child drools or spits up any of the study product. Study staff will remain with the infant for a minimum of 30 minutes to record any acute serious adverse event post-immunization. Any reactions that occur during this time will be recorded on the standardized Dosing CRF, and appropriate emergency medical treatment will be readily available in case of any reaction following administration of a study product. Breastfeeding 30 minutes before or after administration of the study product will be documented on the Dosing CRF.

If child is found to be unable to swallow or have history of vomiting within 24 hours of a scheduled Dosing Visit (Dose 2 or Dose 3), the study physician can delay administration of the study intervention for a maximum of 8 weeks from administration of the previous dose, respecting a maximum age at last dose of 24 weeks. The caregiver and infant will be asked to return to the Dosing Site 24 hours after symptoms resolve within this interval for the next administration of the study intervention. If a child is found to have gastroenteritis at the Dose 2 or Dose 3 Visit, dosing will not be delayed but a stool sample will be collected for detection of rotavirus antigen.

Study vaccine or placebo will be administered concomitantly with routine EPI immunizations. At each Dosing Visit, the study physician will verify previous receipt of any EPI vaccine through review of the infant's vaccination card, and as needed, concomitantly administer any required routine EPI immunization (see Standard Care below for schedule of EPI vaccines to be provided). Administration of the study intervention will not be delayed if EPI vaccines are unavailable at the time of study dosing. Infants who have already received EPI vaccines at the time of study dosing (e.g. oral polio vaccine administration provided through routine mass vaccination campaigns) will still receive the study intervention as scheduled.

If a child does not present to the health facility for a subsequent Dosing Visit as scheduled, the study team will conduct a home visit to encourage the caregiver to present to the health facility as soon as possible. If the child cannot be seen within -1 to +4 weeks of the scheduled dosing visit, follow up will be handled on a case-by-case basis in coordination with the Medical Coordinator and sponsor.

Assessment of compliance. Study vaccine or placebo will be orally administered by trained study physicians only to infants included in this study. The date, dosage, and time of the vaccinations will be recorded on a standardized Dosing CRF. The Study Field Coordinator will track vaccines received, used and wasted and will manage all unused or expired products.

Follow up. All children will be followed from the time of the first dose until 2 years of age (gastroenteritis and serious adverse events) or from the time of first dose to 28 days post-Dose 3 (adverse events) using both facility- and weekly home-based follow up.

Caregivers will be informed about the signs and symptoms of gastroenteritis and all adverse events, and they will be asked to immediately seek care at a local facility if there is any condition that requires medical attention or is of concern. Upon presentation at the facility, study staff will conduct a medical history of symptoms and medical interventions received for the current illness and perform a clinical exam to document physical signs and clinical indicators of disease severity. Appropriate medical care will be provided in accordance with GCP. If the episode is ongoing at the time of discharge from the health facility, daily home-based follow up by a nurse assistant will organized from the time of discharge until resolution of the episode.

In addition, **Weekly Home Visits** will be performed by a community health agent. At each weekly visit, the community health agent will check discontinuation criteria, and as appropriate, conduct a general physical exam to assess the participant's clinical status, ask caregivers to recall all medical signs or symptoms that are a concern to them at that time, and review the Weekly Diary Card completed by caregivers to confirm information on events since the last visit, including cases of gastroenteritis, adverse events (from first dose to 28 days post-Dose 3), serious adverse events, and medical intervention (including treatments or other vaccinations received, and admission to hospital or therapeutic nutritional programs after 6 months of age). At each weekly visit, the community health agent will remind caregivers to immediately contact the study team in the event of any episode of gastroenteritis or adverse event.

Children found to require medical intervention during the Weekly Home Visit will be referred to a study health facility for management free of charge by study staff and in accordance with GCP. Every 4 weeks, the child's anthropometric status (e.g. weight, height and MUAC), breastfeeding status (e.g. ever vs. never; currently exclusive vs. partial vs. no breastfeeding; frequency of feeds in last 24 hours) and dietary intake will be assessed at home using standard procedures with assistance from the caregiver. If a child is not present at the time of a Weekly Home Visit, the community health agent may contact neighbors or relatives in the area to ascertain the infant's vital and health status and expected time/day of return so that a repeat visit may be scheduled. After all procedures of the Weekly Home Visit have been completed, a reminder will be given about the next scheduled study visit. HIV counseling and testing will not be systematically provided by study staff during the Weekly Home Visit but may be offered as clinically appropriate.

Outcome definition and assessment. Gastroenteritis. Gastroenteritis will be defined as the passage of three of more loose or watery stools within a 24 hour period and/or forceful vomiting. Severe gastroenteritis will be defined clinically as an episode of gastroenteritis that needed overnight treatment in hospital and/or rehydration therapy equivalent to the WHO Plan B (oral rehydration therapy) or Plan C (intravenous rehydration therapy) in health facility, or using the 20-point Vesikari scale (53), where an episode of gastroenteritis with a score of 11 or more is considered severe. A gastroenteritis episode will be considered to be caused by rotavirus if a rotavirus strain is identified in a stool sample collected within 7 days on onset of

symptoms. Gastroenteritis episodes will be classified as two separate episodes if there is an interval of 5 or more consecutive, symptom-free days between the episodes.

Cases of gastroenteritis episodes will be captured through facility- and home-based surveillance from the moment the first dose of vaccine or placebo is administered until 2 years of age. Through facility-based surveillance, one study nurse or physician will be assigned to each health facility able to receive children for gastroenteritis serving the study population of Madarounfa (e.g. 1 hospital, 8 health centers and 25 health posts). Surveillance at all levels of health facilities has been selected for case detection based on available evidence regarding health care seeking behavior in Maradi. In 2009, we conducted a household survey including 2940 children < 5 years of age in 4 districts of Maradi to assess health care utilization practices for the treatment of childhood diarrhea (64). The survey found 37% (95% CI: 34-40%) of children had at least one episode of diarrhea in the previous 1 month and over 70% of cases were managed at a health structure. Lower level health facilities were found to be more frequently visited than hospital for diarrhea management, with 54% of severe diarrhea cases presenting to a health center, 26% to a health post and 11% to a hospital. This evidence suggests that hospital-based case detection alone would be inadequate in this context, and we therefore propose inclusion of health facilities at all levels, in combination with home-based surveillance, for complete case detection.

To encourage presentation at health facilities, caregivers will be informed about the signs and symptoms of gastroenteritis and will be asked to seek care at a local facility if any episode of gastroenteritis is suspected. Compensation for travel to the local facility will be provided for each visit made. Caregivers will be instructed to show the study identification card provided at randomization at the time of presentation to the facility that will identify the infant as a participant in the study. At the facility, the study staff assigned to that facility will collect a stool sample if the onset of the loose or watery stool and/or forceful vomiting is within 7 days, record history of symptoms and medical intervention received for the current illness through caregiver interview, and conduct a clinical exam to document physical signs and clinical indicators of disease severity. Documentation of clinical indicators will include temperature, the quantity and duration of vomiting and/or diarrhea episodes, dehydration status, and treatment in order to assign a Vesikari score. If stool is not available within 2 hours of presentation, a rectal swab will be taken. After discharge from the facility, all continuing gastroenteritis episodes will be followed at home by a nurse assistant on a daily basis until resolution (defined as ≥ 5 consecutive, symptom-free days). If the episode does not resolve within 7 days of discharge, the nurse assistant will refer the child back to a study facility for follow up. Community sensitization and outreach with community leaders, traditional medicine providers and others will be continuously reinforced to facilitate referral of sick children to health facilities throughout the study period. If an episode of gastroenteritis (e.g. ≥ 3 loose or watery stools within a 24 hour period and/or forceful vomiting) is identified before or on the day of presentation to the facility , all data collected during the episode will be transcribed to a standardized Gastroenteritis Surveillance CRF by study physicians after resolution of the episode.

A home-based surveillance system will be used to identify episodes of gastroenteritis for which a caregiver chooses not to present to a health facility. Caregivers will be advised to immediately inform their community health agent whenever there are 3 or more loose or watery stools within 24 hours and/or forceful vomiting. Caregiver communication of gastroenteritis episodes to community health agents will be reinforced through continued education intended to maintain caregiver awareness and interest in study activities and gastroenteritis surveillance. Episodes not immediately reported to the community health agent will be captured during review of the Weekly Diary Card at the Weekly Home Visit with caregivers. Once an episode of gastroenteritis is identified by a community health agent (either through caregiver notification intermediate to a scheduled Weekly Home Visit or during a scheduled Weekly Home Visit), it will be referred to a nurse assistant, who will monitor the episode with daily home visits until resolution (defined as ≥ 5 consecutive, symptom-free days). At each visit, the nurse assistant will complete a standardized Case Surveillance Card with the caregiver to collect information on the number of stools and vomiting episodes, temperature, dehydration status, medication or rehydration administered, and any medical attention sought (defined as medical personnel or facility contact, advice, visit or admission). Information from the Case Surveillance Card will be transcribed to a standardized Gastroenteritis Symptom CRF by study physicians at the resolution of the episode and will be used to determine the severity of gastroenteritis episodes not presented to a health facility using the Vesikari scale. If onset of an episode of gastroenteritis is within 7 days of a nurse assistant's home visit, the nurse assistant will collect the necessary stool sample (one per episode) and ensure appropriate conservation and transport to the Maradi laboratory on the same day. If any gastroenteritis episode is found to require medical attention, the nurse assistant will refer the child to a study physician to ensure appropriate medical care is received.

Adverse events. Adverse events (AEs), including fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in the study SOPs), diarrhea, vomiting, decreased appetite, decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia), will be assessed using facility- and home-based surveillance in all participants from the time of the first dose until 28 days post-Dose 3. Caregivers will be informed about the signs and symptoms of adverse events and will be asked to seek care at a local facility in the event any adverse event is suspected and of concern. At the facility, study staff will record history of symptoms of the current illness through caregiver interview and conduct a clinical exam to document physical signs and clinical condition.

In home-based surveillance, caregivers will be trained to complete an Adverse Event Diary Card to record adverse events and be advised to immediately inform their community health agent when an adverse event is suspected. Events not reported to the community health agent will be captured during the Weekly Home Visit, when the community health agents will review the Adverse Event Diary Card and confirm information on the incidence of all adverse events and related medical interventions received from the first dose until 28 days post-Dose 3. Once an adverse event is identified during this period (either through caregiver notification intermediate to a scheduled Weekly Home Visit or during a scheduled Weekly Home Visit), it will be referred

to a nurse assistant, who will follow the case with daily home visits until resolution. Nurse assistants will refer all adverse events that require medical attention, progress clinically or do not resolve within 7 days to a study physician to ensure appropriate medical care is received.

Serious adverse events (SAE), including any new health-related problem that results in disability, incapacity or death, necessitates hospitalization, or is life-threatening, and according to the Brighton Collaboration, generalized convulsive seizure; hypotonic-hyporesponsive episodes; intussusception (see Intussusception Risks, Assessment and Management below), and persistent crying (54, 55), will be assessed in all participants from the time of first dose until 2 years of age through facility- and home-based surveillance. Caregivers will be informed about the signs of SAEs and will be asked to seek care at a local facility if any is suspected. At the facility, study staff will record history of symptoms of the current illness through caregiver interview and conduct a clinical exam to document physical signs. As with cases of gastroenteritis and adverse events, a standardized Weekly Diary Card will be completed by caregivers and reviewed by community health agents in home-based surveillance to document the incidence of any SAE not attended to at a health facility. SAEs will be followed up until the event resolves, stabilizes, or is otherwise explained by the Medical Coordinator, who will determine whether the event is causally associated with vaccination.

Immunogenicity. Immune response to vaccination will be assessed in a total of 420 infants. Venous blood samples will be collected at 4 time points at a health facility for measurement of concentrations of anti-rotavirus IgA: at inclusion and 28 days following each study dose. Sero-conversion will be defined as ≥ 3 fold rise in serum titre of anti-rotavirus IgA from baseline to 28 days after receipt of vaccine. The primary immunogenicity endpoint will concern sero-conversion between Dose 1 and 28 days post-Dose 3. Sero-conversion after Dose 1 and Dose 2 will be estimated in secondary analyses as the difference between Dose 1 and 28 days post-Dose 2 and post-Dose 3, respectively. Differences in geometric mean titres between baseline and 28 days following each dose will be considered in secondary analyses.

Table 3. Summary of follow up and assessment schedule

	Pre-randomization visit	Randomization and Dose 1 facility visit	Dose 2 facility visit	Dose 3 facility visit	Dose 3 + 28 Days facility visit	Weekly home visit	Facility visit	Year 2 (End of follow-up)
PRE-RANDOMIZATION								
Informed consent for pre-randomization assessment	X							
Birth weight, gestational age and maternal MUAC	X							
ENROLMENT								
Eligibility evaluation		X						
Informed consent for trial participation		X						
Randomization		X						
INTERVENTION								
Vaccine		X	X	X				
Placebo		X	X	X				
ASSESSMENTS								
Background questionnaire		X						
Anthropometry		X	X	X	X	X*		X
Diary card for gastroenteritis, AE, SAE			X	X	X	X		X
Clinical exam and history		X	X	X	X	X	X	X
Post-immunization safety assessment		X	X	X				
Review of discontinuation criteria			X	X	X	X	X	
Stool sample						X**	X**	
Venous blood sample (sub-sample)		X	X	X	X			
Breast milk sample (sub-sample)		X						

*Height, weight and MUAC recorded at home every 4th weekly visit until 2 years of age.
** In the event of gastroenteritis reported within 7 days of onset of illness.

Laboratory assessment. ***Stool***. Stool samples will be collected in health facilities at the time of presentation for gastroenteritis, and at home for any gastroenteritis identified within 7 days of onset. A minimum of 5-10 ml of stool (if watery; 5 gm if semi-solid) will be collected using clean, dry, leak-proof plastic containers or, if no stool is available during the observation period, using a rectal swab. Specimens will be transported in freezer packs at 2-8^{o} C to the Epicentre laboratory in Maradi on the same day. Upon arrival at the Maradi laboratory, designated laboratory personnel will verify the stool container contains an adequate amount of specimen and is adequately labeled before storing at -20^{o} C or colder. If the sample is deemed inadequate, a request will be made to the nurse assistant for an additional sample. Rotavirus

antigen in stool will be detected by enzyme immunoassay (Premier Rotaclone®) by the Epicentre Maradi laboratory. All rotavirus positive stool samples will be shipped to the CERMES laboratory in Niamey for testing by reverse transcriptase PCR followed by reverse hybridization assay and sequencing to identify G and P types. The Study Field Coordinator will monitor specimen handling, storage and transport throughout the trial. Sample SOPs for enzyme immunoassay and reverse transcriptase PCR analysis are provided in Appendix B. Testing for other enteric pathogens will not be systematically conducted during the study period, though stool samples may be stored for later analysis.

Venous blood. For immunogenicity assessment, 3-4 ml of venous blood will be collected from a random sample of 420 infants to determine the serum concentrations of anti-rotavirus IgA antibodies at the Randomization Visit, at the Dose 2 and Dose 3 Visits (± 7 days); and 28 days (± 7 days) after Dose 3 Visit. Participants in the immunogenicity cohort will be randomly selected [35 per month] from the full cohort over a 12-month period to allow inclusion of an approximately equal number of subjects receiving study vaccine and placebo across all seasons Procedures for random selection of the immunogenicity cohort will be provided in the study SOPs. All blood samples will be separated into sera within one hour of arrival from the field and sera will be aliquoted and stored at -20^{o}C until shipment for analysis. Samples will be shipped on dry ice to the Christian Medical College, Vellore, India laboratory, where they will be analyzed for anti-rotavirus IgA by enzyme immunosorvent assay using 1% blotto. Sample SOPs for anti-rotavirus IgA by enzyme immunosorvent analysis in serum are provided in Appendix B.

Interference of the study vaccine with immunogenicity of concomitantly administered oral polio vaccine will also be tested in the immunogenicity sub-sample (n = 420). Anti-poliovirus types 1, 2 and 3 antibody serum titres will be measured using a virus micro-neutralisation test by the Christian Medical College, Vellore, India laboratory. Results will be used to evaluate if sero-conversion for the polio antibody response is non-inferior between the vaccinated and placebo groups.

Breast milk. Immune factors in breast milk at the time of immunization have been hypothesized to contribute to lower immunogenicity of live oral rotavirus vaccines in developing countries(44, 65, 66), therefore we will assess concentrations of maternal anti-rotavirus IgA antibodies in breast milk in order to compare immunogenicity by level of maternal anti-rotavirus IgA concentration. Breastfeeding mothers of children included in the immunogenicity sub-sample (n = 420) will be asked to provide breast milk samples at the time of randomization. Samples of 5 to 10 ml will be requested in order to measure concentrations of maternal anti-rotavirus IgA antibodies by enzyme immunosorvent assay. Samples will be stored at -20^{o}C until shipment to the Christian Medical College, Vellore, India laboratory for analysis.

Participant retention. Once an infant is randomized, the study team will make every reasonable effort to follow the infant for the entire study period. It is projected that the annual rate of loss-to-follow-up will be 10%.

The sponsor will be responsible for developing study SOPs to achieve this level of follow-up. The Pre-randomization Visit is organized to allow caregivers adequate time to consider their participation in the trial and prepare any questions they may have, thereby reducing the number of defaulters once randomization has occurred. At each household contact, study staff will provide a reminder of the next visit, and if a child is ever not present at the time of a Weekly Home Visit, the community health agent can contact neighbors or relatives in the area to ascertain expected time/day of return so that a repeat visit may be scheduled. In addition, study teams will maintain community interest in the study through periodic sensitization of community leaders and provide feedback to caregivers on child growth and development. Preventive interventions (e.g. soap, bed nets) will be regularly provided as in-kind motivation to participating households (see Reimbursement below).

Standard care. During regular study visits, study staff will verify previous receipt of any EPI vaccine through review of the infant's vaccination card, and as needed, administer routine EPI vaccinations free of charge. Vaccines to be provided include: Bacillus Calmette-Guérin (birth); oral poliovirus vaccine (birth, 6, 10 and 14 weeks); and the combined pentavalent vaccine including diphtheria-tetanus-whole cell pertussis, haemophilus influenzae type b and hepatitis B (6, 10, and 14 weeks). Stock of measles and yellow fever vaccination, recommended at 9 months of age, will be insured by the study sponsor and all participants will be invited to receive vaccination at a study facility at 9 months. For all other medical intervention to be provided during the study period, study children will be referred to study staff in a FORSANI-supported health facility for management free of charge and in accordance with GCP. FORSANI currently supports the Ministry of Health to provide pediatric care to all children aged 0-5 y in the Madarounfa Health District.

Sample size. Assuming a 2% attack rate of severe wild-type rotavirus gastroenteritis at 1 year, a 50% true vaccine efficacy and a 20% participant non-assessibility (including withdrawal and loss to follow up), the study will enroll 5138 children to have at least 90% power to detect a 95% confidence interval for vaccine efficacy at 1 year that would be above 0% (67).

Assuming a sero-conversion rate of 30% in the placebo group, 20% non-assessibility (including withdrawal and loss to follow up) and 30% exclusion due to detection of wild-type rotavirus disease between vaccine doses, we will assess immunogenicity in 420 children to have 90% power to detect a 20% difference in the proportion of children that sero-convert (68).

Data analysis. The primary analysis will be a per-protocol analysis of vaccine efficacy of three dose pentavalent vaccine vs. placebo, including children who received the complete vaccination course and entered the efficacy surveillance with no evidence of previous wild-type rotavirus infection. The primary analysis will exclude participants with a laboratory confirmed wild-type rotavirus disease earlier than 28 days post-Dose 3 of vaccine or placebo. Infants who have at least one episode of severe wild-type rotavirus gastroenteritis during the period from 28 days after the last dose until the date the infant reaches 1 year of age will be considered as having achieved the primary outcome. Only gastroenteritis episodes in which wild-type rotavirus (i.e., other than the vaccine strain) is identified in a stool specimen will be included as an event in the

primary efficacy analysis. Gastroenteritis episodes that cannot be classified as rotavirus gastroenteritis or non- rotavirus gastroenteritis with certainty because of incomplete data will be considered as not having achieved the primary outcome. For participants with more than one episode of severe rotavirus gastroenteritis, only the first episode will be counted.

Vaccine efficacy with 95% confidence interval will be calculated as $(1-IR_1/IR_0)*100$, where IR_1 is the person-time incidence rate in the vaccinated group and IR_0 is the person-time incidence rate in the placebo group. 95% confidence intervals will be derived from the exact confidence interval using the conditional binomial distribution. The cumulative hazard of a first episode of severe rotavirus gastroenteritis between groups will be estimated as a minus-log transformation of the Kaplan–Meier survival curve, with the P value calculated using the log-rank test. The proportions of infants having at least one gastroenteritis episode will be compared between groups by Fisher's exact test and expressed in terms of relative risk. The number of events prevented by 100 vaccinated infant-years will be obtained from 100 times the difference in the incidence rate; the associated confidence interval will be derived using the method of Zou and Donner (69).

Secondary analysis of efficacy will be done for efficacy against severe rotavirus gastroenteritis during the second year of life and for the combined period from 28 days after the final dose until 2 years of age; rotavirus gastroenteritis of any severity; rotavirus gastroenteritis with a Vesikari score of ≥ 17; rotavirus gastroenteritis by strain [vaccine-contained and non-vaccine G and P types]; gastroenteritis of any cause; longitudinal prevalence of rotavirus gastroenteritis; hospitalization due to rotavirus gastroenteritis; and hospitalization for any reason. In strain-specific analyses of efficacy, when more than one G type is isolated for an episode, the episode will be counted in every G type category. Exploratory analyses will assess sensitivity of all analyses to the method of case detection (facility- vs. home-based surveillance).

Intention-to-treat (ITT) analyses will be done including all participants who were vaccinated with at least one dose of vaccine or placebo and follow up beginning from the time of enrollment. Sample size allowing, vaccine efficacy will be calculated among children receiving a total of 1 or 2 doses to determine whether vaccine confers protection to infants before completion of the 3-dose regimen.

The analysis of safety will be done using the ITT population, i.e. all infants who received at least one dose of the study vaccine or placebo, and include follow up from the time of enrollment until 28 days post-dose 3 (adverse events) or the end of follow up (serious adverse events). The incidence of adverse and serious adverse events will be compared between groups with the two-sided asymptotic score test for the null hypothesis of identical incidence by group.

The analysis of immunogenicity will be done on the basis of the per-protocol population for whom immunogenicity data are available. Participants with laboratory confirmed wild-type rotavirus disease between vaccine doses will not be included. Sero-conversion rates 28 days following each dose will be calculated with corresponding 95% confidence intervals using the binomial distribution. Differences in geometric mean titres of serum anti-rotavirus IgA

between pre-Dose 1 and 28 days following each dose will be measured with 95% confidence intervals assuming a normal distribution of log-transformed means. To assess individual-level correlates of protection, Receiver Operating Characteristic (ROC) analyses will be used to estimate the IgA threshold most predictive of protection against severe rotavirus gastroenteritis. To explore potential mediating factors of immunogenicity in this setting, sero-conversion and log-transformed geometric mean infant IgA titres 28 days post- Dose 3 will be compared by level of maternal anti-rotavirus IgA antibodies pre-immunization using the chi-square and t-test, respectively. To assess the potential interference of the study vaccine with concomitant oral polio immunization, the statistical difference in anti-poliovirus antibody serum titres 28 days post- Dose 3 will be compared by study intervention using the chi-square test.

There are numerous risk factors for the primary endpoint which will be measured at the time of randomization, including medical history, birth weight, breastfeeding practice, and indicators of socioeconomic status. Randomization ensures that, on average, study groups will be balanced with respect to all of these risk factors, but we realize that this may not be true in any given randomized study. Thus, we will collect additional background data and compare baseline characteristics between groups to identify any significant independent imbalances. Secondary analyses will assess the group effect after adjusting for the risk factors associated with imbalances at the $P < 0.20$ significance level.

This study makes no pre-specified hypotheses regarding *a priori* effect modifiers and is therefore not powered to detect any effect modification which may occur. We acknowledge that unless there is strong modification of an observed intervention effect, the power of our study to detect effect modification will be low. However in analyses for all endpoints, we will consider whether vaccine effects are modified by the following baseline characteristics: child sex; breastfeeding practice (exclusive / partial / none;); gestational age (± 37 weeks); birth weight (± 2500 g) ; breast milk IgA antibody concentration; child serum anti-rotavirus IgA concentration at Dose 1; season of inclusion; concomitant administration of oral polio vaccine; and child anthropometry. To assess the statistical significance of each interaction, we used the Wald test for risk-ratio homogeneity in the risk analyses.

All P values will be 2-sided with $P < 0.05$ considered statistically significant. No adjustment for multiple comparisons will be made as this study considers a single primary endpoint, vaccine efficacy at 1 year at age. All other analyses of secondary endpoints would be best regarded as exploratory, and any significant findings for these endpoints would need to be confirmed. Missing data will be assumed to be missing at random. Sensitivity analyses will be conducted to assess the robustness of trial results under other methods to handling missing data (e.g. missing indicator, last observation carried forward). Data analysis will be conducted using SAS software (version 9.2).

Data collection, management and quality assurance. All the information required by the study protocol will be entered on standardized CRFs provided by the sponsor in French. The Study Field Coordinator and Medical Coordinator will validate all CRFs for completeness and accuracy, signing and dating each to attest to his/her responsibility for the quality of all data recorded and

that the data represents a complete and accurate record of each child's participation in the study. Source documents and CRFs will be maintained at the study site in a secure location to ensure confidentiality and will be available for review by the Study Monitor to ensure all collected data are consistent with the CRFs.

All validated CRFs will be double-entered, compared and verified for accuracy in Maradi, Niger. Data quality will be enforced through a variety of mechanisms, including referential data rules, valid values, range checks, and consistency checks against data already stored in the database (i.e., longitudinal checks).

A data validation plan will be prepared by the sponsor before study initiation. Errors will be detected by programs designed to detect missing data or specific errors in the data. These errors will be summarized along with detailed descriptions of the specific problem in a Data Query Report, which will be sent to the Study Field Coordinator and Medical Coordinator for resolution by checking the original forms for inconsistency, checking other sources to determine the correction, and modifying the original (paper) form as appropriate. Written documentation of changes will be available via electronic logs. A complete back up of the study database will be performed twice a month; incremental data back-ups will be performed on a daily basis. The data manager will provide monthly email reports with information on missing data, missing forms, and missing visits to the sponsor. The primary sponsor alone will have full access to the data.

Timeline. The estimated total duration of the study is approximately 54 months. Given Epicentre's established field presence in Maradi and experience with rotavirus surveillance in the region, we anticipate 6 months will be used for study preparation, including development of SOPs and CRFs, ethical clearance, recruitment and training of field personnel, initiation of the community-based birth registry and facility-based surveillance system and pilot testing. Recruitment of 5138 infants will take 18 months and each child will be followed for two years. Therefore, the duration from enrollment of the first participant until the end of follow-up of the last participant enrolled will be 42 months. Analysis and write-up will be completed in the remaining 6 months after study closure.

Training. Study staff will be trained in GCP, serious adverse event guidelines, clinical assessment of patients, completion of relevant source documents and CRFs, specimen collection and storage of samples. Initial training will be provided by the Study Field Coordinator and Medical Coordinator, with support from the Study Monitor and other partners. Refresher training will be provided on a quarterly basis to all study personnel by the Study Field Coordinator and Medical Coordinator. Routine audits performed by the Study Monitor will be used identify procedures that need to be strengthened and reinforced in routine refresher training.

SAFETY EVALUATION

Standard care. For the duration of the study period, all study children found needing medical intervention will be referred to study staff in a FORSANI-supported health facility for management free of charge and in accordance with GCP. Study staff will verify previous receipt of any EPI vaccine at study visits through review of the infant's vaccination card, and as needed, administer any missing routine vaccination doses free of charge. Vaccines to be provided include: Bacillus Calmette-Guérin (birth); oral poliovirus vaccine (birth, 6, 10 and 14 weeks); and the combined pentavalent vaccine including diphtheria-tetanus-whole cell pertussis, haemophilus influenzae type b and hepatitis B (6, 10, and 14 weeks). Stock of measles and yellow fever vaccination, recommended at 9 months of age, will be insured by the study sponsor and all participants invited to receive vaccination at a study facility at 9 months.

Documenting adverse events. The collection, recording, assessment and reporting of post-immunization reactions, adverse events (AEs) and serious adverse events (SAEs) represent the core activities for the safety evaluation of the vaccine. The participant's caregiver will be instructed to present at a health facility immediately, should the participant manifest any signs or symptoms they perceive as serious during the study period.

Surveillance for adverse events will be conducted from the time of the first dose to 28 days post-Dose 3 in all participants by trained study staff. Solicited adverse events will include fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in study SOPs), diarrhea, vomiting, decreased appetite, decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia).

During the surveillance period, AEs observed by the study team or reported by the participant's caregiver spontaneously or in response to a direct question will be followed on a daily basis by a nurse assistant until resolution. The diagnosis, date and time (where appropriate) of onset, outcome (e.g. resolved; resolved with sequelae; ongoing; died; or lost to follow up/unknown), intensity and relationship to vaccination; and treatment received will be recorded. All data will be transcribed to a standard CRF by study physicians.

Note: All identified gastroenteritis episodes from enrollment to 28 days post-Dose 3 will be reported as an adverse event. After 28 days post-Dose, gastroenteritis will not be reported as an adverse event but will be captured as efficacy outcome. Gastroenteritis episodes requiring hospitalizations for more than 24 hours at any time during study follow up will be considered as a serious adverse event.

Assessment of adverse event severity. The severity of all AEs/SAEs occurring during study follow up will be graded as per the clinical judgment of the study physicians and Medical Coordinator, taking into account information provided by parents. Each event will be assigned to one of 4 categories (intensity grades):

- GRADE 1 Mild - Transient or mild discomfort (< 48 hours); no medical intervention/therapy required

- GRADE 2 Moderate - Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required

- GRADE 3 Severe - Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible

- Grade 4 Life Threatening - any adverse experience that places the child, in the view of the investigator, at immediate risk of death from the reaction as it occurred. (The investigator should not grade a reaction as life-threatening if had it occurred in a more severe form then it might have caused death.)

Note: An AE that is assessed as severe should not be confused with the term SAE. Severity is a category utilized for rating the intensity of an event; both AEs and SAEs can be assessed as severe.

Documenting serious adverse events. Surveillance for serious adverse events (SAEs) will continue from the time of the first dose until 2 years of age. SAEs will be defined as:

(1) Death during the period of protocol-defined surveillance.
(2) Any new event that results in a persistent or significant disability/incapacity.
(3) Any new event requiring inpatient hospitalization. This will be related to hospitalization other than that related to management of diarrhea.
(4) Any new life-threatening event (defined as a study participant at immediate risk of death at the time of the event).

According to the Brighton Collaboration for adverse events following immunization for oral vaccines, SAEs will also include generalized convulsive seizure, hypotonic-hyporesponsive episodes, intussusception, or persistent crying (54, 55).

Children that die in a facility will have events before and at the time of death transcribed from hospital records to determine the cause of death. If death occurs outside of the hospital, cause of death will be established using a standard verbal autopsy form administered to determine the clinical picture and treatment received before death.

SAEs identified through facility- or home-based surveillance will be reported to the Medical Coordinator and PI within 24 hours of becoming aware of the SAE, whether considered to be associated with the study intervention or not. The field team will assemble specific documentation, including medical records and other supporting documents, and will record on the CRF: type of SAE; description of event with time of onset in relation to vaccination and severity; any medical actions taken and outcome; and preliminary assessment of causality. Where applicable, hospital records and verbal autopsies should be obtained. The dossier will be

submitted to the Medical Coordinator for adjudication and determination whether the cases were vaccine-related. All SAEs will be followed up until the event resolves, stabilizes, or is otherwise explained. If an SAE remains unresolved at the time of study closure, a clinical assessment will be made by the Medical Coordinator and sponsor to determine if continued follow up of the SAE is warranted. All relevant field personnel will be appropriately trained in the reporting and treatment of serious adverse events according to GCP. The DSMB and the National Ethical Committee of Niger will receive a quarterly status report including all notified SAEs. Copies of each report and documentation of IRB notification will be kept in the Trial Master File by the Medical Coordinator.

HIV status may be made known to the Medical Coordinator through review of existing medical records during assessment of SAE causality. If the HIV status of an infant experiencing a SAE is unknown but deemed necessary for appropriate clinical management of the SAE, HIV counseling and testing may be recommended by the Medical Coordinator at the time of SAE documentation.

Intussusception risks, assessment and management. In 1998, the rotavirus vaccine, RotaShield®, was recommended for use in the United States. However, within less than a year and more than 500,000 children vaccinated, RotaShield® was found to cause a transient increased risk of intussusception (estimated to occur in 1 child in 10,000) in the first 10 days after initial vaccination. RotaShield® was subsequently withdrawn from the market before detailed public discussion of the risks and benefits surrounding its use (70, 71). Post-licensure studies of the 2 second-generation rotavirus vaccines currently WHO-prequalified (RotaTeq® and Rotarix®) have monitored the risk of intussusception in the United States, Latin America, Mexico and some countries in sub-Saharan Africa. These studies suggest that all orally administered live rotavirus vaccines carry some detectable risk of intussusception (e.g. the risks associated with Rotashield® are not unique) but the risk of intussusception seems to be small (72). The Global Advisory Committee on Vaccine Safety (GACVS), an expert clinical and scientific advisory body, has concluded that currently available rotavirus vaccines continue to exhibit a good safety profile but may be associated with an increased (up to 6-fold) risk of intussusception after the first dose of vaccine in some populations (73). The levels of risk observed are substantially less than those observed with the previous vaccine, RotaShield®, and the benefits of rotavirus vaccination to all infants are thought to exceed the risks, particularly in developing countries with moderate and high mortality from rotavirus disease. Active surveillance of intussusception in African and Asian countries that plan to introduce rotavirus vaccines is warranted to eventually provide additional benefit–risk information related to rotavirus vaccination (73).

A definitive diagnosis of intussusception is based on the demonstration of invagination of the intestine on contrast enema (air or liquid), ultrasound or surgery. However, a clinical case definition for the diagnosis of acute intussusception in infants and children has been developed following recommendations of a WHO consultation and through consensus of the Brighton Collaboration Intussusception Working Group (74). This definition provides a case definition that is suitable for use in studies conducted in different geographical regions with different health

care facilities and resources, and has been validated in a developed and developing country setting (75). The Brighton clinical case definition for intussusception has been endorsed by the CIOMS/WHO Working Group on Vaccine Pharmacovigilance.

Parents and study staff will be trained on screening tools to promptly identify and assess any suspected cases of intussusception, including bloody stools, continuous vomiting, abdominal distension and/or abdominal "lumps". Children presenting with any suspected symptoms or signs at the time of a home visit or presentation to a study facility will be promptly referred to the Regional Reference Hospital of the Region of Maradi, where radiological and clinical examination will be performed before evaluating the risks and benefits of surgical intervention. All subsequent surgical interventions to resolve the invagination will be performed on site. Any patient deemed complicated by the attending physician based on their clinical judgment will be referred to the National Hospital of Niamey for management. Resolved cases of intussusception will be followed with home visits 2, 6 and 14 days after discharge from hospital.

This study will use the clinical case definition of intussusception from the Brighton Collaboration Working Group on Intussusception (74). All cases of suspected or confirmed intussusception identified among children ≤24 months at the time of diagnosis will be analyzed by the Medical Coordinator following WHO guidance (76). The Medical Coordinator will define cases of intussusception as Level 1, 2, or 3 according to the certainty of the diagnosis and the Brighton Collaboration clinical case definition. A case will be considered a potential rotavirus vaccine-related intussusception case if a rotavirus vaccine was received prior to the episode of intussusception. Cases for which there is insufficient information to establish the diagnosis according to the Brighton Collaboration clinical case definition will be classified as an unconfirmed case of intussusception and analyzed accordingly with other adverse events. Cases for which a diagnosis of intussusception is excluded on the basis of clinical assessment and/or appropriate investigations defined in the Brighton Collaboration clinical case definition will also be considered as another potential adverse event.

Assessment of causality. Every effort will be made by the study team to explain each SAE and assess its causal relationship, if any, to administration of the study vaccine or placebo. Appropriate medical judgment will be used to determine the causal relationship, considering all relevant factors including the pattern of reaction, temporal relationship, re-challenge, biological plausibility, and confounding factors such as concomitant medication, concomitant disease and relevant history.

The causality of all SAEs will be classified as:

> **Related,** when there is a reasonable possibility that the study vaccine contributed to the SAE.
>
> **Unrelated,** when administration of the study vaccine is not suspected to have contributed to the SAE.

All post-immunization reactions (e.g. those occurring within 30 minutes of administration) will be considered related to vaccination. SAEs that occur after informed consent is obtained from the parent/guardian, but prior to first vaccination, will be documented on the CRF but not considered as an SAE or related to the study. Hospitalization related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures (including hospitalization for "social" reasons) that are not the result of an SAE, will be recorded on the CRF but not considered related to the vaccine.

Dose modification. No modification of the study intervention dose is allowed. Subjects who experience unacceptable adverse events attributed to the study should not receive further vaccination and should be treated under the Medical Coordinator's discretion.

Data and Safety Monitoring Board. An independent Data and Safety Monitoring Board (DSMB) composed of 5 experts in operational, medical, and biostatistical aspects of clinical trials will be set up prior to initiating the study. All members of the DSMB will be completely uninvolved in the running of the trial and cannot be unfairly influenced by people or institutions involved in the trial. It is anticipated that the first DSMB review will occur approximately 6 months after the first participant is enrolled, with subsequent reviews every 6 months thereafter. Each meeting will include an administrative review to assess accrual, retention, and the progress of the study, as well as interim analyses of efficacy and safety, including any serious adverse events. Guidelines for trial review and modification, including statistical and non-statistical criteria for early stopping or modification based on interim analysis, will be determined at the first DSMB meeting and outlined in the DSMB charter to be established before the first meeting and made available upon request through the study sponsor.

The DSMB will be provided with the following summary of blinded accruing safety data on a monthly basis:

- Accrual and subject status data with regard to completion/discontinuation of study vaccinations
- Summaries of solicited adverse event data by severity grade, duration, body system and relation to study intervention
- Reported SAEs, including cases of intussusception, hospitalization and deaths

DSMB reviews will be summarized with recommendations to the sponsor, including recommendations regarding safety concerns and if the study should continue without change, be modified, or terminated. The DSMB will discuss potential stopping or any modification to the trial with the Scientific Committee and sponsor. The sponsor will have final authority to stop or modify the trial for any reason.

MONITORING AND AUDITING. The sponsor will permit trial-related monitoring, audits, Institutional Review Board review, and regulatory inspection(s), providing direct access to source data and documents.

A qualified and appropriately GCP-trained Study Monitor will be designated by the sponsor to carefully monitor all aspects of the study.

The Study Monitor will perform an initial audit before the start of the study to insure all necessary tools and supports are in place for study implementation in accordance with GCP. During the project period, the Study Monitor will routinely contact study sites and perform on-site visits to inspect facilities and documentation, observe performance of study procedures, discuss the protocol in detail and identify and clarify any areas of weakness. The extent, nature and frequency of site visits during the project period will be based on considerations of study objectives, study design and complexity, and enrollment rate. Periodicity and nature of monitoring activities will be described in the Monitoring Plan, with a minimum of 3 formal audits (e.g. study start, 6 months after first enrollment and study closure).

Monitoring will be conducted according to GCP and study SOPs. The Study Monitor will have access to all records necessary to ensure the integrity/validity of the recorded data and will periodically review the progress of the study. During site visits and contacts, the Study Monitor will specifically:

1. Check and assess the progress of the study
2. Review study data collected
3. Perform source data verification to verify compliance with study SOPs, including adherence to inclusion/exclusion criteria, dosing schedule, and recording of concomitant medications
4. Verify correct storage, distribution and inventory of study products
5. Verify compliance with human subjects protection and research guidelines, including confidentiality procedures and informed consent process
6. Identify any issues and address their resolution

This will be done in order to verify that:

1. The data are authentic, accurate and complete
2. The safety and rights of participants are being protected
3. The study is conducted in accordance with the approved protocol (and any subsequent amendment), GCP and all applicable regulatory requirements

Representatives of the Scientific Committee and DSMB authorized by the sponsors may accompany the Study Monitor during site visits to conduct independent audits as needed and with due consideration to relevant security issues. The processes to be reviewed can relate to participant enrolment, consent, eligibility, and allocation to study groups; adherence to trial interventions and policies to protect participants, including reporting of harms; completeness,

accuracy, and timeliness of data collection; and adherence to the International Conference on Harmonization GCP.

ETHICAL CONSIDERATIONS

Summary of known and potential risks. There is no data on the safety of BRV-PV in sub-Saharan Africa. However, based on documentation of safety of the vaccine in other settings, the risks associated with the use of the vaccine, placebo and various other study procedures proposed in this trial are expected to be minimal. All clinical and immunization procedures (oral vaccine administration, venous blood collection, rectal swab collection) will be performed by adequately trained and experienced personnel under regular supervision to minimize any risk or discomfort to participants. There is a very small risk of anal/rectal area skin abrasion while taking a swab from the rectal area. Additionally, there is also a small risk associated with phlebotomy for participants who are requested to give a blood sample. This may include pain, redness and, very rarely, local infection at the phlebotomy area.

In 1998, the rotavirus vaccine, RotaShield®, was recommended for use in the United States. However, within less than a year, it was found to cause a transient increased risk of intussusception (estimated to occur in 1 child in 10,000) in the first 10 days after initial vaccination. Intussusception is a rare event, and rates of intussusception vary between countries and populations, as the rotavirus vaccine–intussusception association may be modified by environmental or genetic factors that differ between populations. Factors that are hypothesized to affect risk of intussusception or the immune response to rotavirus vaccines—including differences in infant diet, breastfeeding, concomitant administration of oral poliovirus vaccine vs. inactivated poliovirus vaccine, and maternal antibody levels—may also contribute to the variation in intussusception risk by country (44, 47). Given biological differences between the various rotavirus vaccine strains, including rates of intestinal vaccine virus replication and shedding in fecal specimens, any potential risk of intussusception may also vary between vaccine formulations.

Post-licensure studies of the 2 second-generation rotavirus vaccines currently WHO-prequalified (RotaTeq® and Rotarix®) have monitored the risk of intussusception in North America, Latin America, and sub-Saharan Africa. With almost 800,000 doses of pentavalent rotavirus vaccine delivered, these analyses found that any orally administered live rotavirus vaccine carries some detectable risk of intussusception but conclude that the risk of intussusception is small: an excess risk of 1 intussusception event per 65,287 RV5 vaccines following dose 1 can be reliably excluded (77). Based on available evidence, the benefits of rotavirus vaccination to all infants greatly exceed any potential low-level risk for intussusception, particularly in countries with moderate and high mortality from rotavirus disease.

Risk minimization and benefits. All personnel involved in taking biological samples are trained health care personnel, who will be provided with additional training to avoid or minimize the possibility of any unplanned side effects of these procedures. The trial will be conducted in compliance with protocol, GCP, and the applicable regulatory requirements. Sterile techniques and disposable sterile needles and syringes will be utilized to obtain blood.

The direct benefit individual subjects may expect from participating in this study is regularly seeing a member of the study team and the assurance of the best available medical care with close and regular surveillance. Recipients of the vaccine will also potentially benefit from the probable protective effect of the vaccine against severe rotavirus gastroenteritis. Rotavirus vaccine has not yet been introduced into the national immunization program of Niger and individual access to one of the two available vaccines [e.g. Rotarix® and Rotateq®] is unlikely for all except the disproportionately wealthy of the study population. At the population-level, an important benefit of obtaining data on the efficacy of the BRV-PV vaccine, if proven protective, is the vaccine potentially being made available for a larger population of children in Niger.

Informed consent. Information about the study aims, procedures and informed consent process will be provided to community leaders in all study villages before recruitment begins. Written informed consent will be obtained from each subject's parent/guardian prior to any study-related screening procedures being performed on the subject. The consent forms to be signed by the parent/guardian will include information on the purpose of the study, the study intervention, procedures to be followed and the risks and benefits of participation. All informed consent procedures and documents will be in the local language.

Informed consent will be sought at 2 times during the study. During the Pre-randomization Visit (at birth), informed consent will be sought by a study nurse to allow recording of maternal MUAC, as well as infant birth weight and gestational age at birth. At this time, the study nurse will discuss details of the study with a parent/guardian and provide an Information Sheet (Appendix A) including information on the purpose of the full study and a description of procedures to be conducted that day. The Pre-randomization Visit will allow the parent/guardian adequate time to consider their participation in the full trial and prepare any questions they may have.

During the Randomization Visit (6-8 weeks of age), informed consent for participation in the full study will be sought. A study physician will inform the parent/guardian of all pertinent aspects pertaining to the study, including study aims, methods and potential risk and benefits, and then seek informed consent for participation in the full study in writing in accordance with GCP, the Declaration of Helsinki and all applicable regulatory requirements. Materials consent will be obtained at this time to address the collection of venous blood and breast milk specimens for the designated immunogenicity sub-studies. Materials consent will relate to the use of data and specimens in the specified protocols and in future unspecified research.

The informed consent process of the Pre-randomization Visit and the Randomization Visit will give individuals all of the relevant information they need to decide whether to allow their children to participate. The informed consent process will begin with study staff describing the study protocol and procedures to the parent/guardian using a standardized Information Sheet (Appendix A). Study staff will give the parent/guardian ample opportunity to inquire about details of the study and ask any questions. Illiterate individuals will have the Information Sheet and Informed Consent Form (Appendix A) read to them in their native language in the presence of a literate and impartial witness. The witness will sign and date the consent form to attest the

consent process appears to be fair and the parent/guardian voluntarily allows the subject to be included. The study physician who administered the consent procedure will countersign to confirm that the consent has been obtained following the procedure described in the study protocol and in accordance with GCP. An infant will not be enrolled into the study and given a randomization number until written informed consent for participation in the full trial is provided during the Randomization Visit.

The original Informed Consent Form will be kept on file by the Medical Coordinator for possible inspection by regulatory authorities and the sponsor. The participant's parent/guardian will receive a copy of the Information Sheet and signed and dated Informed Consent Form.

Confidentiality. Participants will be identified by a unique individual identification number in all CRFs and laboratory specimens. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. The participant's caregiver will be informed that representatives of the sponsor or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in the strictest confidence. Participants' study information will not otherwise be released outside of the study without the written permission of the participant's caregiver. The Study Field Coordinator will maintain a personal list of participant identification numbers and names to enable records to be found at a later date in the event of a medical need. This information will be destroyed upon study completion. Personal identifiers will not be included in any study report.

All study records and data will be kept confidentially under lock and key and/or electronic password protection, as appropriate and in accordance with local data protection laws for 5 years. Only senior study personnel will have access to these records.

Reimbursement. There are no plans to provide monetary payment for participation in this study. However, as the study will require caregivers to visit a study facility for scheduled and interim visits, the study will provide compensation for all facility visits at the time of the visit. In addition, in recognition of the burden associated with the study requirement to be present for all home visits, the study will provide an in-kind motivation for subject participation and retention. This will include 3 pieces of soap each month for the duration of the study and one mosquito net prior to the first peak malaria transmission season following inclusion (e.g. first June / July following inclusion). Distribution of soap and mosquito nets will take place at home upon completion of the designated Weekly Home Visit e.g. one time per month.

Storage of specimens. Stored study research samples will be labeled by a unique individual identification number code that can only be linked to the participant by senior study staff. All stored research samples will be entered into a secure database and all uses will be documented. Samples may be stored at several different laboratories in order to complete the analyses required to meet study primary, secondary and exploratory analyses.

Institutional Review Board approval. The study will be approved by the research ethics committee of the Ministry of Health Niger, the Comité de Protection des Personnes Ile-de-France, Commission d'Ethique de la Recherche sur l'Etre Humain, Hôpitaux Universitaires de Genève and the Western Institutional Review Board, and will be done in accordance with the Declaration of Helsinki and guidelines for GCP.

Declaration of conflict of interests. The primary and secondary sponsors declare no conflict of interests.

STUDY ADMINISTRATION

Protocol amendments. Any modifications to the protocol which may impact the conduct of the study or may affect patient safety / benefit, including changes of study objectives, study design, patient population, sample sizes, and study procedures, will require a formal amendment to the protocol. Such amendment will be agreed upon by the Scientific Committee and sponsors and approved by the appropriate ethics committee prior to implementation. The sponsor will be responsible to notify the appropriate regulatory agencies and trial registry. Administrative changes of the protocol, including minor corrections and/or clarifications that have no effect on the way the study is to be conducted, will be agreed upon by Scientific Committee and sponsors and will be documented in a memorandum.

Protocol deviations and violations. A protocol violation is any departure from the approved protocol, trial documents or any other information relating to the conduct of the study which may affect the safety of trial participants or the study outcomes. Examples include failure to obtain informed consent (i.e. no documentary evidence) or enrolment of participants that do not meet inclusion/exclusion criteria.

A protocol deviation is any departure from the approved protocol, trial documents or any other information relating to the conduct of the trial that does not result in harm to the trial participants and does not significantly affect the study outcomes. Examples of deviations include missed protocol visits or a protocol visit date outside the study visit window or an isolated incident of a missed or incomplete study procedure or study evaluation. Serious or repeated protocol violations or deviations will require assessment of the root cause and implementation of corrective and preventive action plans. They may constitute grounds to interrupt the trial at a study site.

Any changes from protocol-specified procedures and study-related SOPs occurring during the conduct of the trial will be documented and reported as protocol violations or deviations. Protocol violations will be reported to the sponsor and reviewing ethical committees, as appropriate and in accordance with the requirements of the involved committees.

Ancillary care and insurance. In the event that a subject suffers injury attributable to participation in this study, appropriate medical management and treatment will be paid for by the study and provided by study staff with support from FORSANI. The study sponsor will have insurance to cover non-negligent harm associated with the protocol.

Data storage and archival. The Study Monitor will provide the Medical Coordinator with a Site Master File, which will be used to file the protocol, drug accountability records, correspondence with the IRB and sponsor, and other study-related documents. The Study Field Coordinator and Medical Coordinator will maintain, and store securely, complete, accurate and current study records throughout the study.

The sponsor will keep essential documents, including subject's medical records, until at least 5 years after study closure. No data will be destroyed without the permission of the sponsor.

Dissemination and authorship policy. When the clinical study report is completed, the investigators will share the summary results with local, regional and national immunization program representatives to provide results and answer any questions.

The findings from this study will also be published in a peer-reviewed scientific journal and disseminated at appropriate national and international conferences. Each paper or abstract will be submitted to the Scientific Committee for review of its appropriateness and scientific merit prior to submission. The Scientific Committee may recommend changes to the authors but the ultimate decision to submit will remain with the primary sponsor. Every attempt will be made to reduce to an absolute minimum the interval between the completion of data collection and the release of the study results. A period of 3 to 6 months is expected to compile the final results paper for an appropriate journal. Trial results will be disseminated to key stakeholders regardless of the direction or magnitude of effect.

The sponsors and Scientific Committee will determine the specific topics and numbers of publications, with rights to authorship being determined by intellectual contribution to the study design, implementation, and analysis, as is specified by most major scientific journals. Preference will be given for publication in peer-reviewed, open-access journals with appropriate readership and high impact factors.

Data sharing policy. The research data will be the property of the sponsors, though we realize that the data collected from this study may provide other investigators with the opportunity to answer scientific questions about a number of ancillary issues. Therefore, data will be made as widely and freely available as possible, in a timely fashion, while safeguarding the privacy of participants and protecting confidential data. A de-identified data set can be made available under a data-sharing agreement that provides for a commitment to using the data only for research purposes and securing data using appropriate technology.

STUDY MANAGEMENT

Study sponsors. The primary sponsor will develop the study protocol, with substantial input from Serum Institute of India, Limited (SIIL), and other partners. The primary sponsor will hold the data and conduct all analyses. The final report will be written by the primary sponsor, who will have full access the data and final responsibility for the data analysis and decision to submit for publication. SIIL will have no direct oversight, participation in field activities, DSMB meetings, or data analysis. Primary sponsor staff will independently monitor study execution at field sites but not participate in closed sessions of the Data and Safety Monitoring Board meetings. The study will be managed by a primary sponsor-investigator who both initiates and conducts the trial. The obligations of a sponsor-investigator include both those of a sponsor and those of an investigator.

The secondary sponsor is Médecins Sans Frontières- Operational Center Geneva (MSF-OCG). MSF-OCG has agreed with the primary sponsor to act as the Primary Sponsor's legal representative in relation to the trial site and provide funding for the trial.

Serum Institute of India, Limited. Vaccine and placebo are to be provided in kind by SIIL.

Scientific Committee. The Scientific Committee (see Appendix C for Terms of Reference) will be asked to contribute to the following activities:

- Review drafts of the trial protocol, and agree on a final version;
- Review the trial Standard Operating Procedures, and agree on a final version;
- Advise and agree on an Analysis Plan covering data generated by the trial;
- Reply promptly to specific queries regarding the trial methods, practical implementation, analysis and interpretation;
- Review drafts of the final trial report, and agree on a final version.

Reporting procedures of the Scientific Committee to the sponsor are as outlined in the Terms of Reference (Appendix C). The sponsor-investigator assumes responsibility that the Scientific Committee is informed of all components of the trial protocol.

Data and Safety Monitoring Board. An independent Data and Safety Monitoring Board (DSMB) composed of 5 experts in operational, medical, and biostatistical aspects of clinical trials will be set up prior to initiating the study. All members of the DSMB will be completely uninvolved in the running of the trial and cannot be unfairly influenced by people or institutions involved in the trial. It is anticipated that the first DSMB review will occur approximately 6 months after the first participant is enrolled, with a second review 6 months thereafter. Each meeting will include an administrative review to assess accrual, retention, and the progress of the study, as well as interim analyses of efficacy and safety, including any serious adverse events. Guidelines for trial review and modification, including statistical and non-statistical criteria for early stopping or modification based on interim analysis, will be determined at the first DSMB meeting and outlined in the DSMB charter to be established before the first meeting and made

available upon request through the study sponsor. Reporting procedures to the study sponsor will be outlined in the DSMB charter.

Human resources. Overall study development and direction will be provided by a team of 4 investigators based in Paris and Geneva (Figure 1). Rebecca Freeman Grais will serve as the sponsor-investigator for the study. Dr. Grais is an international expert in vaccination in sub-Saharan Africa, and as the sponsor-investigator, will be the guarantor of the trial. Sheila Isanaka, Principal Investigator, is a recognized epidemiologist with extensive experience in conducting large-scale trials of high burden pediatric morbidities. Dr. Isanaka will be responsible for ensuring appropriate study design and procedures. Dominique Legros, Co-investigator, representative of the secondary sponsor (MSF-OCG) and former division head at the WHO headquarters in Geneva, will be responsible for ensuring that this trial adheres to international medical standards as recognized by the WHO and the Ministry of Health of Niger. Emmanuel Baron, Co-investigator, General Director of Epicentre and former Medical Director of Médecins Sans Frontières-Operational Center Paris, will ensure the appropriate medical management of all study participants. Dr. Baron has over 15 years of experience in providing and managing medical care throughout sub-Saharan Africa.

In addition, the study will be supported a permanent GCP-trained Study Monitor based in Niamey, Niger. Through routine contact and site visits, the Study Monitor will insure all necessary tools and supports are in place for study implementation, review collected data and perform source data verification in accordance with GCP.

All day-to-day study activities will take place in the Madarounfa Health District, Maradi, Niger, and interaction with participants will be primarily carried out by the study field team. The field team will be comprised of a Study Field Coordinator and Medical Coordinator based in Maradi, and specialized health care personnel based in rural sites throughout the Madarounfa Health District and responsible for the enrollment and medical follow-up of all study subjects. The Study Field Coordinator and Medical Coordinator will be under the supervision of the Principal Investigator and the Director, Epicentre Niger, with whom there will be at least weekly communication by email, teleconference or videoconference to discuss study activities and challenges.

The Study Field Coordinator will be responsible for coordination of all field worker and supervisor schedules, standardization of follow up methods and assurance that activities are conducted according to protocol. The Medical Coordinator will be responsible for overseeing the medical management and follow-up of all study children. The Study Field Coordinator and Medical Coordinator will together supervise 4 teams:

- Community-based recruitment team will be comprised of village representatives, including village chiefs, midwifes or health workers. These individuals will create and maintain a population registry for their village, recording births, deaths and migration in order to provide a basis for study inclusion. There will be 1-2 recruitment monitors per study village, depending on village size.

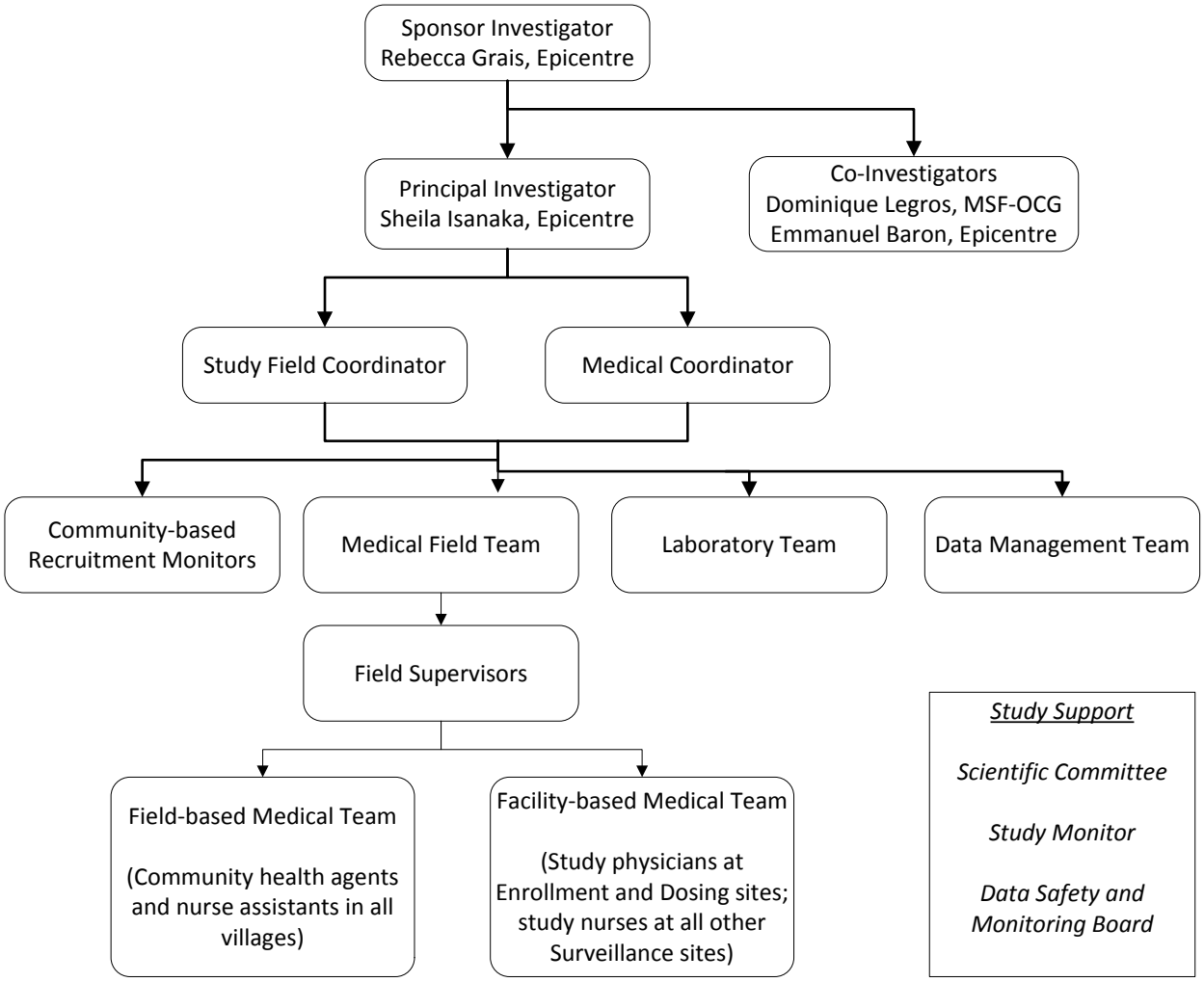
- Medical field team will include field-based and facility-based personnel. Field-based personnel will include community health agents and nurse assistants. The community health agent (n = 1 per 20-25 children; 4-5 home visits per day) will conduct the Weekly Home Visits to review the Weekly Diary Card with caregivers and notify the nurse assistant when an episode of gastroenteritis or adverse event is identified. The nurse assistant (n = 1 per 100 children; 4-5 visits per day) will follow children at home with gastroenteritis or any adverse event to collect information regarding the severity of the episode, and as appropriate stool samples. Facility-based personnel (n = 1 per site during normal facility operating hours) will include study physicians and study nurses. One study physician will be based in each designated Enrollment and Dosing Site and will be responsible for recruitment (including the informed consent process), administration of study vaccine and placebo and post-immunization observation, receiving children presenting to the facility with symptoms of gastroenteritis or other medical complication, and recording all CRFs. One study nurse will be based in all remaining facilities in the Health District and will be responsible for receiving children presenting to the facility with symptoms of gastroenteritis or adverse events and recording pertinent medical information for transcription to standardized CRFs by the study physicians. Field supervisors (n= 1 per 10-15 agent) will directly oversee field-based and facility-based personnel by tracking follow up logs and attending home and facility visits to ensure procedures are standardized and conducted according to protocol.

- Laboratory team, including 2 laboratory technicians and 1 laboratory supervisor, will be responsible for the preparation and storage of all biological samples collected during the study period; analysis of collected stool for the presence of rotavirus antigen by enzyme immunoassay; and preparation all rotavirus positive stool samples for shipment to the CERMES laboratory in Niamey for further testing by reverse transcriptase PCR.

- Data management team, including 5 data entry operators and 1 Data Manager, will be responsible for the double-entry and validation of all data collected on standard CRFs.

All teams will be adequately staffed with sufficient back-up personnel to allow continuity of study activities over standard annual and sick leaves.

Figure 1. Study organogram



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Efficacy and safety of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis in Niger

Study Protocol

Version 6

September 8, 2013

Centre Collaborateur de l'OMS
pour la Recherche en Epidémiologie
et la Réponse aux Maladies Emergentes

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Title	Efficacy and safety of a pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in Niger
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Secondary Sponsor	Médecins Sans Frontières-Operational Center Geneva 78 rue de Lausanne CP 116 1211 Geneva 21, Switzerland
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Protocol Version	Version 6 (Issue date: September 8, 2013)
Revision Chronology (Date and Reason for Amendment)	N/A

INVESTIGATORS' SIGNATURE PAGE

By my signature below, I hereby confirm that I will conduct the study described in this protocol in compliance with ICH/GCP and the version of such protocol agreed to by the applicable regulatory authorities and approved by all Institutional Review Board and Ethical Committees.

Primary Sponsor, Epicentre
Rebecca Grais

_____ _____

Signature **Date**

Secondary Sponsor, Médecins Sans Frontières-Operational Center Geneva
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Signature **Date**

Serum Institute of India, Limited
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TRIAL REGISTRATION DATA

Primary Registry and Trial Identifying Number: To be completed with ClinicalTrials.gov

Date of Registration in Primary Registry: To be completed with ClinicalTrials.gov

Secondary Identifying Numbers: None

Sources of Monetary and Material Support
Médecins Sans Frontières-Operational Center Geneva will provide funding for the trial. Vaccine and placebo are to be provided in-kind by the Serum Institute of India, Limited.

Primary Sponsor
Epicentre takes responsibility for initiating, registering and conducting the trial, and as such, will be involved in the study design; collection, management and analysis, and interpretation of data; and writing of the report. Epicentre takes responsibility for ensuring the trial is properly monitored and results made available.

Primary sponsor contact: Dr. Rebecca Freeman Grais, Director, Department of Epidemiology and Population Health, Epicentre (Rebecca.Grais@epicentre.msf.org; Tel: +33 (0)1 4021 5475; Address: Epicentre, 8 rue Saint Sabin, 75011 Paris France)

Secondary Sponsor
Médecins Sans Frontières- Operational Center Geneva has agreed with the primary sponsor to act as the secondary sponsor and the primary sponsor's legal representative in relation to the trial site and provide funding for the trial. The secondary sponsor will be involved in the study design, interpretation of data and writing of the report.

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Public Title

Efficacy and safety of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis in Niger

Scientific Title

Randomized, double-blind, placebo-controlled phase III trial to assess the efficacy and safety of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis among infants in Niger

Country of Recruitment

Niger

Health Condition(s) or Problem(s) Studied

Severe rotavirus gastroenteritis

Interventions

Active comparator: Live attenuated bovine-human [UK] reassortant rotavirus vaccine manufactured by the Serum Institute of India, Limited (SIIL). The pentavalent vaccine (BRV-PV) contains rotavirus serotypes G1, G2, G3, G4, and G9 (≥5.6 log10 FFU/serotype/dose). The vaccine is in lyophilized form and supplied with 2.5 ml of citrate bicarbonate buffer that is added for reconstitution just before oral administration.

Placebo comparator: Same constituents as the active vaccine but without the viral antigens; manufactured by SIIL.

Key Inclusion and Exclusion Criteria

The study will be performed in infants in Madarounfa, Niger. Healthy male and female infants meeting the following inclusion criteria are eligible for enrollment:

(1) aged 6-8 weeks at the time of inclusion
(2) able to swallow and no history of vomiting within 24 hours
(3) resident in Madarounfa Health District within 15 km of central health facility
(4) intending to remain in the study area for 2 years
(5) parent/guardian providing informed consent

Exclusion criteria will include the following:

(1) unable to swallow or history of vomiting within 24 hours
(2) known history of congenital abdominal disorders, intussusception, or abdominal surgery
(3) receipt of intramuscular, oral, or intravenous corticosteroid treatment within 2 wks
(4) receipt of a blood transfusion or blood products, including immunoglobulins
(5) non-resident in Madarounfa Health District within 15 km of central health facility
(6) not intending to remain in the study area for 2 years
(7) parent/guardian informed consent not provided
(8) any other condition in which, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or the parent/guardian's ability to give informed consent

Study Type

Interventional, individually randomized, double (e.g. investigator and participant) blinded, parallel two-arm, phase III trial to assess the efficacy, safety and immunogenicity of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis when administered concomitantly with EPI vaccines in Niger. Infants will be individually randomized in a 1:1 ratio to receive three doses of the vaccine or placebo administered orally. The initial dose will be at 6-8 weeks of age. Each subsequent dose will be administered after a 4-week intervals (-1 to +4 weeks), with a maximum age at last dose of 24 weeks.

Unique identification numbers will be allocated by SIIL using a computer-generated random number list using permuted blocks of random sizes. Block sizes will not be disclosed to reduce predictability of the random sequence and ensure allocation concealment. Study physicians who oversee randomization will be given a subset of sequentially numbered, sealed, opaque envelopes that will be prepared by SIIL and contain printed randomization numbers and a corresponding group code. The study physician will be instructed to assign the randomization number and group code noted in the next envelope to each eligible infant as (s)he is enrolled.

Vaccine and placebo packages will be labeled with an assigned code and delivered to the study site in otherwise identical presentations. Group assignment will remain concealed from study personnel, investigators and caregivers of participating infants for the whole study period. The Data and Safety Monitoring Board (DSMB) and a sponsor statistician not involved in the rest of the trial will be masked to the group assignment. Investigators conducting the final analysis will remain masked to the group assignment until the end of the analysis.

The study code will be broken only in case of a medical event in which the Medical Coordinator deems the participant cannot be appropriately treated without knowing his/her group assignment. A set of sealed envelopes with group assignment will be securely held at the field site with the Medical Coordinator. Any such case will be fully documented by the Medical Coordinator and written notification will be provided to the sponsor within 48 hours.

Date of First Enrollment
December 2013

Target Sample Size
Assuming a 2% attack rate of severe rotavirus gastroenteritis at 1 year, a 50% true vaccine efficacy and a 20% participant non-assessibility (including withdrawal and loss to follow up), the study will enroll 5138 children to have at least 90% power to detect a 95% confidence interval for vaccine efficacy at 1 year that would be above 0%.

Recruitment Status
Pending: participants are not yet being recruited or enrolled at any site.

Primary Outcome
The primary endpoint is vaccine efficacy of three doses of the SIIL pentavalent rotavirus vaccine vs. placebo against a first episode of severe wild-type rotavirus gastroenteritis at 1 year of age. Gastroenteritis events will be detected through facility- and weekly home-based surveillance and will be defined as the passage of three of more loose or watery stools within a 24 hour period and/or forceful vomiting. Severe gastroenteritis will be defined clinically as an episode of gastroenteritis that needed overnight treatment in hospital and/or rehydration therapy equivalent to the WHO Plan B (oral rehydration therapy) or Plan C (intravenous rehydration therapy) in health facility, or using the 20-point Vesikari scale, where an episode of gastroenteritis with a score of 11 or more is considered severe. In the primary per-protocol analysis, infants who have at least one episode of severe wild-type rotavirus gastroenteritis during the period from 28 days post-Dose 3 until the date the infant reaches 1 year of age will be considered as having achieved the primary outcome. A secondary intention-to-treat (ITT) analysis of the primary endpoint will be conducted including all infants vaccinated with at least one dose of vaccine or placebo and follow up beginning from the time of randomization.

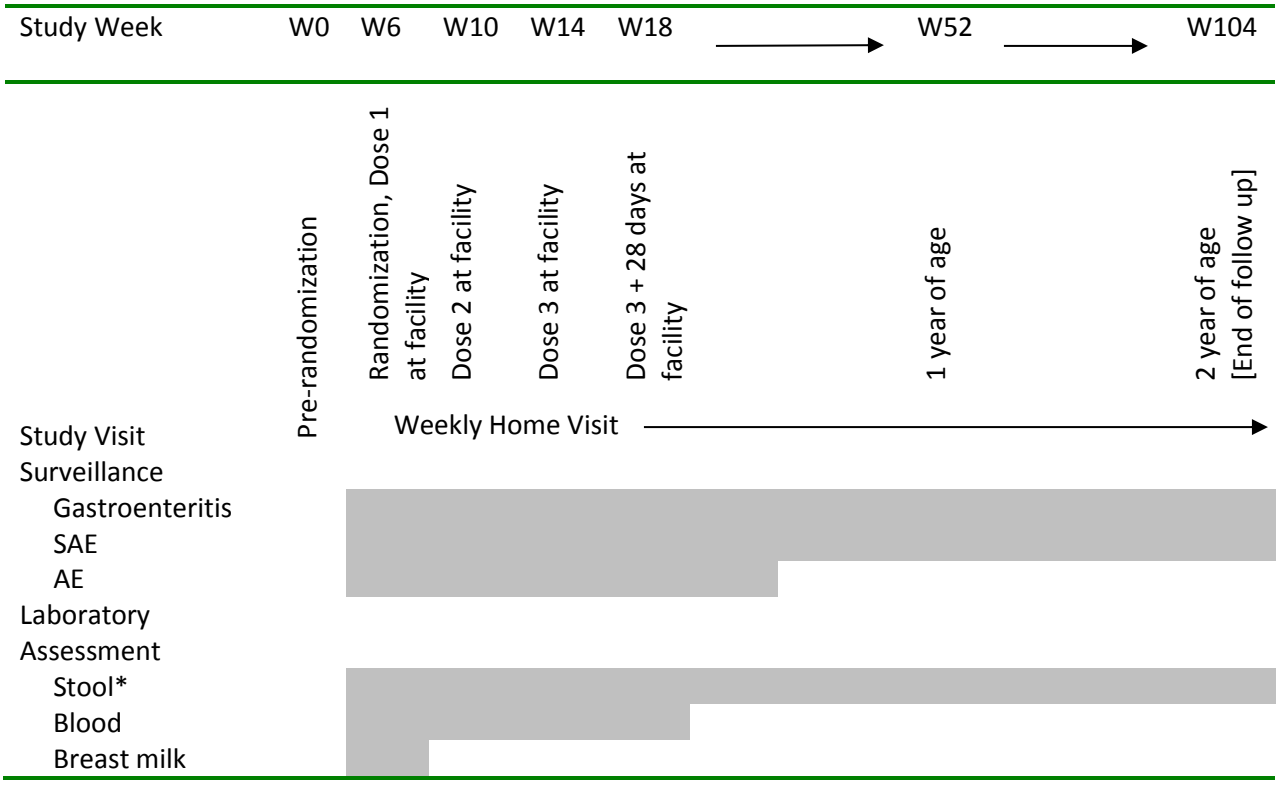
Key Secondary Outcomes
Secondary efficacy endpoints include: vaccine efficacy against severe rotavirus gastroenteritis during the second year of life and for the combined period for the first from 28 days post-Dose 3 until 2 years of age; rotavirus gastroenteritis of any severity; rotavirus gastroenteritis with a Vesikari score of ≥ 17; rotavirus gastroenteritis by serotype; gastroenteritis of any cause; longitudinal prevalence of rotavirus gastroenteritis; hospitalization due to rotavirus gastroenteritis; and hospitalization for any reason. The rates of all secondary efficacy endpoints will be compared by study intervention as in the per-protocol and ITT analyses of the primary efficacy endpoint.

Secondary safety endpoints include: the risk of adverse events from the time of the first dose to 28 days post-Dose 3 and the risk of serious adverse events from the time of the first dose until 2 years of age. Adverse events will include fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in Standard Operating Procedures), diarrhea, vomiting, decreased appetite, decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia). Serious adverse

events will be defined as any new health-related problem that results in disability, incapacity or death; necessitates hospitalization or is life-threatening, and according to the Brighton Collaboration for adverse events following immunization for oral vaccines, will also include generalized convulsive seizure, hypotonic-hyporesponsive episodes, intussusception, and persistent crying. The rates of all adverse and serious adverse events will be compared by study intervention in the ITT population, i.e. all infants who received at least one dose of the study vaccine or placebo, and include follow up from the time of enrollment until 28 days post-dose 3 (adverse events) or the end of follow up (serious adverse events).

The secondary immunogenicity endpoints will be assessed in a sub-sample of participants and include sero-conversion, defined as at least a three-fold serum titre rise of anti-rotavirus IgA, and differences in geometric mean titres from the first dose to 28 days (± 7 days) after the third dose of vaccine or placebo. Immune factors in breast milk at the time of immunization have been hypothesized to contribute to lower immunogenicity of live oral rotavirus vaccines in developing countries, therefore secondary immunogenicity analyses will assess concentrations of maternal anti-rotavirus IgA antibodies in breast milk in order to compare immunogenicity by level of maternal anti-rotavirus IgA concentration. Statistical differences in the risk of sero-conversion by level of maternal IgA concentration in breast milk will be assessed using the chi-square test. As the study vaccine will be administered concomitantly with the oral polio vaccine as part of the standard EPI, interference of the study vaccine with anti-poliovirus antibody serum titres will be assessed in the immunogenicity sub-sample. Differences in the concentration of anti-polio antibodies by study intervention will be compared using the chi-square test.

Table A. Schema of overall study design



* Stool collected at facility or home one time per episode of loose or watery stools and/or forceful vomiting within 7 days onset.

ABBREVIATIONS

AE	Adverse Event
BRV-PV	Bovine-human reassortant rotavirus vaccine-pentavalent
CERMES	Centre de Recherche Médicale et Sanitaire
CRF	Case Report Form
DHS	Demographic and Health Survey
DSMB	Data and Safety Monitoring Board
EPI	Expanded Programme on Immunization
FORSANI	Forum Santé Niger
GAVI	Global Alliance for Vaccines and Immunization
GEMS	Global Enteric Multicenter Study
HIV	Human Immunodeficiency Virus
GCP	Good Clinical Practice
IMCI	Integrated Management of Childhood Illness
ITT	Intention To Treat
MEM	Minimum Essential Medium
MSF	Médecins Sans Frontières
NGO	Non-governmental organization
ORS	Oral rehydration salts
PATH	Program for Appropriate Technology in Health
PI	Principal Investigator
ROC	Receiver Operating Characteristic
SAE	Serious adverse event
SIIL	Serum Institute of India, Limited
SOP	Standard Operating Procedure
UN	United Nations
UNICEF	United Nations Children's Fund
WHO	World Health Organization

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BACKGROUND AND RATIONALE

Epidemiology of childhood diarrhea. Acute diarrhea remains one of the major causes of morbidity and mortality among children, accounting for 11% of child deaths worldwide, more than malaria (7%) and far greater than HIV (2%) (1). Diarrhea-specific mortality has decreased in recent decades, but worldwide it is estimated to still account for 800,000 deaths in children < 5 years of age each year (2) and there has been little progress in reducing the incidence of diarrheal illness (3). There remains an estimated 2.5 billion episodes of childhood diarrhea per year (4). Children < 2 years of age are thought to experience an average of three to five episodes of diarrhea per year, with the highest rates (six to eight episodes per year) among infants 6-11 months old (5).

Acute diarrhea in children is often caused by a diverse group of infectious agents, including viruses, bacteria, and parasites. Transmission may occur through fecal-oral routes, respiratory secretions, or fomites (inanimate objects such as kitchen utensils). The recently completed Global Enteric Multicenter Study (GEMS) provides new evidence regarding the incidence, etiology and clinical outcome of moderate-to-severe diarrhea in seven sites throughout sub-Saharan Africa and south Asia (6). Using a comprehensive panel of microbiological assays to identify the etiology of moderate-to-severe diarrhea, a substantial proportion of diarrheal disease was attributed to 4 pathogens: rotavirus, *cryptosporidium*, and heat stable toxin producing *enterotoxigenic e.coli*, and *shigella*. In the first two years of life, the attributable incidence of moderate-to-severe diarrhea was dominated by rotavirus: incidence in infancy (7 episodes per 100 child-years) was more than double that of any other pathogen.

Current strategies for the management of childhood diarrhea. Acute diarrhea is rapidly dehydrating and can be life-threatening unless fluid therapy is initiated. In 1978, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) adopted oral rehydration salts (ORS) solution as the primary tool to fight dehydration. For more than 25 years, ORS was the only drug recommended by WHO and UNICEF for the prevention and treatment of dehydration. Rehydration therapy, however, does not decrease stool output, nor the duration or incidence of diarrhea, and ORS uptake is low: it is estimated that only one-third of children with diarrhea in developing countries currently receive ORS for treatment of their illness(4).

In 2004, WHO and UNICEF released revised recommendations for the management of diarrhea that also included therapeutic zinc supplementation for 10 to 14 days and incorporated zinc in the WHO Essentials Medicines List making it possible for zinc to be stocked in UNICEF warehouses (7). Despite the extensive body of scientific evidence supporting zinc for diarrhea management (8), many developing countries have been slow to adopt zinc as an explicit component of their diarrhea control programs, and zinc tablets appropriate for young children remain largely unavailable (4).

To accelerate progress towards reducing childhood diarrhea, calls for continued scaling up of proven interventions continue (9). In 2009, UNICEF and WHO issued the report, 'Diarrhea: why

are children still dying and what can be done,' and called for the large-scale implementation of 7 interventions for comprehensive diarrhea control in developing countries. The plan includes a treatment package: fluid replacement (with low-osmolarity ORS and continued breastfeeding or feeding) and zinc treatment to decrease diarrhea severity and duration; and a prevention package: rotavirus and measles vaccination, promotion of early and exclusive breast-feeding and vitamin A supplementation, promotion of hand washing with soap, improved water supply and quality including household water treatment and safe storage of household water, and community wide sanitation (4).

Epidemiology of rotavirus and public health response. Rotavirus is the leading cause of severe gastroenteritis in children and is responsible for an estimated 450,000 deaths per year in children < 5 years of age, with most of the deaths occurring in developing countries (10). In sub-Saharan Africa, the proportion of hospitalizations for diarrhea associated with rotavirus ranges from 29% to 52% (11), with a global estimate of 34% in a meta-analysis of recent studies published between 2006 and 2008 (12).

Rotavirus genotypes are based on the two structural proteins of the virus outer capsid, VP7 (G glycoprotein) and VP4 (P protein). Four genotypes (G1P[8], G2P[4], G3P[8], G4P[8]) were historically recognized to be the most frequent, representing 88% of all strains worldwide in a meta-analysis from 2005, with the single G1P[8] genotype responsible for over 70% of infection in North America, Europe and Australia (13). More recently, G9P[8] has emerged worldwide and other rare genotypes, such as G12 and G6, are also emerging in Asia and Africa (14-16). Overall, recent studies have shown a wider variety of rotavirus genotypes circulating in developing countries compared to industrialized countries (11, 17).

Two rotavirus vaccines are currently available and prequalified by the WHO. Rotarix® (GlaxoSmithKline) is a live, attenuated vaccine derived from the human 89-12 strain which belongs to G1P[8] type, while Rotateq® (Merck) is a pentavalent human-bovine reassortant strain containing the G1, G2, G3, G4 and P[8] proteins. These vaccines have been shown to be safe and efficacious in high and middle-income countries (18, 19), where the impact of their introduction in routine vaccination programs on rotavirus-related hospital admissions and deaths has been demonstrated (20-24). Recent vaccine trials in Africa and Asia have shown substantially lower vaccine efficacy in low-income countries than in high or middle-income countries, with efficacies ranging from 50% to 64% (25-27). Despite these reduced efficacies, WHO has extended their recommendation for rotavirus vaccine introduction to all countries based on the higher predicted number of deaths averted in low-income countries (28).

Most of the difference in vaccine efficacy between high and low-income countries may be due to lower immunogenicity of oral live vaccines in developing countries (29, 30). Enteropathy and malnutrition are thought to be major factors responsible for the reduced immune response to oral vaccines (31, 32). Whether the greater variety of locally circulating genotypes and lower correlation with vaccine genotypes also contribute to the reduced efficacy remains a question for debate. Pooled data from randomized controlled trials show that the G1P[8]-containing Rotarix® vaccine offers some protection against non-G1 and non-P[8] strain (33), but vaccine

effectiveness studies in countries that have seen the emergence of the fully heterotypic G2P[4] genotype after introduction of Rotarix® have shown contradictory results on heterotypic protection (34, 35).

Countries that have introduced rotavirus vaccines into their immunization programs have seen an improvement in child health. Recent studies show the swift and significant impact of rotavirus vaccines following introduction in national immunization programs. In Mexico, diarrheal deaths in children < 5 years of age decreased by 46% during 2007-2009 (36). In Australia, Belgium, El Salvador and the United States, hospitalizations and clinic visits for rotavirus-related diarrhea in children < 5 years of age declined by 60-94% between 2007 and 2010 (22). This reduction of severe diarrhea underscores the potential for rotavirus vaccines to save children's lives. Since 2011, Sudan, Ghana, Rwanda, Moldova, Yemen, Malawi, Armenia and Tanzania have introduced rotavirus vaccines into their national immunization programs. At present, 22 other countries in sub-Saharan Africa are expected to introduce rotavirus vaccines over the next several years (37).

Ongoing support from the Global Alliance for Vaccines and Immunization (GAVI) allows eligible countries to purchase vaccine at reduced cost, but the sustainability of time-limited vaccine subsidies remains a concern. Further, introducing current presentations of rotavirus vaccine into existing immunization programs may substantially disrupt the vaccine supply and cold chains. The added volume of new vaccines could displace other Expanded Programme on Immunization (EPI) vaccines from storage and transport space, overwhelm transport and storage at lower levels of the supply chain, and reduce the availability of all EPI vaccines at health centers where they are delivered. This scenario was born out during the 2006 to 2007 RotaTeq® and Rotarix® introductions in 7 Latin American countries. RotaTeq® and Rotarix® were too large for many of the existing supply chains, surpassing refrigerator capacities of many health centers and forcing health care workers to carry extra thermoses and cold boxes (38). Because no contingency plans were in place, these unexpected consequences resulted in the expiration of large stocks of vaccines. While this experience compelled manufacturers to re-design their vaccine packaging, it also underscores the possibility for new vaccines to not fit smoothly into supply chains, failing to reach their target populations and preventing other vaccines from reaching clinics.

Rotavirus in Niger. Since 2009, Epicentre has led a large-scale surveillance effort to gather data on the epidemiology of rotavirus in urban and rural Niger (39). From December 2009 to March 2012, 10,597 children aged 0-59 months presenting to health facilities in Niamey and Maradi with watery diarrhea and signs of dehydration were enrolled. Stool specimens were systematically collected at presentation, with a rapid test performed on-site to determine the presence of rotavirus and genotyping performed on a subsample of rotavirus-positive specimens to determine genotype distribution and evolution during the study period. Surveillance identified 30.4% (95% CI: 29.6-31.3) of diarrhea to be rotavirus-positive, with 80% of all rotavirus cases found among children < 1 year of age and 96% of all cases found among children < 18 months. A higher proportion of rotavirus was found among cases in rural health centers than urban hospitals (32.4% versus 23.3%). Severe rotavirus-positive diarrhea represented 1.2% of cases among children < 5 years of age and 3.0% among children < 1 year of

age. Cases were seen year-round, with a consistent peak in the dry and cool season (October to December) and a natural switch from G2P[4] to G12P[8] genotype predominance observed during the study period. With 30% of children aged 0-59 months with diarrhea and dehydration positive for rotavirus, these results confirm the high burden of rotavirus in Niger, particularly in children < 18 months of age.

The Ministry of Health of Niger has been formally approved for a GAVI-subsidized introduction of rotavirus vaccine, but has chosen to delay introduction until at least 2015 due to concerns about vaccine presentation, storage and cold chain requirements among others.

Serum Institute of India, Limited rotavirus vaccine. The currently in-development rotavirus vaccine (live attenuated bovine-human [UK] reassortant rotavirus vaccine) manufactured by the Serum Institute of India, Limited (SIIL) holds great promise for immunization programs in countries like Niger. The pentavalent vaccine (BRV-PV) contains rotavirus serotypes G1, G2, G3, G4 and G9 ($\geq$5.6 $\log_{10}$ FFU/serotype/dose) and is delivered in lyophilized form supplied with 2.5 ml of citrate bicarbonate buffer that is added for reconstitution just before oral administration. The proposed schedule includes a three-dose series of oral vaccine administered , concomitantly with EPI vaccines. The initial dose is given at 6-8 weeks of age, with each subsequent dose given following a 4-week interval.

Compared to the 2 WHO prequalified vaccines, the SIIL formulation introduces important advantages for immunization programs (Table 1). First, BRV-PV is expected to be more affordable than available vaccines, with the potential for dramatic cost savings after 2015 when GAVI subsidies can expire. Second, BRV-PV offers the unique potential to be delivered out of cold chain. BRV-PV was tested and found to be stable at 37°C for 1 year and 40°C for 6 months; if delivered out of cold chain, this formulation could introduce significant logistical advantages for national programs in sub-Saharan Africa where cold chain capacity is limited.

Pre-clinical evaluation of BRV vaccine. Because of the promising safety and immunogenicity profile observed in a phase I clinical trial, SIIL conducted a two-center, double-blind, randomized, placebo-controlled phase II clinical trial with three doses of the pentavalent rotavirus vaccine in Pune, India. Sixty healthy infants received three administrations containing Log_{10} 5.6-5.8 FFU/dose or placebo at 8-10 wks, 12-14 wks & 16-18 wks of age, with at least 4 weeks interval between each dose. Safety parameters included recording of solicited symptoms in the 14-day follow-up period after each dose, monitoring and recording of adverse events (AEs) and serious adverse events (SAEs) in the post-dose 28 day follow-up period, and changes in laboratory parameters including hematology, biochemistry, changes noted during physical examination and vital signs assessment. In total, 132 AEs were reported during the study, and 26 (86.7%) participants in the vaccine arm reported at least one AE during the study compared to 21 (67.7%) participants in placebo arm. Most reported adverse events were mild in intensity (97.4% in vaccine group and 94.6% in placebo group), and all events recovered without any sequelae before the study completion. No SAEs were reported during the entire period of the study. Overall, as the type, frequency, and severity of adverse events observed in vaccine-

treated participants appeared similar to those of placebo-treated participants, the SIIL rotavirus vaccine at 3 doses (Log_{10} 5.6-5.8 FFU/dose) was found to be safe and tolerable in infants.

Table 1. Summary of rotavirus vaccines

	Rotarix® (GlaxoSmithKline)	**Rotateq® (Merck)**	**BRV-PV (SIIL)**
Origin	Human monovalent	Bovine pentavalent	Bovine pentavalent
Genotype(s)	G1, P[8]	G1, G2, G3, G4, P[8], G6P[7]	G1, G2, G3, G4, G9
Vaccine course	2 doses – oral	3 doses – oral	3 doses – oral
Schedule	With DTP1 / 2	With DTP1 / 2 / 3	With DTP1 / 2 / 3
Age restrictions	First dose at 6-15 wk of age; Max age for last dose at 32 wk	First dose at 6-15 wk of age; Max age for last dose at 32 wk	First dose at 6-8 wk of age; Max age for last dose at 24 wk
Intussusception risk	None observed	None observed	None observed
Presentation	Lyophilized and reconstituted; or liquid	Liquid, single dose pouch	Lyophilized and reconstituted
Volume per dose	259.8 cm^3/1 dose box	798 cm^3/10 dose box	202.5 cm^3/1 dose box
Storage	2-8^oC; diluent at room temp	2-8^oC	< 25 ^oC to central facility and out of cold chain to distribution; diluent at room temp
Price (USD)	$2.5 / dose (GAVI)	$5 / dose (GAVI)	< $ 2 / dose (expected)
WHO pre-qualification	2007	2008	

Sero-conversion rates at 28 days post-Dose 3 in the phase II trial were 60.0% in the vaccine arm and 7.7% in the placebo arm, indicating that the vaccine is highly immunogenic as compared to placebo. The net sero-conversion rate of 52.3% [60.0% (BRV vaccine) – 7.7% (placebo)] observed in this study is similar to that observed with Rotarix® and Rotateq® vaccines in India and other developing countries (40-49). At baseline, 23.3% of participants in the vaccine arm and 38.5% of participants in the placebo arm had an IgA concentration of ≥20.00 units/ml, indicating an early exposure to natural rotavirus infection. These results were not unexpected, as similarly high initial sero-conversion rates (in the absence of prior vaccination) have been observed in South Asia in past surveillance and previous rotavirus vaccine studies (40, 41, 50). Nevertheless, the good immunogenic response seen in initially sero-positive infants (57.1%) implies that the vaccine can successfully colonize the infant gut, induce a robust immune response and significantly increase initial antibody levels, even in the presence of pre-existing IgA antibodies.

In summary, preliminary study has demonstrated that, compared to placebo, the SIIL rotavirus vaccine has a similar reactogenicity profile and is immunogenic in an environment where a substantial proportion of infants are initially sero-positive. These encouraging results justify conducting a phase III clinical trial to evaluate the protective efficacy of the SIIL rotavirus vaccine with dose Log_{10} 5.6-5.8 FFU/serotype/dose.

Permission for a phase III clinical trial was granted by the Drug Controller General of India in May 2013. This will be a double-blind, placebo-controlled study to assess efficacy of the SIIL pentavalent vaccine in prevention of severe rotavirus gastroenteritis. The multi-centric study will be conducted in 6 sites across India in collaboration with the Program for Appropriate Technology in Health (PATH) and was approved by the Western Institutional Review Board, USA. The study is expected to start in September 2013; 7500 children will be enrolled and followed until September 2016.

Study rationale. Sub-Saharan Africa carries the largest burden of rotavirus-related mortality, but immunization against rotavirus presents unique challenges. Current supply of the 2 WHO prequalified vaccines is constrained (51), and in many African settings, national immunization programs are challenged by supply shortages and a lack of trained health workers. Unreliable transportation systems and storage facilities also make it difficult to preserve vaccines that require refrigeration. If rotavirus vaccine is to be brought to the infants that need it most through national immunization programs in the region, new vaccines that address these challenges are urgently needed. The BRV-PV vaccine is a relatively low-cost and heat-stable formulation whose introduction into national immunization programs may help minimize the burden on already-strained national programs throughout sub-Saharan Africa.

The WHO Expert Committee on Biological Standardization has recommended that the efficacy of new rotavirus vaccines be demonstrated in diverse geographical regions including developing countries before widespread implementation (52). The Ministry of Health of Niger, Médecins Sans Frontières (MSF) – Operational Center Geneva and Epicentre along with other partners have formed a research consortium to bring additional evidence to inform public health decision making on the potential value of the BRV-PV vaccine in an African setting. The goal of the present study is to collect additional data on the efficacy profile of BRV-PV vaccine in a randomized controlled setting, while gaining further experience with vaccine-related adverse events. This will be conducted through the performance of a phase III trial in Niger conducted in compliance with the version of the protocol agreed to by the applicable regulatory authorities and Good Clinical Practice (GCP).

Evidence supporting the efficacy and safety of this formulation in an African setting would support the pre-qualification and increased global access to the BRV-PV vaccine. If shown to be efficacious and pre-qualified, the government of Niger would benefit from a low cost vaccine adapted to the logistical and supply demands of the national immunization program.

STUDY OBJECTIVES AND ENDPOINTS

Primary objective:

(1) To estimate the efficacy of three doses of the SIIL pentavalent rotavirus vaccine vs. placebo against severe rotavirus gastroenteritis at 1 year of age.

Secondary objectives:

(2) To estimate the efficacy of three doses of the SIIL pentavalent rotavirus vaccine vs. placebo against severe rotavirus gastroenteritis at 2 years of age.

(3) To estimate the safety of three doses of the SIIL pentavalent rotavirus vaccine in terms of adverse and serious adverse events from the time of the first dose until 28 days post-Dose 3 (adverse events) or 2 years of age (serious adverse events).

(4) To estimate the immunogenicity of three doses of the SIIL pentavalent rotavirus vaccine in terms of anti-rotavirus IgA sero-conversion in a sub-sample of participants.

(5) To assess the difference in immunogenicity by level of breast milk concentration of rotavirus-specific IgA in a sub-sample of participants.

(6) To assess the difference of anti-poliovirus antibody titres at 28 days post-Dose 3 by study group in a sub-sample of participants.

Primary endpoint. The primary endpoint is vaccine efficacy against a first episode of severe wild-type rotavirus gastroenteritis from 28 days after the final dose until 1 year of age. Severe gastroenteritis will be defined clinically as an episode of gastroenteritis that needed overnight treatment in hospital and/or rehydration therapy equivalent to the WHO Plan B (oral rehydration therapy) or Plan C (intravenous rehydration therapy) in health facility, or using the 20-point Vesikari scale (53), where an episode of gastroenteritis with a score of 11 or more is considered severe.

Secondary endpoints. Secondary efficacy endpoints include vaccine efficacy against a first episode of severe wild-type rotavirus gastroenteritis during the second year of life and for the combined period from 28 days after the final dose until 2 years of age; rotavirus gastroenteritis of any severity; rotavirus gastroenteritis with a Vesikari score of ≥ 17; rotavirus gastroenteritis by serotype; gastroenteritis of any cause; longitudinal prevalence of rotavirus gastroenteritis; hospitalization due to rotavirus gastroenteritis; and hospitalization for any reason.

Safety endpoints include the risk of adverse events from the time of the first dose to 28 days post-Dose 3 and the risk of serious adverse events from the time of the first dose until 2 years of age. Adverse events will include fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in Standard Operating Procedures), diarrhea, vomiting, decreased appetite,

decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia). Serious adverse events will be defined as any new health-related problem that results in disability, incapacity or death; necessitates hospitalization; or is life-threatening, and according to the Brighton Collaboration for adverse events following immunization for oral vaccines will also include generalized convulsive seizure, hypotonic-hyporesponsive episodes, intussusception, and persistent crying (54, 55).

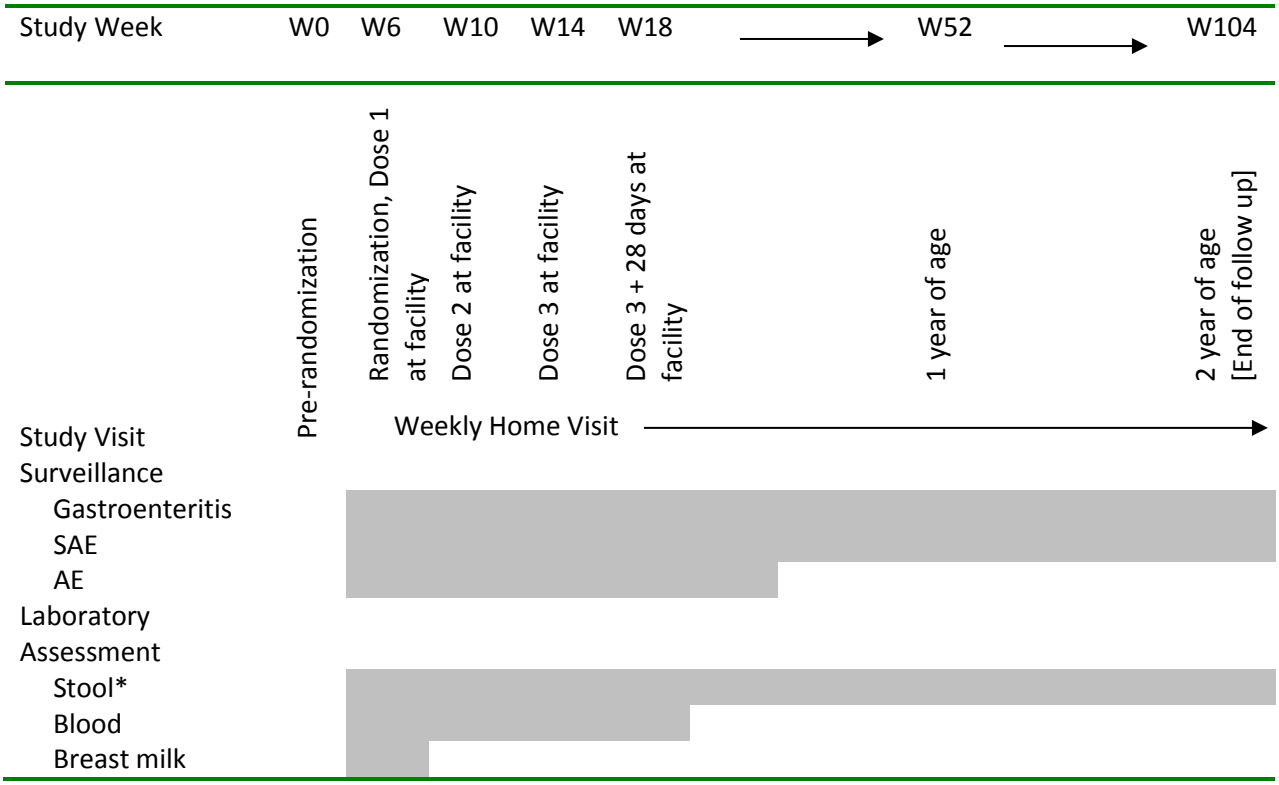
Immunogenicity endpoints will be assessed in a sub-sample of participants. The principal endpoint will include three-dose sero-conversion, defined as at least a three-fold serum titre rise for anti-rotavirus IgA from the first dose to 28 days post-Dose 3. Sero-conversion following Dose 1 and Dose 2, as well as geometric mean titres following all doses, will be considered secondary immunogenicity endpoints. Immunogenicity analyses will consider concentrations of maternal anti-rotavirus IgA antibodies in breast milk in order to compare immunogenicity by level of maternal anti-rotavirus IgA concentration, as well as anti-poliovirus antibody serum titres to assess interference of the study vaccine with anti-polio immunogenicity.

METHODOLOGY

Study design. We propose a double-blind, placebo-controlled randomized phase III trial with two parallel groups in Madarounfa, Niger to assess the efficacy, safety and immunogenicity of BRV-PV, a pentavalent rotavirus vaccine manufactured by SIIL. The primary aim of the study is to assess the efficacy of three doses of pentavalent rotavirus vaccine vs. placebo against severe wild-type rotavirus gastroenteritis at 1 year of age when administered concomitantly with EPI vaccines. Infants aged approximately 6-8 weeks will be individually randomized in a 1:1 ratio using permuted blocks of random sizes to receive three doses BRV-PV or placebo and followed until 2 years of age. Episodes of gastroenteritis, adverse events and serious adverse events will be identified through facility- and home-based surveillance until 2 years of age (28 days post-Dose 3 for adverse events). Immunogenicity will be assessed in a sub-sample of study participants 28 days following each dose.

Placebo, instead of an active comparator, will be used in this trial as no other licensed rotavirus vaccine is yet available in Niger and data from an African setting are needed to inform WHO prequalification of BRV-PV (see Choice of Placebo Comparator below).

Table 2. Schema of overall study design



* Stool collected at facility or home one time per episode of loose or watery stools and/or forceful vomiting within 7 days of onset.

Study setting. Niger is one of the poorest countries in the world, ranking 186 of 187 in 2011 on the Human Development Index(56). Fertility is high, estimated by the 2006 Demographic and Health Survey (DHS) at 7·1 children per woman(57). While rates of child and neonatal mortality in Niger have been among the highest in the world, significant progress has been made with investments in maternal, child and newborn program and policy. Recent data suggests child mortality in Niger declined 43% between 1998 and 2009, from 226 to 128 deaths per 1000 live births(58). Progress in reducing neonatal mortality however has been slower, with the same data showing high levels of neonatal mortality and no significant reduction in neonatal mortality between 1998 and 2009 (39 vs. 33 neonatal deaths per 1000 live births).

The health system in Niger is a pyramidal system in line with the 1985 Lusaka agreements, based on health structures with increasing levels of service capacity: health posts (cases de santé) provide basic care and preventive services and are most often staffed by community health workers who are helped by community representatives. Health centers (centres de santé intégrés) are staffed by nurses and ensure the provision of all services not requiring hospitalization. Complications are referred to the district hospital and to the regional hospital from the district hospital. Integrated Management of Childhood Illness (IMCI) algorithms provide the basis for the organization of care and referral system for children. In this organization, severity signs are assessed at the level of health centers and only severe cases are referred to the district hospital, with the exception of severe dehydration, which should be treated immediately at the health center.

The current organization of the health system is based on several international initiatives launched in 1995-1996. In Niger, this period was marked by the creation of Health Districts for decentralization of care, the gradual introduction of IMCI in all districts, and the implementation of cost recovery systems following the Bamako Initiative. Despite these efforts, the most recent final report from the national DHS in Niger in 2006 showed that only 17% of caretakers of children < 5 years of age had sought advice or treatment in case of diarrhea in their child (57). With the aim of reducing child mortality in line with the UN Millennium Development Goals, free care for children under 5 years of age was introduced in April 2007.

The study will take place in the region of Maradi, in south-central Niger along the Nigerian border. The region of Maradi is comprised of seven Health Districts with a total estimated population in 2009 of about 3 million inhabitants. The proposed site for this study is the Madarounfa Health District, a rural area of 4700 km^2 largely representative of the Sahel region of Niger and sub-Saharan Africa (59). In 2009, the average number of public health structures per 100 km^2 was 1.5 and the public health system coverage, defined as the proportion of the population with access to any health structure within a distance of less than 5 km, was 83% (60).

In collaboration with the Ministry of Health, MSF has been supporting pediatric care in the Madarounfa Health District of Maradi since 2001. Since 2009, project activities have been transferred to local control and implemented through a Nigerien non-governmental organization, Forum Santé Niger (FORSANI) in collaboration with the Ministry of Health. FORSANI provides care and treatment to over 30,000 children in the Madarounfa Health District

each year. Epicentre, the epidemiologic and research organization affiliated with MSF has been present in Niger since 2009. In close partnership with the Ministry of Health, MSF, the CERMES and other NGOs working in the area, Epicentre develops and conducts research aimed at responding to the medical and operational objectives of local and regional public health actors. A team of medical professionals, epidemiologists, biologists and data management specialists work in Maradi and Niamey.

Study intervention (vaccine and placebo). The study vaccine, BRV-PV, is a pentavalent rotavirus vaccine containing rotavirus serotypes G1, G2, G3, G4, and G9 manufactured by SIIL. Each dose of vaccine contains an estimated potency of ≥ 5.6 $\log_{10}$ infectious units / serotype per dose; dose selection was based on demonstrated immunogenicity in phase II trials. Placebo, also manufactured by SIIL, will contain the same constituents as the active vaccine but without the viral antigens; both are lyophilized and will be reconstituted with 2.5 ml of liquid citrate bicarbonate buffer before administration. The initial dose of study vaccine or placebo will be administered orally at a health facility by a study physician at approximately 6-8 weeks of age. The second and third doses of study vaccine or placebo will each be administered following a 4 week internal (-1 to +4 weeks), with a maximum age at last dose of 24 weeks.

Study vaccine and placebo will be administered concomitantly with EPI vaccines, however, administration of the study intervention will not be delayed if EPI vaccines are unavailable at the time of study dosing. Infants who have already received EPI vaccines at the time of study dosing can still receive the study intervention.

Table 2. Characteristics of study interventions

Study Vaccine	Live attenuated Bovine-Human (UK) reassortant pentavalent rotavirus vaccine containing ≥ $\log_{10}$5.6 FFU/dose of each serotype G1, G2, G3, G4 and G9
Placebo	Placebo, consisting of the lyophilized MEM culture medium used to grow the vaccine, excipients and stabilizers
Diluent	Buffered Diluent (containing 25.6 mg/mL of sodium bicarbonate and 9.6 mg/mL citric acid per liter)
Manufacturer	Serum Institute of India Limited, India

Preparation and administration. The process for preparation and administration of vaccine and placebo will be fully detailed in the study Standard Operating Procedures (SOP). In brief, the vaccine and placebo are dispensed as single dose and are for one time use only. The reconstituted vaccine and placebo, as well as the vial containing buffered diluent, should be inspected visually for any foreign particulate matter and/or abnormal physical appearance prior to administration. In the event either is observed, the vaccine or placebo will be discarded.

BRV-PV and placebo are for oral use only and should under no circumstances be injected. Neither vaccine nor placebo should be mixed with other medicinal products. Procedures for reconstitution are as follows:

- Remove plastic caps from the vials containing buffered diluent and lyophilized powder.
- Fix transfer adapter to 5 ml disposable syringe (without needle).
- Connect syringe/adapter to the vial containing buffered diluent.
- Pull the plunger back and aspirate 2.5 ml of buffered diluent from vial into the syringe.
- Remove the assembly from buffer diluent vial and connect it with the vaccine or placebo vial containing lyophilized powder.
- Inject the entire contents of the syringe into the vial.
- With syringe still attached shake the vial and examine for complete suspension of the powder. The reconstituted vaccine or placebo will appear as a clear, pinkish solution.
- Pull the plunger back and aspirate reconstituted solution from vial into the syringe.
- Remove the syringe from the transfer adapter.

Study vaccine and placebo must be administered within one hour of reconstitution.

Packaging and labeling. Each single oral dose of study vaccine and placebo will be approximately 2.5 ml in volume. Packaging for both study vaccine and placebo will contain 1 vial of lyophilized vaccine/placebo, 1 vial of citrate bicarbonate buffer, 1 adapter and 1 syringe (without needle) of 5ml capacity for vaccine reconstitution. Only the specific buffer diluent provided must be used for reconstitution.

The vials used for administration of the study drugs will be labeled according to the local regulations and requirements of the study protocol. Label text will be approved by the sponsor prior to label printing. All labels will contain the following minimum information**:**

- Name of Sponsor/Manufacturer
- Imprint “For Clinical Trial Use Only”
- Imprint “For Oral use only”
- Blind code to identify content

Finally, a vaccine vial monitor will be affixed to each vial to allow a visual indication of whether the product has been kept at the recommended temperature.

Storage. The study vaccine and placebo will be stored below 25°C up to the central facility. Changes in temperature outside the allowed range before arrival at the central facility-level will be immediately reported and any lots experiencing such out of range changes will be brought to the attention of the sponsor for determination of appropriate action.

After arrival at the central facility-level, study vaccine and placebo will be stored out of cold chain until administration. Ambient temperatures will be recorded on a daily basis at the Epicentre Maradi Weather Station to describe the conditions observed out of cold chain during the study period. Procedures for proper storage from the manufacturer (SIIL) to Niamey, the central facility level (Maradi) and to administration at the rural health facility (Madarounfa) will be fully detailed in the study SOPs.

Study supply and accountability. All study vaccine and placebo will be provided by SIIL. The sponsor will acknowledge receipt of the vaccine and placebo indicating shipment content and condition. The sponsor will maintain an inventory record of study product received and account for all study products used in the study using appropriate accountability records.

Temperature will be monitored during shipment, and the sponsor will check and maintain temperature records on file. The sponsor will inform SIIL immediately of any shipment that is out of range. The lot number of used study products will be recorded at the time of administration for accountability and used vials destroyed on site. All unused or partially used study products and packaging will be returned to SIIL or destroyed on site. The process for disposing of unused or partially used materials will be fully detailed in the study SOPs.

Choice of placebo comparator. Two vaccines are currently WHO prequalified, however, several issues arise with respect to their use in the evaluation of BRV-PV. A placebo-control, instead of an active comparator, has been selected for this trial given the following issues and ethical considerations (61, 62):

- It is known that the current oral vaccines have lower efficacy in low-resource settings than in high-resource settings. To inform eventual WHO pre-qualification and increased access to rotavirus vaccine, it will be important to demonstrate the absolute efficacy of new formulations in low-resource settings. A placebo-controlled design is needed to directly demonstrate absolute efficacy; an active-comparator design would determine relative efficacy and be less informative in the process towards WHO pre-qualification.

- There is no efficacy data on any licensed rotavirus vaccine in Niger, and a placebo-controlled trial remains defensible and appropriate where there is no “proven” intervention, as per the Declaration of Helsinki (Appendix D).

- Rotavirus vaccine is not part of EPI in Niger. As rotavirus vaccination is not a standard of care, ethical considerations permit the use of a placebo, instead of an active comparator (61). Although children in the placebo group will not potentially benefit from vaccination during the study period, all children will benefit from prompt, free and appropriate primary care, minimizing the risks and enhancing benefits to participants. If shown to be safe and efficacious and pre-qualified, the vaccine should be made available to all children in Niger through the national immunization program.

- A non-inferiority trial using an active comparator would require a prohibitively large sample size and involve the disproportionate use of time and resources. It would be practically infeasible to conduct such a trial, and the resulting impact of such an investment has been subject to debate and criticism due to the potential delay in bringing rotavirus vaccines to children who stand to benefit most.

- There is no rotavirus vaccine currently registered in Niger by the National Regulatory Authority, and current supply of the 2 WHO prequalified vaccines is constrained (51), suggesting no practical active alternative to placebo in this setting.

Randomization and blinding procedures. Infants will be individually randomized in a 1:1 ratio to receive three doses of the vaccine or placebo. The initial dose will be orally administered concomitantly with EPI vaccines at approximately 6-8 weeks of age. The second and third doses of study vaccine or placebo will each be administered following a 4 week interval (-1 to + 4 weeks), with a maximum age at last dose of 24 weeks.

A list of unique identification numbers from 1 to 5138 will be allocated to vaccine or placebo by SIIL using a computer-generated random number list using permuted blocks of random sizes. Block sizes will not be disclosed to reduce predictability of the random sequence and ensure allocation concealment. Study physicians who will oversee randomization will be given a subset of sequentially numbered, sealed, opaque envelopes that will be prepared by SIIL and contain printed randomization numbers and a corresponding group code. The study physician will be instructed to assign the randomization number and group code noted in the next envelope to each eligible infant as (s)he is enrolled. Adherence to the randomization list will be periodically verified by the Study Monitor. The randomization list will remain with SIIL for the duration of the study; randomization will therefore be conducted without any influence of sponsors or field personnel.

Vaccine and placebo packages will be labeled with the assigned code and delivered to the study site in otherwise identical presentations. Vaccine and placebo presentation and packaging will be indistinguishable to insure that caregivers and investigators can not identify to which group the infant has been assigned. Group assignment will remain concealed from study team, investigators and parents of participating infants for the whole study period. The Data and Safety Monitoring Board (DSMB) and sponsor statistician will be masked to the group assignment. The DSMB will remain masked unless otherwise deemed necessary by the DSMB members. Investigators conducting the final analysis will remain masked to the group assignment until the end of the analysis.

The study code will be broken only in case of a medical event in which the Medical Coordinator deems the participant cannot be appropriately treated without knowing his/her group assignment. A set of sealed envelopes with group assignment will be held at the field site with the Medical Coordinator. All code breaks will be fully documented and reported to the sponsor within 48 hours, and the date, time and reason for unblinding will be noted. Codes will not be freely available to the sponsors or other study personnel until after the completion of the trial and final data review.

Unblinding will not be sufficient reason for individual discontinuation from the study.

Target population. The study will be performed in infants in Madarounfa, Niger. Healthy male and female infants meeting the following inclusion criteria are eligible for enrollment:

(1) aged 6-8 weeks at the time of inclusion
(2) able to swallow and no history of vomiting within 24 hours
(3) resident in Madarounfa Health District within 15 km of central health facility
(4) intending to remain in the study area for 2 years
(5) parent/guardian providing informed consent

There will be no restriction based on breast feeding around the time of vaccination, receipt of routine pediatric vaccinations, prematurity, low birth weight or HIV status (63). Inability to swallow and history of vomiting within the last 24 hours are the only conditions based on the infant's immediate clinical status to delay oral administration of the study vaccine or placebo.

Exclusion criteria will include the following:

(1) unable to swallow or history of vomiting within 24 hours
(2) known history of congenital abdominal disorders, intussusception, or abdominal surgery
(3) receipt of intramuscular, oral, or intravenous corticosteroid treatment within 2 wks
(4) receipt or planned administration during the study period of a blood transfusion or blood products, including immunoglobulins
(5) non-resident in Madarounfa Health District within 15 km of central health facility
(6) not intending to remain in the study area for 2 years
(7) parent/guardian informed consent not provided
(8) any other condition in which, in the judgment of the investigator, would interfere with or serves as a contraindication to protocol adherence or the parent/guardian's ability to give informed consent*.

*While determining eligibility, study physicians will be asked to use good clinical judgment in considering a participant's overall fitness for inclusion. Some participants may not be appropriate for the study even if they meet all inclusion criteria. For instance, medical, occupational or other conditions of the caregiver may make routine home visits and evaluation difficult or make the child a poor candidate for retention.

Discontinuation criteria. The following criteria will be checked at each Weekly Home Visit and if any becomes applicable the participant will be required to discontinue vaccination and/or follow up:

(9) Serious adverse event related to the study intervention
(10) Use of any investigational or non-registered vaccine other than the study vaccine during the study period
(11) Administration of immunosuppressant for > 14 days during the study period
(12) Administration of any blood products during the study period
(13) Parent/guardian consent withdrawal
(14) Permanently migrated or moved from the study area
(15) Loss to follow up

(16) Opinion of the study sponsor that it is not within the subject's best interest to continue vaccination or follow up

The Medical Coordinator will discuss any potential discontinuation with the PI and sponsor. The sponsor will have final authority to discontinue vaccination and/or follow up of any participant according to the above criteria.

In the event of a participant's discontinuation in the study, an Early Termination Visit will be performed by the Medical Coordinator. The Early Termination Visit will include a review of address and contact details; physical examination; update of relevant medical history and intervention; review of vaccination history; and documentation of reason(s) for termination. In the event of a Serious Adverse Event leading to discontinuation, the child will be followed until resolution of the event and/or the end of the study (whichever is earlier). A participant's parents/guardian can withdraw consent for participation in the study at any time without prejudice.

After discontinuation, follow up for all endpoints will cease, and any participant discontinued from the study will not be replaced. Data collected up to the point of last contact will be included in the analysis. Subjects who are discontinued due to migration or lost to follow up but later present and are willing to continue participation will continue to be followed until 2 years of age, and all available data will be included in the analysis.

Study sites. Study activities will take place in the Madarounfa Health District, Maradi, Niger. Health facilities in the Madarounfa Health District, including 1 hospital, 8 health centers and 25 health posts in the 8 rural health zones of Gabi, Safo, Madarounfa, Dan Issa, Tofa, Serkin Yama, Djirataoua, and Moulé will be included as Surveillance Sites, where a study physician or nurse will be present to assess symptoms of gastroenteritis and adverse events. Of the 34 Surveillance Sites, a limited number of facilities will be designated as Enrolment and Dosing Sites. Enrolment and Dosing Sites will be staffed by study physicians trained to seek informed consent and equipped with appropriate emergency medical treatment in the event of any acute serious adverse event post-immunization. The number and location of Enrolment and Dosing Sites will be made to insure reasonable access by the study population and in consideration of available human resources.

Additional study activities will be take place at the participants' home (see Recruitment and Follow up for further detail).

Recruitment. Rotavirus in Niger is known to circulate year-round in the Maradi region (with a consistent peak in October to November), therefore enrolment will occur year-round. Eligible children will be continuously identified during an 18-month enrollment period using community-based recruitment monitors selected from each village by village representatives, often being a women's group leader or midwife. These individuals selected as recruitment monitors are intended to be well regarded and trusted members of the community with broad knowledge of community events, such as births, deaths and migration. Beginning two months

before study initiation, the recruitment monitor will begin to record all pregnancies in his/her village. The registry will be routinely updated, and notice of the time and place of any live birth will be immediately transferred to study staff as they occur during the enrollment period.

Within 48 hours of a live birth, a study nurse will visit mothers at home for a **Pre-randomization Visit**, at which time the nurse will provide the household with information about the study. An informational sheet with details of the study will be provided and discussed with caregivers and heads of households. The Pre-randomization Visit will allow caregivers adequate time to consider their participation in the trial and prepare any questions they may have, thereby reducing the number of defaulters once randomization has occurred. At this time, informed consent will be sought specifically to allow recording of maternal mid-upper arm circumference (MUAC), as well as infant birth weight and gestational age at birth, using standard field procedures. Informed consent to participate in the full trial will be sought at the time of randomization. Infants < 6 wks of age who migrate into the study area will be identified by recruitment monitors and similarly visited at home by a study nurse for a Pre-randomization Visit and confirmation of the child's age. At all Pre-randomization Visits, the study nurse will confirm receipt of routine vaccinations scheduled for administration at birth [e.g. Bacillus Calmette-Guérin and oral poliovirus vaccine] through review of the infant's vaccination card, and as needed, invite the caregiver to have the child vaccinated at home or the nearest health facility.

At 6-8 weeks of age, the recruitment monitors will ask interested caregivers to present with their child to the nearest Enrollment and Dosing Site for the **Randomization Visit**. At this time, a study physician will confirm eligibility with an assessment of age, residence and medical history and will seek informed consent from the parent/guardian for the child's participation in the full study. Issues of confidentiality will be underscored. No financial or non-financial incentives will be provided to study staff for enrolment.

If during the eligibility evaluation a child is found to be unable to swallow or to have history of vomiting within 24 hours but is otherwise eligible for study inclusion, the study physician can delay randomization. The caregiver and infant will be asked to return to the Enrollment and Dosing Site 24 hours after symptoms resolve but before 8 weeks of age for randomization and administration of Dose 1. If a child is found to be entirely ineligible for inclusion, the child will be recorded as an exclusion on an Eligibility Evaluation Case Report Form (CRF), with the reason for exclusion noted.

Once informed consent for participation in the full trial is provided by the parent/guardian during the Randomization Visit, the child will be enrolled in the study and a randomization number assigned. Study procedures, to be detailed in the study SOPs, are as follows:

- Administration of study intervention (Dose 1): The first study dose will be administered at the health facility according to the study's randomization procedures. A Dosing CRF will be completed to record information on the study product received, date and time. If a child vomits any of the study product immediately after administration, a new dose will be

administered and the event will be recorded on the appropriate CRF. A repeat dose will not be administered if the child drools or spits up any of the study product. Study staff will remain with the infant for a minimum of 30 minutes to record any acute serious adverse event post-immunization. Any reactions that occur during this time will be recorded on the standardized Dosing CRF, and appropriate emergency medical treatment will be readily available in case of a rare reaction following administration of a study product. Breastfeeding 30 minutes before or after administration of the study product will be documented on the Dosing CRF.

- Administration of EPI vaccines: During the Randomization Visit, the study physician will verify previous receipt of any EPI vaccine through review of the infant's vaccination card. As needed, the study physician will concomitantly administer any required routine EPI immunization and update the child's vaccination records (see Standard Care below for schedule of EPI vaccines to be provided). Note: Supply of EPI vaccines will be supported by the study sponsor, but if any EPI vaccine is not available at the time of study dosing, administration of the study intervention will not be delayed. Children who have received EPI vaccines prior to randomization remain eligible to receive the study intervention at this time.

- Collection of background information: A detailed background questionnaire will be administered to the caregiver, including information on maternal age, nutritional status (e.g. weight, height and MUAC) and reproductive history (e.g. parity, age of first delivery); household size and socio-demographic characteristics; infant's clinical and breast feeding history; and infant anthropometry.

- Collection of biological specimens (sub-sample of participants and caregivers): Blood and breast milk specimens will be collected for testing of anti-rotavirus IgA concentration from a sub-sample of infants and breastfeeding mothers, respectively (see Laboratory Assessment below).

Note: If a child is found to have gastroenteritis at the time of randomization, randomization and administration of Dose 1 will not be delayed but a stool sample will be collected for detection of rotavirus antigen. HIV status will not be systematically assessed by study staff during the Randomization Visit.

After all procedures of the Randomization Visit have been completed, the caregiver will receive a study identification card, which should be shown at the time of presentation to any health facility and will include study contact information in case of emergency. At this time, the study physician will remind the participant's caregiver of the next scheduled home visit.

The recruitment period will extend over 18 months. We anticipate recruiting 65 infants per week in 8 rural zones of the Madarounfa Health District [e.g. Gabi, Safo, Madarounfa, Dan Issa, Tofa, Serkin Yama, Djirataoua, and Moulé], where there were 9021 live births in 2013(59).

Dosing Visits. A three-dose series of study vaccine or placebo will be orally administered to infants with the initial dose given at time of the Randomization Visit (6-8 weeks of age) and each

subsequent administration given after a 4-week interval (-1 to +4 weeks) . Maximum age at last dose is 24 weeks. All doses of study vaccine or placebo will be administered at a designated Enrollment and Dosing Site by a trained study physician. If a child vomits any of the study product immediately after administration, a new dose will be administered and the event will be recorded on the appropriate Dosing CRF. A repeat dose will not be administered if the child drools or spits up any of the study product. Study staff will remain with the infant for a minimum of 30 minutes to record any acute serious adverse event post-immunization. Any reactions that occur during this time will be recorded on the standardized Dosing CRF, and appropriate emergency medical treatment will be readily available in case of any reaction following administration of a study product. Breastfeeding 30 minutes before or after administration of the study product will be documented on the Dosing CRF.

If child is found to be unable to swallow or have history of vomiting within 24 hours of a scheduled Dosing Visit (Dose 2 or Dose 3), the study physician can delay administration of the study intervention for a maximum of 8 weeks from administration of the previous dose, respecting a maximum age at last dose of 24 weeks. The caregiver and infant will be asked to return to the Dosing Site 24 hours after symptoms resolve within this interval for the next administration of the study intervention. If a child is found to have gastroenteritis at the Dose 2 or Dose 3 Visit, dosing will not be delayed but a stool sample will be collected for detection of rotavirus antigen.

Study vaccine or placebo will be administered concomitantly with routine EPI immunizations. At each Dosing Visit, the study physician will verify previous receipt of any EPI vaccine through review of the infant's vaccination card, and as needed, concomitantly administer any required routine EPI immunization (see Standard Care below for schedule of EPI vaccines to be provided). Administration of the study intervention will not be delayed if EPI vaccines are unavailable at the time of study dosing. Infants who have already received EPI vaccines at the time of study dosing (e.g. oral polio vaccine administration provided through routine mass vaccination campaigns) will still receive the study intervention as scheduled.

If a child does not present to the health facility for a subsequent Dosing Visit as scheduled, the study team will conduct a home visit to encourage the caregiver to present to the health facility as soon as possible. If the child cannot be seen within -1 to +4 weeks of the scheduled dosing visit, follow up will be handled on a case-by-case basis in coordination with the Medical Coordinator and sponsor.

Assessment of compliance. Study vaccine or placebo will be orally administered by trained study physicians only to infants included in this study. The date, dosage, and time of the vaccinations will be recorded on a standardized Dosing CRF. The Study Field Coordinator will track vaccines received, used and wasted and will manage all unused or expired products.

Follow up. All children will be followed from the time of the first dose until 2 years of age (gastroenteritis and serious adverse events) or from the time of first dose to 28 days post-Dose 3 (adverse events) using both facility- and weekly home-based follow up.

Caregivers will be informed about the signs and symptoms of gastroenteritis and all adverse events, and they will be asked to immediately seek care at a local facility if there is any condition that requires medical attention or is of concern. Upon presentation at the facility, study staff will conduct a medical history of symptoms and medical interventions received for the current illness and perform a clinical exam to document physical signs and clinical indicators of disease severity. Appropriate medical care will be provided in accordance with GCP. If the episode is ongoing at the time of discharge from the health facility, daily home-based follow up by a nurse assistant will organized from the time of discharge until resolution of the episode.

In addition, **Weekly Home Visits** will be performed by a community health agent. At each weekly visit, the community health agent will check discontinuation criteria, and as appropriate, conduct a general physical exam to assess the participant's clinical status, ask caregivers to recall all medical signs or symptoms that are a concern to them at that time, and review the Weekly Diary Card completed by caregivers to confirm information on events since the last visit, including cases of gastroenteritis, adverse events (from first dose to 28 days post-Dose 3), serious adverse events, and medical intervention (including treatments or other vaccinations received, and admission to hospital or therapeutic nutritional programs after 6 months of age). At each weekly visit, the community health agent will remind caregivers to immediately contact the study team in the event of any episode of gastroenteritis or adverse event.

Children found to require medical intervention during the Weekly Home Visit will be referred to a study health facility for management free of charge by study staff and in accordance with GCP. Every 4 weeks, the child's anthropometric status (e.g. weight, height and MUAC), breastfeeding status (e.g. ever vs. never; currently exclusive vs. partial vs. no breastfeeding; frequency of feeds in last 24 hours) and dietary intake will be assessed at home using standard procedures with assistance from the caregiver. If a child is not present at the time of a Weekly Home Visit, the community health agent may contact neighbors or relatives in the area to ascertain the infant's vital and health status and expected time/day of return so that a repeat visit may be scheduled. After all procedures of the Weekly Home Visit have been completed, a reminder will be given about the next scheduled study visit. HIV counseling and testing will not be systematically provided by study staff during the Weekly Home Visit but may be offered as clinically appropriate.

Outcome definition and assessment. Gastroenteritis. Gastroenteritis will be defined as the passage of three of more loose or watery stools within a 24 hour period and/or forceful vomiting. Severe gastroenteritis will be defined clinically as an episode of gastroenteritis that needed overnight treatment in hospital and/or rehydration therapy equivalent to the WHO Plan B (oral rehydration therapy) or Plan C (intravenous rehydration therapy) in health facility, or using the 20-point Vesikari scale (53), where an episode of gastroenteritis with a score of 11 or more is considered severe. A gastroenteritis episode will be considered to be caused by rotavirus if a rotavirus strain is identified in a stool sample collected within 7 days on onset of

symptoms. Gastroenteritis episodes will be classified as two separate episodes if there is an interval of 5 or more consecutive, symptom-free days between the episodes.

Cases of gastroenteritis episodes will be captured through facility- and home-based surveillance from the moment the first dose of vaccine or placebo is administered until 2 years of age. Through facility-based surveillance, one study nurse or physician will be assigned to each health facility able to receive children for gastroenteritis serving the study population of Madarounfa (e.g. 1 hospital, 8 health centers and 25 health posts). Surveillance at all levels of health facilities has been selected for case detection based on available evidence regarding health care seeking behavior in Maradi. In 2009, we conducted a household survey including 2940 children < 5 years of age in 4 districts of Maradi to assess health care utilization practices for the treatment of childhood diarrhea (64). The survey found 37% (95% CI: 34-40%) of children had at least one episode of diarrhea in the previous 1 month and over 70% of cases were managed at a health structure. Lower level health facilities were found to be more frequently visited than hospital for diarrhea management, with 54% of severe diarrhea cases presenting to a health center, 26% to a health post and 11% to a hospital. This evidence suggests that hospital-based case detection alone would be inadequate in this context, and we therefore propose inclusion of health facilities at all levels, in combination with home-based surveillance, for complete case detection.

To encourage presentation at health facilities, caregivers will be informed about the signs and symptoms of gastroenteritis and will be asked to seek care at a local facility if any episode of gastroenteritis is suspected. Compensation for travel to the local facility will be provided for each visit made. Caregivers will be instructed to show the study identification card provided at randomization at the time of presentation to the facility that will identify the infant as a participant in the study. At the facility, the study staff assigned to that facility will collect a stool sample if the onset of the loose or watery stool and/or forceful vomiting is within 7 days, record history of symptoms and medical intervention received for the current illness through caregiver interview, and conduct a clinical exam to document physical signs and clinical indicators of disease severity. Documentation of clinical indicators will include temperature, the quantity and duration of vomiting and/or diarrhea episodes, dehydration status, and treatment in order to assign a Vesikari score. If stool is not available within 2 hours of presentation, a rectal swab will be taken. After discharge from the facility, all continuing gastroenteritis episodes will be followed at home by a nurse assistant on a daily basis until resolution (defined as ≥ 5 consecutive, symptom-free days). If the episode does not resolve within 7 days of discharge, the nurse assistant will refer the child back to a study facility for follow up. Community sensitization and outreach with community leaders, traditional medicine providers and others will be continuously reinforced to facilitate referral of sick children to health facilities throughout the study period. If an episode of gastroenteritis (e.g. ≥ 3 loose or watery stools within a 24 hour period and/or forceful vomiting) is identified before or on the day of presentation to the facility , all data collected during the episode will be transcribed to a standardized Gastroenteritis Surveillance CRF by study physicians after resolution of the episode.

A home-based surveillance system will be used to identify episodes of gastroenteritis for which a caregiver chooses not to present to a health facility. Caregivers will be advised to immediately inform their community health agent whenever there are 3 or more loose or watery stools within 24 hours and/or forceful vomiting. Caregiver communication of gastroenteritis episodes to community health agents will be reinforced through continued education intended to maintain caregiver awareness and interest in study activities and gastroenteritis surveillance. Episodes not immediately reported to the community health agent will be captured during review of the Weekly Diary Card at the Weekly Home Visit with caregivers. Once an episode of gastroenteritis is identified by a community health agent (either through caregiver notification intermediate to a scheduled Weekly Home Visit or during a scheduled Weekly Home Visit), it will be referred to a nurse assistant, who will monitor the episode with daily home visits until resolution (defined as ≥ 5 consecutive, symptom-free days). At each visit, the nurse assistant will complete a standardized Case Surveillance Card with the caregiver to collect information on the number of stools and vomiting episodes, temperature, dehydration status, medication or rehydration administered, and any medical attention sought (defined as medical personnel or facility contact, advice, visit or admission). Information from the Case Surveillance Card will be transcribed to a standardized Gastroenteritis Symptom CRF by study physicians at the resolution of the episode and will be used to determine the severity of gastroenteritis episodes not presented to a health facility using the Vesikari scale. If onset of an episode of gastroenteritis is within 7 days of a nurse assistant's home visit, the nurse assistant will collect the necessary stool sample (one per episode) and ensure appropriate conservation and transport to the Maradi laboratory on the same day. If any gastroenteritis episode is found to require medical attention, the nurse assistant will refer the child to a study physician to ensure appropriate medical care is received.

Adverse events. Adverse events (AEs), including fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in the study SOPs), diarrhea, vomiting, decreased appetite, decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia), will be assessed using facility- and home-based surveillance in all participants from the time of the first dose until 28 days post-Dose 3. Caregivers will be informed about the signs and symptoms of adverse events and will be asked to seek care at a local facility in the event any adverse event is suspected and of concern. At the facility, study staff will record history of symptoms of the current illness through caregiver interview and conduct a clinical exam to document physical signs and clinical condition.

In home-based surveillance, caregivers will be trained to complete an Adverse Event Diary Card to record adverse events and be advised to immediately inform their community health agent when an adverse event is suspected. Events not reported to the community health agent will be captured during the Weekly Home Visit, when the community health agents will review the Adverse Event Diary Card and confirm information on the incidence of all adverse events and related medical interventions received from the first dose until 28 days post-Dose 3. Once an adverse event is identified during this period (either through caregiver notification intermediate to a scheduled Weekly Home Visit or during a scheduled Weekly Home Visit), it will be referred

to a nurse assistant, who will follow the case with daily home visits until resolution. Nurse assistants will refer all adverse events that require medical attention, progress clinically or do not resolve within 7 days to a study physician to ensure appropriate medical care is received.

Serious adverse events (SAE), including any new health-related problem that results in disability, incapacity or death, necessitates hospitalization, or is life-threatening, and according to the Brighton Collaboration, generalized convulsive seizure; hypotonic-hyporesponsive episodes; intussusception (see Intussusception Risks, Assessment and Management below), and persistent crying (54, 55), will be assessed in all participants from the time of first dose until 2 years of age through facility- and home-based surveillance. Caregivers will be informed about the signs of SAEs and will be asked to seek care at a local facility if any is suspected. At the facility, study staff will record history of symptoms of the current illness through caregiver interview and conduct a clinical exam to document physical signs. As with cases of gastroenteritis and adverse events, a standardized Weekly Diary Card will be completed by caregivers and reviewed by community health agents in home-based surveillance to document the incidence of any SAE not attended to at a health facility. SAEs will be followed up until the event resolves, stabilizes, or is otherwise explained by the Medical Coordinator, who will determine whether the event is causally associated with vaccination.

Immunogenicity. Immune response to vaccination will be assessed in a total of 420 infants. Venous blood samples will be collected at 4 time points at a health facility for measurement of concentrations of anti-rotavirus IgA: at inclusion and 28 days following each study dose. Sero-conversion will be defined as ≥ 3 fold rise in serum titre of anti-rotavirus IgA from baseline to 28 days after receipt of vaccine. The primary immunogenicity endpoint will concern sero-conversion between Dose 1 and 28 days post-Dose 3. Sero-conversion after Dose 1 and Dose 2 will be estimated in secondary analyses as the difference between Dose 1 and 28 days post-Dose 2 and post-Dose 3, respectively. Differences in geometric mean titres between baseline and 28 days following each dose will be considered in secondary analyses.

Table 3. Summary of follow up and assessment schedule

	Pre-randomization visit	Randomization and Dose 1 facility visit	Dose 2 facility visit	Dose 3 facility visit	Dose 3 + 28 Days facility visit	Weekly home visit	Facility visit	Year 2 (End of follow-up)
PRE-RANDOMIZATION								
Informed consent for pre-randomization assessment	X							
Birth weight, gestational age and maternal MUAC	X							
ENROLMENT								
Eligibility evaluation		X						
Informed consent for trial participation		X						
Randomization		X						
INTERVENTION								
Vaccine		X	X	X				
Placebo		X	X	X				
ASSESSMENTS								
Background questionnaire		X						
Anthropometry		X	X	X	X	X*		X
Diary card for gastroenteritis, AE, SAE			X	X	X	X		X
Clinical exam and history		X	X	X	X	X	X	X
Post-immunization safety assessment		X	X	X				
Review of discontinuation criteria			X	X	X	X	X	
Stool sample						X**	X**	
Venous blood sample (sub-sample)		X	X	X	X			
Breast milk sample (sub-sample)		X						

*Height, weight and MUAC recorded at home every 4th weekly visit until 2 years of age.
** In the event of gastroenteritis reported within 7 days of onset of illness.

Laboratory assessment. ***Stool***. Stool samples will be collected in health facilities at the time of presentation for gastroenteritis, and at home for any gastroenteritis identified within 7 days of onset. A minimum of 5-10 ml of stool (if watery; 5 gm if semi-solid) will be collected using clean, dry, leak-proof plastic containers or, if no stool is available during the observation period, using a rectal swab. Specimens will be transported in freezer packs at 2-8^{o} C to the Epicentre laboratory in Maradi on the same day. Upon arrival at the Maradi laboratory, designated laboratory personnel will verify the stool container contains an adequate amount of specimen and is adequately labeled before storing at -20^{o} C or colder. If the sample is deemed inadequate, a request will be made to the nurse assistant for an additional sample. Rotavirus

antigen in stool will be detected by enzyme immunoassay (Premier Rotaclone®) by the Epicentre Maradi laboratory. All rotavirus positive stool samples will be shipped to the CERMES laboratory in Niamey for testing by reverse transcriptase PCR followed by reverse hybridization assay and sequencing to identify G and P types. The Study Field Coordinator will monitor specimen handling, storage and transport throughout the trial. Sample SOPs for enzyme immunoassay and reverse transcriptase PCR analysis are provided in Appendix B. Testing for other enteric pathogens will not be systematically conducted during the study period, though stool samples may be stored for later analysis.

Venous blood. For immunogenicity assessment, 3-4 ml of venous blood will be collected from a random sample of 420 infants to determine the serum concentrations of anti-rotavirus IgA antibodies at the Randomization Visit, at the Dose 2 and Dose 3 Visits (± 7 days); and 28 days (± 7 days) after Dose 3 Visit. Participants in the immunogenicity cohort will be randomly selected [35 per month] from the full cohort over a 12-month period to allow inclusion of an approximately equal number of subjects receiving study vaccine and placebo across all seasons Procedures for random selection of the immunogenicity cohort will be provided in the study SOPs. All blood samples will be separated into sera within one hour of arrival from the field and sera will be aliquoted and stored at -20^{o}C until shipment for analysis. Samples will be shipped on dry ice to the Christian Medical College, Vellore, India laboratory, where they will be analyzed for anti-rotavirus IgA by enzyme immunosorvent assay using 1% blotto. Sample SOPs for anti-rotavirus IgA by enzyme immunosorvent analysis in serum are provided in Appendix B.

Interference of the study vaccine with immunogenicity of concomitantly administered oral polio vaccine will also be tested in the immunogenicity sub-sample (n = 420). Anti-poliovirus types 1, 2 and 3 antibody serum titres will be measured using a virus micro-neutralisation test by the Christian Medical College, Vellore, India laboratory. Results will be used to evaluate if sero-conversion for the polio antibody response is non-inferior between the vaccinated and placebo groups.

Breast milk. Immune factors in breast milk at the time of immunization have been hypothesized to contribute to lower immunogenicity of live oral rotavirus vaccines in developing countries(44, 65, 66), therefore we will assess concentrations of maternal anti-rotavirus IgA antibodies in breast milk in order to compare immunogenicity by level of maternal anti-rotavirus IgA concentration. Breastfeeding mothers of children included in the immunogenicity sub-sample (n = 420) will be asked to provide breast milk samples at the time of randomization. Samples of 5 to 10 ml will be requested in order to measure concentrations of maternal anti-rotavirus IgA antibodies by enzyme immunosorvent assay. Samples will be stored at -20^{o}C until shipment to the Christian Medical College, Vellore, India laboratory for analysis.

Participant retention. Once an infant is randomized, the study team will make every reasonable effort to follow the infant for the entire study period. It is projected that the annual rate of loss-to-follow-up will be 10%.

The sponsor will be responsible for developing study SOPs to achieve this level of follow-up. The Pre-randomization Visit is organized to allow caregivers adequate time to consider their participation in the trial and prepare any questions they may have, thereby reducing the number of defaulters once randomization has occurred. At each household contact, study staff will provide a reminder of the next visit, and if a child is ever not present at the time of a Weekly Home Visit, the community health agent can contact neighbors or relatives in the area to ascertain expected time/day of return so that a repeat visit may be scheduled. In addition, study teams will maintain community interest in the study through periodic sensitization of community leaders and provide feedback to caregivers on child growth and development. Preventive interventions (e.g. soap, bed nets) will be regularly provided as in-kind motivation to participating households (see Reimbursement below).

Standard care. During regular study visits, study staff will verify previous receipt of any EPI vaccine through review of the infant's vaccination card, and as needed, administer routine EPI vaccinations free of charge. Vaccines to be provided include: Bacillus Calmette-Guérin (birth); oral poliovirus vaccine (birth, 6, 10 and 14 weeks); and the combined pentavalent vaccine including diphtheria-tetanus-whole cell pertussis, haemophilus influenzae type b and hepatitis B (6, 10, and 14 weeks). Stock of measles and yellow fever vaccination, recommended at 9 months of age, will be insured by the study sponsor and all participants will be invited to receive vaccination at a study facility at 9 months. For all other medical intervention to be provided during the study period, study children will be referred to study staff in a FORSANI-supported health facility for management free of charge and in accordance with GCP. FORSANI currently supports the Ministry of Health to provide pediatric care to all children aged 0-5 y in the Madarounfa Health District.

Sample size. Assuming a 2% attack rate of severe wild-type rotavirus gastroenteritis at 1 year, a 50% true vaccine efficacy and a 20% participant non-assessibility (including withdrawal and loss to follow up), the study will enroll 5138 children to have at least 90% power to detect a 95% confidence interval for vaccine efficacy at 1 year that would be above 0% (67).

Assuming a sero-conversion rate of 30% in the placebo group, 20% non-assessibility (including withdrawal and loss to follow up) and 30% exclusion due to detection of wild-type rotavirus disease between vaccine doses, we will assess immunogenicity in 420 children to have 90% power to detect a 20% difference in the proportion of children that sero-convert (68).

Data analysis. The primary analysis will be a per-protocol analysis of vaccine efficacy of three dose pentavalent vaccine vs. placebo, including children who received the complete vaccination course and entered the efficacy surveillance with no evidence of previous wild-type rotavirus infection. The primary analysis will exclude participants with a laboratory confirmed wild-type rotavirus disease earlier than 28 days post-Dose 3 of vaccine or placebo. Infants who have at least one episode of severe wild-type rotavirus gastroenteritis during the period from 28 days after the last dose until the date the infant reaches 1 year of age will be considered as having achieved the primary outcome. Only gastroenteritis episodes in which wild-type rotavirus (i.e., other than the vaccine strain) is identified in a stool specimen will be included as an event in the

primary efficacy analysis. Gastroenteritis episodes that cannot be classified as rotavirus gastroenteritis or non- rotavirus gastroenteritis with certainty because of incomplete data will be considered as not having achieved the primary outcome. For participants with more than one episode of severe rotavirus gastroenteritis, only the first episode will be counted.

Vaccine efficacy with 95% confidence interval will be calculated as $(1-IR_1/IR_0)*100$, where IR_1 is the person-time incidence rate in the vaccinated group and IR_0 is the person-time incidence rate in the placebo group. 95% confidence intervals will be derived from the exact confidence interval using the conditional binomial distribution. The cumulative hazard of a first episode of severe rotavirus gastroenteritis between groups will be estimated as a minus-log transformation of the Kaplan–Meier survival curve, with the P value calculated using the log-rank test. The proportions of infants having at least one gastroenteritis episode will be compared between groups by Fisher's exact test and expressed in terms of relative risk. The number of events prevented by 100 vaccinated infant-years will be obtained from 100 times the difference in the incidence rate; the associated confidence interval will be derived using the method of Zou and Donner (69).

Secondary analysis of efficacy will be done for efficacy against severe rotavirus gastroenteritis during the second year of life and for the combined period from 28 days after the final dose until 2 years of age; rotavirus gastroenteritis of any severity; rotavirus gastroenteritis with a Vesikari score of ≥ 17; rotavirus gastroenteritis by strain [vaccine-contained and non-vaccine G and P types]; gastroenteritis of any cause; longitudinal prevalence of rotavirus gastroenteritis; hospitalization due to rotavirus gastroenteritis; and hospitalization for any reason. In strain-specific analyses of efficacy, when more than one G type is isolated for an episode, the episode will be counted in every G type category. Exploratory analyses will assess sensitivity of all analyses to the method of case detection (facility- vs. home-based surveillance).

Intention-to-treat (ITT) analyses will be done including all participants who were vaccinated with at least one dose of vaccine or placebo and follow up beginning from the time of enrollment. Sample size allowing, vaccine efficacy will be calculated among children receiving a total of 1 or 2 doses to determine whether vaccine confers protection to infants before completion of the 3-dose regimen.

The analysis of safety will be done using the ITT population, i.e. all infants who received at least one dose of the study vaccine or placebo, and include follow up from the time of enrollment until 28 days post-dose 3 (adverse events) or the end of follow up (serious adverse events). The incidence of adverse and serious adverse events will be compared between groups with the two-sided asymptotic score test for the null hypothesis of identical incidence by group.

The analysis of immunogenicity will be done on the basis of the per-protocol population for whom immunogenicity data are available. Participants with laboratory confirmed wild-type rotavirus disease between vaccine doses will not be included. Sero-conversion rates 28 days following each dose will be calculated with corresponding 95% confidence intervals using the binomial distribution. Differences in geometric mean titres of serum anti-rotavirus IgA

between pre-Dose 1 and 28 days following each dose will be measured with 95% confidence intervals assuming a normal distribution of log-transformed means. To assess individual-level correlates of protection, Receiver Operating Characteristic (ROC) analyses will be used to estimate the IgA threshold most predictive of protection against severe rotavirus gastroenteritis. To explore potential mediating factors of immunogenicity in this setting, sero-conversion and log-transformed geometric mean infant IgA titres 28 days post- Dose 3 will be compared by level of maternal anti-rotavirus IgA antibodies pre-immunization using the chi-square and t-test, respectively. To assess the potential interference of the study vaccine with concomitant oral polio immunization, the statistical difference in anti-poliovirus antibody serum titres 28 days post- Dose 3 will be compared by study intervention using the chi-square test.

There are numerous risk factors for the primary endpoint which will be measured at the time of randomization, including medical history, birth weight, breastfeeding practice, and indicators of socioeconomic status. Randomization ensures that, on average, study groups will be balanced with respect to all of these risk factors, but we realize that this may not be true in any given randomized study. Thus, we will collect additional background data and compare baseline characteristics between groups to identify any significant independent imbalances. Secondary analyses will assess the group effect after adjusting for the risk factors associated with imbalances at the $P < 0.20$ significance level.

This study makes no pre-specified hypotheses regarding *a priori* effect modifiers and is therefore not powered to detect any effect modification which may occur. We acknowledge that unless there is strong modification of an observed intervention effect, the power of our study to detect effect modification will be low. However in analyses for all endpoints, we will consider whether vaccine effects are modified by the following baseline characteristics: child sex; breastfeeding practice (exclusive / partial / none;); gestational age (± 37 weeks); birth weight (± 2500 g) ; breast milk IgA antibody concentration; child serum anti-rotavirus IgA concentration at Dose 1; season of inclusion; concomitant administration of oral polio vaccine; and child anthropometry. To assess the statistical significance of each interaction, we used the Wald test for risk-ratio homogeneity in the risk analyses.

All P values will be 2-sided with $P < 0.05$ considered statistically significant. No adjustment for multiple comparisons will be made as this study considers a single primary endpoint, vaccine efficacy at 1 year at age. All other analyses of secondary endpoints would be best regarded as exploratory, and any significant findings for these endpoints would need to be confirmed. Missing data will be assumed to be missing at random. Sensitivity analyses will be conducted to assess the robustness of trial results under other methods to handling missing data (e.g. missing indicator, last observation carried forward). Data analysis will be conducted using SAS software (version 9.2).

Data collection, management and quality assurance. All the information required by the study protocol will be entered on standardized CRFs provided by the sponsor in French. The Study Field Coordinator and Medical Coordinator will validate all CRFs for completeness and accuracy, signing and dating each to attest to his/her responsibility for the quality of all data recorded and

that the data represents a complete and accurate record of each child's participation in the study. Source documents and CRFs will be maintained at the study site in a secure location to ensure confidentiality and will be available for review by the Study Monitor to ensure all collected data are consistent with the CRFs.

All validated CRFs will be double-entered, compared and verified for accuracy in Maradi, Niger. Data quality will be enforced through a variety of mechanisms, including referential data rules, valid values, range checks, and consistency checks against data already stored in the database (i.e., longitudinal checks).

A data validation plan will be prepared by the sponsor before study initiation. Errors will be detected by programs designed to detect missing data or specific errors in the data. These errors will be summarized along with detailed descriptions of the specific problem in a Data Query Report, which will be sent to the Study Field Coordinator and Medical Coordinator for resolution by checking the original forms for inconsistency, checking other sources to determine the correction, and modifying the original (paper) form as appropriate. Written documentation of changes will be available via electronic logs. A complete back up of the study database will be performed twice a month; incremental data back-ups will be performed on a daily basis. The data manager will provide monthly email reports with information on missing data, missing forms, and missing visits to the sponsor. The primary sponsor alone will have full access to the data.

Timeline. The estimated total duration of the study is approximately 54 months. Given Epicentre's established field presence in Maradi and experience with rotavirus surveillance in the region, we anticipate 6 months will be used for study preparation, including development of SOPs and CRFs, ethical clearance, recruitment and training of field personnel, initiation of the community-based birth registry and facility-based surveillance system and pilot testing. Recruitment of 5138 infants will take 18 months and each child will be followed for two years. Therefore, the duration from enrollment of the first participant until the end of follow-up of the last participant enrolled will be 42 months. Analysis and write-up will be completed in the remaining 6 months after study closure.

Training. Study staff will be trained in GCP, serious adverse event guidelines, clinical assessment of patients, completion of relevant source documents and CRFs, specimen collection and storage of samples. Initial training will be provided by the Study Field Coordinator and Medical Coordinator, with support from the Study Monitor and other partners. Refresher training will be provided on a quarterly basis to all study personnel by the Study Field Coordinator and Medical Coordinator. Routine audits performed by the Study Monitor will be used identify procedures that need to be strengthened and reinforced in routine refresher training.

SAFETY EVALUATION

Standard care. For the duration of the study period, all study children found needing medical intervention will be referred to study staff in a FORSANI-supported health facility for management free of charge and in accordance with GCP. Study staff will verify previous receipt of any EPI vaccine at study visits through review of the infant's vaccination card, and as needed, administer any missing routine vaccination doses free of charge. Vaccines to be provided include: Bacillus Calmette-Guérin (birth); oral poliovirus vaccine (birth, 6, 10 and 14 weeks); and the combined pentavalent vaccine including diphtheria-tetanus-whole cell pertussis, haemophilus influenzae type b and hepatitis B (6, 10, and 14 weeks). Stock of measles and yellow fever vaccination, recommended at 9 months of age, will be insured by the study sponsor and all participants invited to receive vaccination at a study facility at 9 months.

Documenting adverse events. The collection, recording, assessment and reporting of post-immunization reactions, adverse events (AEs) and serious adverse events (SAEs) represent the core activities for the safety evaluation of the vaccine. The participant's caregiver will be instructed to present at a health facility immediately, should the participant manifest any signs or symptoms they perceive as serious during the study period.

Surveillance for adverse events will be conducted from the time of the first dose to 28 days post-Dose 3 in all participants by trained study staff. Solicited adverse events will include fever (axillary temperature of ≥37.5^{o}C; procedures for assessment outlined in study SOPs), diarrhea, vomiting, decreased appetite, decreased activity level, otitis media, nasopharyngitis, upper respiratory infection, bronchospasm, irritability, and gastrointestinal bleeding (hematochezia).

During the surveillance period, AEs observed by the study team or reported by the participant's caregiver spontaneously or in response to a direct question will be followed on a daily basis by a nurse assistant until resolution. The diagnosis, date and time (where appropriate) of onset, outcome (e.g. resolved; resolved with sequelae; ongoing; died; or lost to follow up/unknown), intensity and relationship to vaccination; and treatment received will be recorded. All data will be transcribed to a standard CRF by study physicians.

Note: All identified gastroenteritis episodes from enrollment to 28 days post-Dose 3 will be reported as an adverse event. After 28 days post-Dose, gastroenteritis will not be reported as an adverse event but will be captured as efficacy outcome. Gastroenteritis episodes requiring hospitalizations for more than 24 hours at any time during study follow up will be considered as a serious adverse event.

Assessment of adverse event severity. The severity of all AEs/SAEs occurring during study follow up will be graded as per the clinical judgment of the study physicians and Medical Coordinator, taking into account information provided by parents. Each event will be assigned to one of 4 categories (intensity grades):

- GRADE 1 Mild - Transient or mild discomfort (< 48 hours); no medical intervention/therapy required

- GRADE 2 Moderate - Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required

- GRADE 3 Severe - Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible

- Grade 4 Life Threatening - any adverse experience that places the child, in the view of the investigator, at immediate risk of death from the reaction as it occurred. (The investigator should not grade a reaction as life-threatening if had it occurred in a more severe form then it might have caused death.)

Note: An AE that is assessed as severe should not be confused with the term SAE. Severity is a category utilized for rating the intensity of an event; both AEs and SAEs can be assessed as severe.

Documenting serious adverse events. Surveillance for serious adverse events (SAEs) will continue from the time of the first dose until 2 years of age. SAEs will be defined as:

(5) Death during the period of protocol-defined surveillance.
(6) Any new event that results in a persistent or significant disability/incapacity.
(7) Any new event requiring inpatient hospitalization. This will be related to hospitalization other than that related to management of diarrhea.
(8) Any new life-threatening event (defined as a study participant at immediate risk of death at the time of the event).

According to the Brighton Collaboration for adverse events following immunization for oral vaccines, SAEs will also include generalized convulsive seizure, hypotonic-hyporesponsive episodes, intussusception, or persistent crying (54, 55).

Children that die in a facility will have events before and at the time of death transcribed from hospital records to determine the cause of death. If death occurs outside of the hospital, cause of death will be established using a standard verbal autopsy form administered to determine the clinical picture and treatment received before death.

SAEs identified through facility- or home-based surveillance will be reported to the Medical Coordinator and PI within 24 hours of becoming aware of the SAE, whether considered to be associated with the study intervention or not. The field team will assemble specific documentation, including medical records and other supporting documents, and will record on the CRF: type of SAE; description of event with time of onset in relation to vaccination and severity; any medical actions taken and outcome; and preliminary assessment of causality. Where applicable, hospital records and verbal autopsies should be obtained. The dossier will be

submitted to the Medical Coordinator for adjudication and determination whether the cases were vaccine-related. All SAEs will be followed up until the event resolves, stabilizes, or is otherwise explained. If an SAE remains unresolved at the time of study closure, a clinical assessment will be made by the Medical Coordinator and sponsor to determine if continued follow up of the SAE is warranted. All relevant field personnel will be appropriately trained in the reporting and treatment of serious adverse events according to GCP. The DSMB and the National Ethical Committee of Niger will receive a quarterly status report including all notified SAEs. Copies of each report and documentation of IRB notification will be kept in the Trial Master File by the Medical Coordinator.

HIV status may be made known to the Medical Coordinator through review of existing medical records during assessment of SAE causality. If the HIV status of an infant experiencing a SAE is unknown but deemed necessary for appropriate clinical management of the SAE, HIV counseling and testing may be recommended by the Medical Coordinator at the time of SAE documentation.

Intussusception risks, assessment and management. In 1998, the rotavirus vaccine, RotaShield®, was recommended for use in the United States. However, within less than a year and more than 500,000 children vaccinated, RotaShield® was found to cause a transient increased risk of intussusception (estimated to occur in 1 child in 10,000) in the first 10 days after initial vaccination. RotaShield® was subsequently withdrawn from the market before detailed public discussion of the risks and benefits surrounding its use (70, 71). Post-licensure studies of the 2 second-generation rotavirus vaccines currently WHO-prequalified (RotaTeq® and Rotarix®) have monitored the risk of intussusception in the United States, Latin America, Mexico and some countries in sub-Saharan Africa. These studies suggest that all orally administered live rotavirus vaccines carry some detectable risk of intussusception (e.g. the risks associated with Rotashield® are not unique) but the risk of intussusception seems to be small (72). The Global Advisory Committee on Vaccine Safety (GACVS), an expert clinical and scientific advisory body, has concluded that currently available rotavirus vaccines continue to exhibit a good safety profile but may be associated with an increased (up to 6-fold) risk of intussusception after the first dose of vaccine in some populations (73). The levels of risk observed are substantially less than those observed with the previous vaccine, RotaShield®, and the benefits of rotavirus vaccination to all infants are thought to exceed the risks, particularly in developing countries with moderate and high mortality from rotavirus disease. Active surveillance of intussusception in African and Asian countries that plan to introduce rotavirus vaccines is warranted to eventually provide additional benefit–risk information related to rotavirus vaccination (73).

A definitive diagnosis of intussusception is based on the demonstration of invagination of the intestine on contrast enema (air or liquid), ultrasound or surgery. However, a clinical case definition for the diagnosis of acute intussusception in infants and children has been developed following recommendations of a WHO consultation and through consensus of the Brighton Collaboration Intussusception Working Group (74). This definition provides a case definition that is suitable for use in studies conducted in different geographical regions with different health

care facilities and resources, and has been validated in a developed and developing country setting (75). The Brighton clinical case definition for intussusception has been endorsed by the CIOMS/WHO Working Group on Vaccine Pharmacovigilance.

Parents and study staff will be trained on screening tools to promptly identify and assess any suspected cases of intussusception, including bloody stools, continuous vomiting, abdominal distension and/or abdominal "lumps". Children presenting with any suspected symptoms or signs at the time of a home visit or presentation to a study facility will be promptly referred to the Regional Reference Hospital of the Region of Maradi, where radiological and clinical examination will be performed before evaluating the risks and benefits of surgical intervention. All subsequent surgical interventions to resolve the invagination will be performed on site. Any patient deemed complicated by the attending physician based on their clinical judgment will be referred to the National Hospital of Niamey for management. Resolved cases of intussusception will be followed with home visits 2, 6 and 14 days after discharge from hospital.

This study will use the clinical case definition of intussusception from the Brighton Collaboration Working Group on Intussusception (74). All cases of suspected or confirmed intussusception identified among children ≤24 months at the time of diagnosis will be analyzed by the Medical Coordinator following WHO guidance (76). The Medical Coordinator will define cases of intussusception as Level 1, 2, or 3 according to the certainty of the diagnosis and the Brighton Collaboration clinical case definition. A case will be considered a potential rotavirus vaccine-related intussusception case if a rotavirus vaccine was received prior to the episode of intussusception. Cases for which there is insufficient information to establish the diagnosis according to the Brighton Collaboration clinical case definition will be classified as an unconfirmed case of intussusception and analyzed accordingly with other adverse events. Cases for which a diagnosis of intussusception is excluded on the basis of clinical assessment and/or appropriate investigations defined in the Brighton Collaboration clinical case definition will also be considered as another potential adverse event.

Assessment of causality. Every effort will be made by the study team to explain each SAE and assess its causal relationship, if any, to administration of the study vaccine or placebo. Appropriate medical judgment will be used to determine the causal relationship, considering all relevant factors including the pattern of reaction, temporal relationship, re-challenge, biological plausibility, and confounding factors such as concomitant medication, concomitant disease and relevant history.

The causality of all SAEs will be classified as:

> **Related,** when there is a reasonable possibility that the study vaccine contributed to the SAE.
>
> **Unrelated,** when administration of the study vaccine is not suspected to have contributed to the SAE.

All post-immunization reactions (e.g. those occurring within 30 minutes of administration) will be considered related to vaccination. SAEs that occur after informed consent is obtained from the parent/guardian, but prior to first vaccination, will be documented on the CRF but not considered as an SAE or related to the study. Hospitalization related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures (including hospitalization for "social" reasons) that are not the result of an SAE, will be recorded on the CRF but not considered related to the vaccine.

Dose modification. No modification of the study intervention dose is allowed. Subjects who experience unacceptable adverse events attributed to the study should not receive further vaccination and should be treated under the Medical Coordinator's discretion.

Data and Safety Monitoring Board. An independent Data and Safety Monitoring Board (DSMB) composed of 5 experts in operational, medical, and biostatistical aspects of clinical trials will be set up prior to initiating the study. All members of the DSMB will be completely uninvolved in the running of the trial and cannot be unfairly influenced by people or institutions involved in the trial. It is anticipated that the first DSMB review will occur approximately 6 months after the first participant is enrolled, with subsequent reviews every 6 months thereafter. Each meeting will include an administrative review to assess accrual, retention, and the progress of the study, as well as interim analyses of efficacy and safety, including any serious adverse events. Guidelines for trial review and modification, including statistical and non-statistical criteria for early stopping or modification based on interim analysis, will be determined at the first DSMB meeting and outlined in the DSMB charter to be established before the first meeting and made available upon request through the study sponsor.

The DSMB will be provided with the following summary of blinded accruing safety data on a monthly basis:

- Accrual and subject status data with regard to completion/discontinuation of study vaccinations
- Summaries of solicited adverse event data by severity grade, duration, body system and relation to study intervention
- Reported SAEs, including cases of intussusception, hospitalization and deaths

DSMB reviews will be summarized with recommendations to the sponsor, including recommendations regarding safety concerns and if the study should continue without change, be modified, or terminated. The DSMB will discuss potential stopping or any modification to the trial with the Scientific Committee and sponsor. The sponsor will have final authority to stop or modify the trial for any reason.

MONITORING AND AUDITING. The sponsor will permit trial-related monitoring, audits, Institutional Review Board review, and regulatory inspection(s), providing direct access to source data and documents.

A qualified and appropriately GCP-trained Study Monitor will be designated by the sponsor to carefully monitor all aspects of the study.

The Study Monitor will perform an initial audit before the start of the study to insure all necessary tools and supports are in place for study implementation in accordance with GCP. During the project period, the Study Monitor will routinely contact study sites and perform on-site visits to inspect facilities and documentation, observe performance of study procedures, discuss the protocol in detail and identify and clarify any areas of weakness. The extent, nature and frequency of site visits during the project period will be based on considerations of study objectives, study design and complexity, and enrollment rate. Periodicity and nature of monitoring activities will be described in the Monitoring Plan, with a minimum of 3 formal audits (e.g. study start, 6 months after first enrollment and study closure).

Monitoring will be conducted according to GCP and study SOPs. The Study Monitor will have access to all records necessary to ensure the integrity/validity of the recorded data and will periodically review the progress of the study. During site visits and contacts, the Study Monitor will specifically:

1. Check and assess the progress of the study
2. Review study data collected
3. Perform source data verification to verify compliance with study SOPs, including adherence to inclusion/exclusion criteria, dosing schedule, and recording of concomitant medications
4. Verify correct storage, distribution and inventory of study products
5. Verify compliance with human subjects protection and research guidelines, including confidentiality procedures and informed consent process
6. Identify any issues and address their resolution

This will be done in order to verify that:

1. The data are authentic, accurate and complete
2. The safety and rights of participants are being protected
3. The study is conducted in accordance with the approved protocol (and any subsequent amendment), GCP and all applicable regulatory requirements

Representatives of the Scientific Committee and DSMB authorized by the sponsors may accompany the Study Monitor during site visits to conduct independent audits as needed and with due consideration to relevant security issues. The processes to be reviewed can relate to participant enrolment, consent, eligibility, and allocation to study groups; adherence to trial interventions and policies to protect participants, including reporting of harms; completeness,

accuracy, and timeliness of data collection; and adherence to the International Conference on Harmonization GCP.

ETHICAL CONSIDERATIONS

Summary of known and potential risks. There is no data on the safety of BRV-PV in sub-Saharan Africa. However, based on documentation of safety of the vaccine in other settings, the risks associated with the use of the vaccine, placebo and various other study procedures proposed in this trial are expected to be minimal. All clinical and immunization procedures (oral vaccine administration, venous blood collection, rectal swab collection) will be performed by adequately trained and experienced personnel under regular supervision to minimize any risk or discomfort to participants. There is a very small risk of anal/rectal area skin abrasion while taking a swab from the rectal area. Additionally, there is also a small risk associated with phlebotomy for participants who are requested to give a blood sample. This may include pain, redness and, very rarely, local infection at the phlebotomy area.

In 1998, the rotavirus vaccine, RotaShield®, was recommended for use in the United States. However, within less than a year, it was found to cause a transient increased risk of intussusception (estimated to occur in 1 child in 10,000) in the first 10 days after initial vaccination. Intussusception is a rare event, and rates of intussusception vary between countries and populations, as the rotavirus vaccine–intussusception association may be modified by environmental or genetic factors that differ between populations. Factors that are hypothesized to affect risk of intussusception or the immune response to rotavirus vaccines—including differences in infant diet, breastfeeding, concomitant administration of oral poliovirus vaccine vs. inactivated poliovirus vaccine, and maternal antibody levels—may also contribute to the variation in intussusception risk by country (44, 47). Given biological differences between the various rotavirus vaccine strains, including rates of intestinal vaccine virus replication and shedding in fecal specimens, any potential risk of intussusception may also vary between vaccine formulations.

Post-licensure studies of the 2 second-generation rotavirus vaccines currently WHO-prequalified (RotaTeq® and Rotarix®) have monitored the risk of intussusception in North America, Latin America, and sub-Saharan Africa. With almost 800,000 doses of pentavalent rotavirus vaccine delivered, these analyses found that any orally administered live rotavirus vaccine carries some detectable risk of intussusception but conclude that the risk of intussusception is small: an excess risk of 1 intussusception event per 65,287 RV5 vaccines following dose 1 can be reliably excluded (77). Based on available evidence, the benefits of rotavirus vaccination to all infants greatly exceed any potential low-level risk for intussusception, particularly in countries with moderate and high mortality from rotavirus disease.

Risk minimization and benefits. All personnel involved in taking biological samples are trained health care personnel, who will be provided with additional training to avoid or minimize the possibility of any unplanned side effects of these procedures. The trial will be conducted in compliance with protocol, GCP, and the applicable regulatory requirements. Sterile techniques and disposable sterile needles and syringes will be utilized to obtain blood.

The direct benefit individual subjects may expect from participating in this study is regularly seeing a member of the study team and the assurance of the best available medical care with close and regular surveillance. Recipients of the vaccine will also potentially benefit from the probable protective effect of the vaccine against severe rotavirus gastroenteritis. Rotavirus vaccine has not yet been introduced into the national immunization program of Niger and individual access to one of the two available vaccines [e.g. Rotarix® and Rotateq®] is unlikely for all except the disproportionately wealthy of the study population. At the population-level, an important benefit of obtaining data on the efficacy of the BRV-PV vaccine, if proven protective, is the vaccine potentially being made available for a larger population of children in Niger.

Informed consent. Information about the study aims, procedures and informed consent process will be provided to community leaders in all study villages before recruitment begins. Written informed consent will be obtained from each subject's parent/guardian prior to any study-related screening procedures being performed on the subject. The consent forms to be signed by the parent/guardian will include information on the purpose of the study, the study intervention, procedures to be followed and the risks and benefits of participation. All informed consent procedures and documents will be in the local language.

Informed consent will be sought at 2 times during the study. During the Pre-randomization Visit (at birth), informed consent will be sought by a study nurse to allow recording of maternal MUAC, as well as infant birth weight and gestational age at birth. At this time, the study nurse will discuss details of the study with a parent/guardian and provide an Information Sheet (Appendix A) including information on the purpose of the full study and a description of procedures to be conducted that day. The Pre-randomization Visit will allow the parent/guardian adequate time to consider their participation in the full trial and prepare any questions they may have.

During the Randomization Visit (6-8 weeks of age), informed consent for participation in the full study will be sought. A study physician will inform the parent/guardian of all pertinent aspects pertaining to the study, including study aims, methods and potential risk and benefits, and then seek informed consent for participation in the full study in writing in accordance with GCP, the Declaration of Helsinki and all applicable regulatory requirements. Materials consent will be obtained at this time to address the collection of venous blood and breast milk specimens for the designated immunogenicity sub-studies. Materials consent will relate to the use of data and specimens in the specified protocols and in future unspecified research.

The informed consent process of the Pre-randomization Visit and the Randomization Visit will give individuals all of the relevant information they need to decide whether to allow their children to participate. The informed consent process will begin with study staff describing the study protocol and procedures to the parent/guardian using a standardized Information Sheet (Appendix A). Study staff will give the parent/guardian ample opportunity to inquire about details of the study and ask any questions. Illiterate individuals will have the Information Sheet and Informed Consent Form (Appendix A) read to them in their native language in the presence of a literate and impartial witness. The witness will sign and date the consent form to attest the

consent process appears to be fair and the parent/guardian voluntarily allows the subject to be included. The study physician who administered the consent procedure will countersign to confirm that the consent has been obtained following the procedure described in the study protocol and in accordance with GCP. An infant will not be enrolled into the study and given a randomization number until written informed consent for participation in the full trial is provided during the Randomization Visit.

The original Informed Consent Form will be kept on file by the Medical Coordinator for possible inspection by regulatory authorities and the sponsor. The participant's parent/guardian will receive a copy of the Information Sheet and signed and dated Informed Consent Form.

Confidentiality. Participants will be identified by a unique individual identification number in all CRFs and laboratory specimens. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. The participant's caregiver will be informed that representatives of the sponsor or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in the strictest confidence. Participants' study information will not otherwise be released outside of the study without the written permission of the participant's caregiver. The Study Field Coordinator will maintain a personal list of participant identification numbers and names to enable records to be found at a later date in the event of a medical need. This information will be destroyed upon study completion. Personal identifiers will not be included in any study report.

All study records and data will be kept confidentially under lock and key and/or electronic password protection, as appropriate and in accordance with local data protection laws for 5 years. Only senior study personnel will have access to these records.

Reimbursement. There are no plans to provide monetary payment for participation in this study. However, as the study will require caregivers to visit a study facility for scheduled and interim visits, the study will provide compensation for all facility visits at the time of the visit. In addition, in recognition of the burden associated with the study requirement to be present for all home visits, the study will provide an in-kind motivation for subject participation and retention. This will include 3 pieces of soap each month for the duration of the study and one mosquito net prior to the first peak malaria transmission season following inclusion (e.g. first June / July following inclusion). Distribution of soap and mosquito nets will take place at home upon completion of the designated Weekly Home Visit e.g. one time per month.

Storage of specimens. Stored study research samples will be labeled by a unique individual identification number code that can only be linked to the participant by senior study staff. All stored research samples will be entered into a secure database and all uses will be documented. Samples may be stored at several different laboratories in order to complete the analyses required to meet study primary, secondary and exploratory analyses.

Institutional Review Board approval. The study will be approved by the research ethics committee of the Ministry of Health Niger, the Comité de Protection des Personnes Ile-de-France, Commission d'Ethique de la Recherche sur l'Etre Humain, Hôpitaux Universitaires de Genève and the Western Institutional Review Board, and will be done in accordance with the Declaration of Helsinki and guidelines for GCP.

Declaration of conflict of interests. The primary and secondary sponsors declare no conflict of interests.

STUDY ADMINISTRATION

Protocol amendments. Any modifications to the protocol which may impact the conduct of the study or may affect patient safety / benefit, including changes of study objectives, study design, patient population, sample sizes, and study procedures, will require a formal amendment to the protocol. Such amendment will be agreed upon by the Scientific Committee and sponsors and approved by the appropriate ethics committee prior to implementation. The sponsor will be responsible to notify the appropriate regulatory agencies and trial registry. Administrative changes of the protocol, including minor corrections and/or clarifications that have no effect on the way the study is to be conducted, will be agreed upon by Scientific Committee and sponsors and will be documented in a memorandum.

Protocol deviations and violations. A protocol violation is any departure from the approved protocol, trial documents or any other information relating to the conduct of the study which may affect the safety of trial participants or the study outcomes. Examples include failure to obtain informed consent (i.e. no documentary evidence) or enrolment of participants that do not meet inclusion/exclusion criteria.

A protocol deviation is any departure from the approved protocol, trial documents or any other information relating to the conduct of the trial that does not result in harm to the trial participants and does not significantly affect the study outcomes. Examples of deviations include missed protocol visits or a protocol visit date outside the study visit window or an isolated incident of a missed or incomplete study procedure or study evaluation. Serious or repeated protocol violations or deviations will require assessment of the root cause and implementation of corrective and preventive action plans. They may constitute grounds to interrupt the trial at a study site.

Any changes from protocol-specified procedures and study-related SOPs occurring during the conduct of the trial will be documented and reported as protocol violations or deviations. Protocol violations will be reported to the sponsor and reviewing ethical committees, as appropriate and in accordance with the requirements of the involved committees.

Ancillary care and insurance. In the event that a subject suffers injury attributable to participation in this study, appropriate medical management and treatment will be paid for by the study and provided by study staff with support from FORSANI. The study sponsor will have insurance to cover non-negligent harm associated with the protocol.

Data storage and archival. The Study Monitor will provide the Medical Coordinator with a Site Master File, which will be used to file the protocol, drug accountability records, correspondence with the IRB and sponsor, and other study-related documents. The Study Field Coordinator and Medical Coordinator will maintain, and store securely, complete, accurate and current study records throughout the study.

The sponsor will keep essential documents, including subject's medical records, until at least 5 years after study closure. No data will be destroyed without the permission of the sponsor.

Dissemination and authorship policy. When the clinical study report is completed, the investigators will share the summary results with local, regional and national immunization program representatives to provide results and answer any questions.

The findings from this study will also be published in a peer-reviewed scientific journal and disseminated at appropriate national and international conferences. Each paper or abstract will be submitted to the Scientific Committee for review of its appropriateness and scientific merit prior to submission. The Scientific Committee may recommend changes to the authors but the ultimate decision to submit will remain with the primary sponsor. Every attempt will be made to reduce to an absolute minimum the interval between the completion of data collection and the release of the study results. A period of 3 to 6 months is expected to compile the final results paper for an appropriate journal. Trial results will be disseminated to key stakeholders regardless of the direction or magnitude of effect.

The sponsors and Scientific Committee will determine the specific topics and numbers of publications, with rights to authorship being determined by intellectual contribution to the study design, implementation, and analysis, as is specified by most major scientific journals. Preference will be given for publication in peer-reviewed, open-access journals with appropriate readership and high impact factors.

Data sharing policy. The research data will be the property of the sponsors, though we realize that the data collected from this study may provide other investigators with the opportunity to answer scientific questions about a number of ancillary issues. Therefore, data will be made as widely and freely available as possible, in a timely fashion, while safeguarding the privacy of participants and protecting confidential data. A de-identified data set can be made available under a data-sharing agreement that provides for a commitment to using the data only for research purposes and securing data using appropriate technology.

STUDY MANAGEMENT

Study sponsors. The primary sponsor will develop the study protocol, with substantial input from Serum Institute of India, Limited (SIIL), and other partners. The primary sponsor will hold the data and conduct all analyses. The final report will be written by the primary sponsor, who will have full access the data and final responsibility for the data analysis and decision to submit for publication. SIIL will have no direct oversight, participation in field activities, DSMB meetings, or data analysis. Primary sponsor staff will independently monitor study execution at field sites but not participate in closed sessions of the Data and Safety Monitoring Board meetings. The study will be managed by a primary sponsor-investigator who both initiates and conducts the trial. The obligations of a sponsor-investigator include both those of a sponsor and those of an investigator.

The secondary sponsor is Médecins Sans Frontières- Operational Center Geneva (MSF-OCG). MSF-OCG has agreed with the primary sponsor to act as the Primary Sponsor's legal representative in relation to the trial site and provide funding for the trial.

Serum Institute of India, Limited. Vaccine and placebo are to be provided in kind by SIIL.

Scientific Committee. The Scientific Committee (see Appendix C for Terms of Reference) will be asked to contribute to the following activities:

- Review drafts of the trial protocol, and agree on a final version;
- Review the trial Standard Operating Procedures, and agree on a final version;
- Advise and agree on an Analysis Plan covering data generated by the trial;
- Reply promptly to specific queries regarding the trial methods, practical implementation, analysis and interpretation;
- Review drafts of the final trial report, and agree on a final version.

Reporting procedures of the Scientific Committee to the sponsor are as outlined in the Terms of Reference (Appendix C). The sponsor-investigator assumes responsibility that the Scientific Committee is informed of all components of the trial protocol.

Data and Safety Monitoring Board. An independent Data and Safety Monitoring Board (DSMB) composed of 5 experts in operational, medical, and biostatistical aspects of clinical trials will be set up prior to initiating the study. All members of the DSMB will be completely uninvolved in the running of the trial and cannot be unfairly influenced by people or institutions involved in the trial. It is anticipated that the first DSMB review will occur approximately 6 months after the first participant is enrolled, with a second review 6 months thereafter. Each meeting will include an administrative review to assess accrual, retention, and the progress of the study, as well as interim analyses of efficacy and safety, including any serious adverse events. Guidelines for trial review and modification, including statistical and non-statistical criteria for early stopping or modification based on interim analysis, will be determined at the first DSMB meeting and outlined in the DSMB charter to be established before the first meeting and made

available upon request through the study sponsor. Reporting procedures to the study sponsor will be outlined in the DSMB charter.

Human resources. Overall study development and direction will be provided by a team of 4 investigators based in Paris and Geneva (Figure 1). Rebecca Freeman Grais will serve as the sponsor-investigator for the study. Dr. Grais is an international expert in vaccination in sub-Saharan Africa, and as the sponsor-investigator, will be the guarantor of the trial. Sheila Isanaka, Principal Investigator, is a recognized epidemiologist with extensive experience in conducting large-scale trials of high burden pediatric morbidities. Dr. Isanaka will be responsible for ensuring appropriate study design and procedures. Dominique Legros, Co-investigator, representative of the secondary sponsor (MSF-OCG) and former division head at the WHO headquarters in Geneva, will be responsible for ensuring that this trial adheres to international medical standards as recognized by the WHO and the Ministry of Health of Niger. Emmanuel Baron, Co-investigator, General Director of Epicentre and former Medical Director of Médecins Sans Frontières-Operational Center Paris, will ensure the appropriate medical management of all study participants. Dr. Baron has over 15 years of experience in providing and managing medical care throughout sub-Saharan Africa.

In addition, the study will be supported a permanent GCP-trained Study Monitor based in Niamey, Niger. Through routine contact and site visits, the Study Monitor will insure all necessary tools and supports are in place for study implementation, review collected data and perform source data verification in accordance with GCP.

All day-to-day study activities will take place in the Madarounfa Health District, Maradi, Niger, and interaction with participants will be primarily carried out by the study field team. The field team will be comprised of a Study Field Coordinator and Medical Coordinator based in Maradi, and specialized health care personnel based in rural sites throughout the Madarounfa Health District and responsible for the enrollment and medical follow-up of all study subjects. The Study Field Coordinator and Medical Coordinator will be under the supervision of the Principal Investigator and the Director, Epicentre Niger, with whom there will be at least weekly communication by email, teleconference or videoconference to discuss study activities and challenges.

The Study Field Coordinator will be responsible for coordination of all field worker and supervisor schedules, standardization of follow up methods and assurance that activities are conducted according to protocol. The Medical Coordinator will be responsible for overseeing the medical management and follow-up of all study children. The Study Field Coordinator and Medical Coordinator will together supervise 4 teams:

- Community-based recruitment team will be comprised of village representatives, including village chiefs, midwifes or health workers. These individuals will create and maintain a population registry for their village, recording births, deaths and migration in order to provide a basis for study inclusion. There will be 1-2 recruitment monitors per study village, depending on village size.

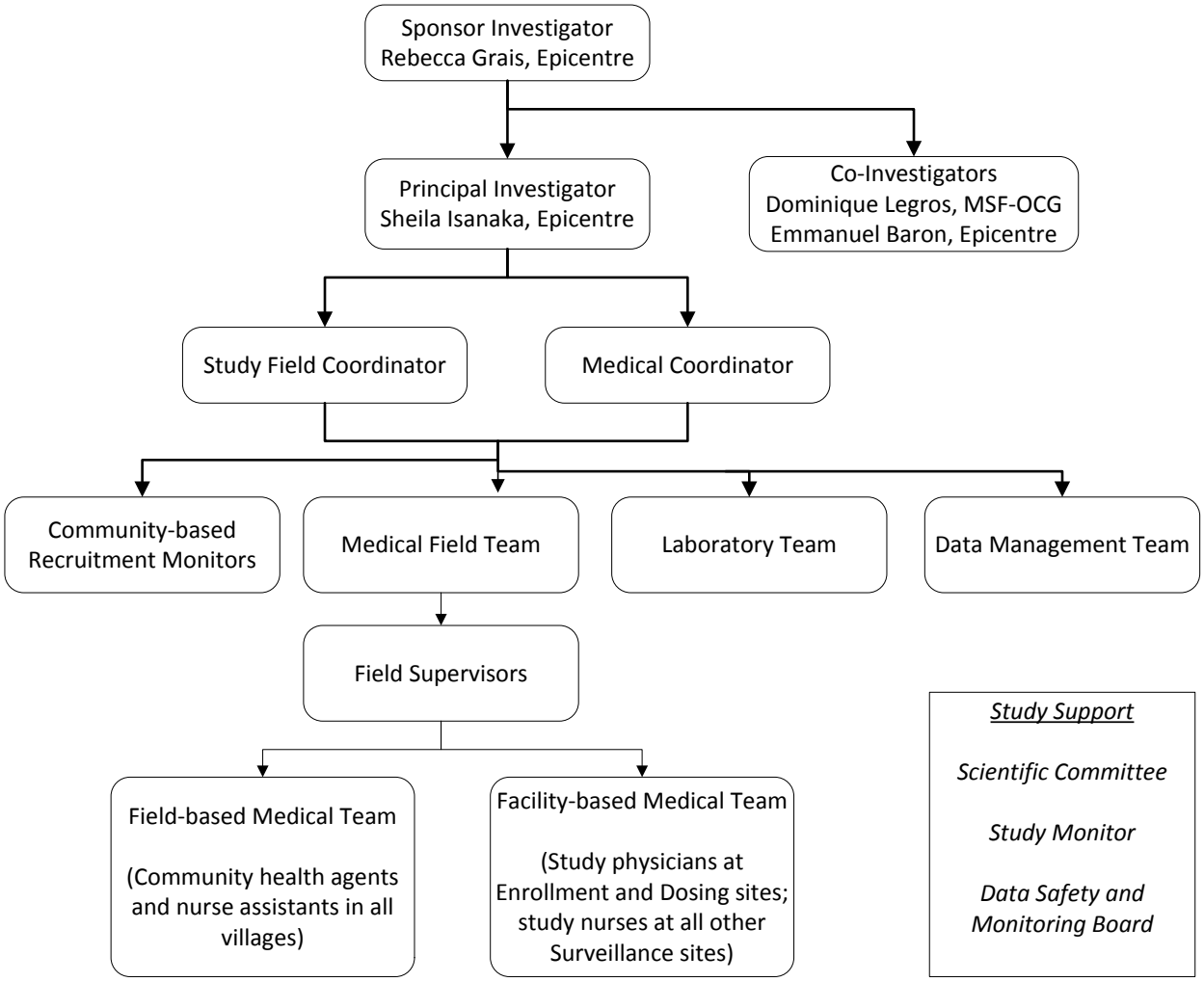
- Medical field team will include field-based and facility-based personnel. Field-based personnel will include community health agents and nurse assistants. The community health agent (n = 1 per 20-25 children; 4-5 home visits per day) will conduct the Weekly Home Visits to review the Weekly Diary Card with caregivers and notify the nurse assistant when an episode of gastroenteritis or adverse event is identified. The nurse assistant (n = 1 per 100 children; 4-5 visits per day) will follow children at home with gastroenteritis or any adverse event to collect information regarding the severity of the episode, and as appropriate stool samples. Facility-based personnel (n = 1 per site during normal facility operating hours) will include study physicians and study nurses. One study physician will be based in each designated Enrollment and Dosing Site and will be responsible for recruitment (including the informed consent process), administration of study vaccine and placebo and post-immunization observation, receiving children presenting to the facility with symptoms of gastroenteritis or other medical complication, and recording all CRFs. One study nurse will be based in all remaining facilities in the Health District and will be responsible for receiving children presenting to the facility with symptoms of gastroenteritis or adverse events and recording pertinent medical information for transcription to standardized CRFs by the study physicians. Field supervisors (n= 1 per 10-15 agent) will directly oversee field-based and facility-based personnel by tracking follow up logs and attending home and facility visits to ensure procedures are standardized and conducted according to protocol.

- Laboratory team, including 2 laboratory technicians and 1 laboratory supervisor, will be responsible for the preparation and storage of all biological samples collected during the study period; analysis of collected stool for the presence of rotavirus antigen by enzyme immunoassay; and preparation all rotavirus positive stool samples for shipment to the CERMES laboratory in Niamey for further testing by reverse transcriptase PCR.

- Data management team, including 5 data entry operators and 1 Data Manager, will be responsible for the double-entry and validation of all data collected on standard CRFs.

All teams will be adequately staffed with sufficient back-up personnel to allow continuity of study activities over standard annual and sick leaves.

Figure 1. Study organogram



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Summary of Changes

September 8, 2013: Protocol Version 6

March 14, 2014: Amendment 1 Version 6.2

- Elaboration of a immunogenicity sub-study to meet reporting requirements for vaccine pre-qualification by the World Health Organization
    - Study objectives and procedures outlined in the protocol have been updated and a separate Information Sheet and Informed Consent form have been developed to reflect the specific activities of the immunogenicity sub-study.
- Increase in sample size for primary endpoint
    - Further statistical consultation has indicated that the original sample of 5138 children would under-power the primary analysis. We have therefore increased the sample size from 5138 to 7770 children in order to ensure adequate power for analysis of the primary endpoint.
- Modification of definition of primary endpoint
    - As per the recommendation of the study's Scientific Committee, we have revised the definition of the primary endpoint. The revision was recommended on the understanding that the previous definition, which restricted to cases of severe wild-type rotavirus gastroenteritis and utilized a clinical definition of severity, was not appropriate for a trial of this nature. We will now consider severe rotavirus gastroenteritis, irrespective of type or strain and defined using the Vesikari score for severity, as the primary endpoint.
- Modification of adverse events definition and follow up
    - The National Ethical Committee of Niger has suggested that it would not be feasible or of clinical interest to consider all proposed adverse events. Therefore as per the recommendation of the National Ethical Committee of Niger and the study Scientific Committee, we have reduced the scope of adverse events to be assessed. Solicited adverse events, including fever, diarrhea, vomiting, decreased appetite, and ¬decreased activity level, will be assessed in all participants for 7 days post vaccination. Unsolicited adverse events (defined as any solicited adverse events plus otitis media, nasopharyngitis, upper respiratory infection, and bronchospasm) will be graded for severity according to International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) / Good Clinical Practice and be assessed from the time of first dose until 28 days post-Dose 3 if severe.
- Modification of definition of serious adverse event
    - As per the recommendation of the study's Scientific Committee, the definition of serious adverse events has been revised to be consistent with ICH guidelines.
- Adoption of an "event-driven" study design
- As per the recommendation of the study's Scientific Committee, we have adopted an event-driven trial design, which will allow an intermediate analysis to be triggered as soon as a sufficient number of cases of the primary endpoint is attained. Investigators will remain masked to the group assignment until the end of the primary analysis.

- Update of cover page
    - To be complete, the cover page has been updated to reflect the addition of new investigators and partners.
- Date of first enrollment
    - The anticipated start date has been updated to reflect current planning.
- Simplification of the Pre-randomization informed consent Information Sheet
    - As per the request of the Commission d'Ethique de la Recherche sur l'Etre Human (CEREH), the Pre-randomization Information Sheet has been simplified and now differentiates the procedures and risks pertinent to the Pre-randomization Visit activities from those of the main trial.

September 12, 2014: Amendment 2 Version 6.4

- Oral consent for participation in Pre-randomization Visit
    - We propose to have a study nurse play a standardized audio recording which will present all information included in the approved letter of information (Appendix A.1), including information on the purpose of the study, procedures to be followed, and the risks and benefits of participation in the local language. We will then audio record the subject providing identifying demographic information (e.g. name, age, village), the subsequent consent discussion with questions and answers between the study nurse and subject, and if granted, the individual's confirmation that she agrees to participate in the Pre-randomization activities. This recording will serve as record that the consenting process was fair, unbiased, and that the subject had a chance to ask questions and received satisfactory responses. The electronic audio file will be kept confidentially under electronic password protection, in accordance with local data protection laws for 5 years.

- Elaboration of informed consent to reflect recent introduction of licensed rotavirus vaccine in the national immunization program of Niger.

    - As per the recommendation of the World Health Organization Research Ethics Review Committee (see meeting summary attached), all parents/guardians should be informed of the introduction of the rotavirus vaccine in the national immunization program once vaccine is available and should be provided the option to participate in the trial or receive vaccine through the national program. All informed consent forms have been revised to provide this new information.

- Addition of acceptability test of lipid-based nutritional supplement for use in the immunogenicity sub-cohort.

    - The immunogenicity sub-study nested within the main trial will employ a new 40g formulation of a lipid-based nutritional supplement (LNS) designed for use by pregnant women. Other LNS formulations have been used safely and with high acceptability in other African settings among pregnant and lactating women and young children, but there is no experience with specific dosage proposed for use in this study and no previous experience of LNS among pregnant women in Niger. We

therefore propose an acceptability test of the study LNS to be conducted among 25 pregnant women in the study zone. The acceptability test will include 2 days of observed tasting and a 2 week home-use period.

June 6, 2015: Amendment 3, Version 6.5

- **Reimbursement about for travel costs for scheduled visits modified**
  - Informed consent forms changed to reflect travel reimbursement cost changes for scheduled visits.

Statistical Analysis Plan (SAP)

Protocol Title:

Randomized, double-blind, placebo-controlled phase III clinical trial to assess the efficacy and safety of a pentavalent rotavirus vaccine (BRV-PV) against severe rotavirus gastroenteritis among infants in Niger

Protocol: ROSE

Phase III

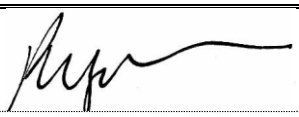
Effective Date: 13 July 2014

Protocol Version: 6.2

Statistical Analysis Plan (SAP)

Signature Page

Type of document	Statistical Analysis Plan	**Identifier**	ROSE
Effective date	13 July 2014	**Version number**	v1
		Supersedes version	None

Rebecca Grais Research Director		01 January 2015
Name and Title	Signature	Date

Revision History

Version:	SOP ID:	Document Name:
Reason for change:		

Table of Contents

List of Abbreviations

AE	Adverse Event
AR	Attack Rate
ARP	Attack Rate in Placebo Group
ARV	Attack Rate in Vaccinated Group
BCG	Bacillus Calmette-Guerin
BRV-PV	Bovine Rotavirus Pentavalent Vaccine
CI	Confidence Interval
CM	Concomitant Medication
CRO	Contract Research Organization
CRF	Case Report Form
DDC	Diarrhea Diary Card
DMC	Data Management Center
DSMB	Data and Safety Monitoring Board
ELISA	Enzyme-Linked Immunosorbent Assay
EW	Early Withdrawal
FFU	Fluorescent Focus Units
GCP	Good Clinical Practice
GE	Gastroenteritis
GMT	Geometric Mean Titre
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMNCI	Integrated Management of Neonatal and Childhood Illnesses
ITT	Intent to Treat
IP	Investigational Product
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
IgA	Immunoglobulin A
Ho	Null Hypothesis
H_1	Alternative Hypothesis
LLN	Lower Limit of Normal
Max	Maximum
MedDRA	Medical Dictionary for Drug Regulatory Activities
MEM	Minimal Essential Medium

mg	milligram
Min	Minimum
mL	milliliter
N	Number of Subjects
OC	Observed Case
PI	Principal Investigator
PIDC	Post-Immunization Diary Card
PPT	Per Protocol
PSRT	Protocol Safety Review Team
RR	Relative Risk
RV	Rotavirus
RVGE	Rotavirus Gastroenteritis
SAE	Serious Adverse Event
SD	Standard Deviation
SII	Serum Institute of India, Limited
SOC	System Organ Class
SOP	Standard Operating Procedure(s)
SAGE	Strategic Advisory Group of Experts on Immunization
SRVGE	Severe Rotavirus Gastroenteritis
ULN	Upper Limit of Normal
VE	Vaccine Efficacy
WHO	World Health Organization
WHO- DD	World Health Organization Drug Dictionary

1. Introduction

1.1 Statistical Analysis Plan

A statistical analysis plan is a comprehensive and detailed description of the methods for data analyses to be used in a clinical trial. A clear detailed SAP will avoid post hoc decisions that may affect the interpretation of the data. This SAP includes details on the procedures for creating Tables, Listings, and Graphs (TLGs) from the results of a phase 3 study, which was carried out to evaluate safety, immunogenicity, and the efficacy of a 3-dose series of BRV-PV administered orally to prevent severe rotavirus gastroenteritis in healthy infants.

This statistical analysis plan will provide full details of the analysis to be presented in the Tables, Listings, and Graphs. This is a separate document apart from statistical section of the protocol. Any changes in the statistical analysis / methods planned in the protocol and even if any additional statistical analysis is planned which is not part of the protocol will be explained in this detailed statistical analysis plan of the study. The SAP was developed before any data was unblinded to the study team.

This SAP:

- Includes a statement of the objectives of the trial, as stated in the protocol.
- Identifies all primary and secondary end-points.
- Specifies the hypotheses to be tested and any parameters that are to be estimated, in order to meet the trial objectives.
- Defines the analysis populations to be used
- Provides a full and detailed description of the methods of analysis including details of handling of missing data, dropouts, derived variables, etc.

By the time this SAP was competed and approval by all parties received, the trial data was still under blind.

1.2 Rationale

Sub-Saharan Africa carries the largest burden of rotavirus-related mortality, but immunization against rotavirus presents unique challenges. Current supply of the 2 WHO prequalified vaccines is constrained, and in many African settings, national immunization programs are challenged by lack of trained health workers. Unreliable transportation systems and storage facilities also make it difficult to preserve vaccines that require refrigeration. If rotavirus vaccine is to be brought to the infants that need it most through national immunization programs in the region, new vaccines that address these challenges are

urgently needed. The BRV-PV vaccine is a relatively low-cost and heat-stable formulation whose introduction into national immunization programs may help minimize the burden on already-strained national programs throughout sub-Saharan Africa.

The WHO Expert Committee on Biological Standardization has recommended that the efficacy of new rotavirus vaccines be demonstrated in diverse geographical regions including developing countries before widespread implementation. The Ministry of Health of Niger, Médecins Sans Frontières (MSF) – Operational Center Geneva and Epicentre along with other partners have formed a research consortium to bring additional evidence to inform public health decision making on the potential value of the BRV-PV vaccine in an African setting. The goal of the present study is to collect additional data on the efficacy profile of BRV-PV vaccine in a randomized controlled setting, while gaining further experience with vaccine-related adverse events. This will be conducted through the performance of a phase III trial in Niger conducted in compliance with the version of the protocol agreed to by the applicable regulatory authorities and Good Clinical Practice (GCP).

Evidence supporting the efficacy and safety of this formulation in an African setting would support the pre-qualification and increased global access to the BRV-PV vaccine. If shown to be efficacious and pre-qualified, the government of Niger would benefit from a low cost vaccine adapted to the logistical and supply demands of the national immunization program.

2. Study Design and Objectives

2.1 Study Objectives

2.1.1 Primary Objective

The Primary objective of the study is:

- To estimate the efficacy of three doses of SIIL BRV-PV vaccine vs. placebo against severe rotavirus gastroenteritis from 28 days post-Dose 3 up to 2 years of age in healthy infants in Niger.

2.1.2 Secondary Objectives

Secondary objectives are:

- To estimate the efficacy of the SIIL BRV-PV vaccine vs. placebo against severe rotavirus gastroenteritis from 28 days post-Dose 3 to 1 year of age and from 1 to 2 years of age.

- To evaluate vaccine efficacy against rotavirus gastroenteritis of any severity.
- To estimate vaccine efficacy against rotavirus gastroenteritis with a Vesikari score ≥ 17.
- To estimate vaccine efficacy against gastroenteritis and severe gastroenteritis of any cause.
- To estimate vaccine efficacy against severe rotavirus gastroenteritis caused by G serotypes included in the vaccine (G1, G2, G3, G4 and G9).
- To estimate longitudinal prevalence of rotavirus gastroenteritis.
- To estimate vaccine efficacy to reduce hospitalizations due to rotavirus gastroenteritis and rotavirus gastroenteritis.
- To estimate vaccine efficacy to reduce hospitalization for any reason.
- To estimate safety of the SIIL BRV-PV vaccine vs. placebo (adverse events [AEs] and serious adverse events [SAEs]).
- To estimate the immunogenicity of BRV PV in a sub-sample of participants.
- To estimate effect of prenatal nutritional supplementation on infant immune response to the vaccine in a sub-sample of participants.
- To demonstrate the immunological non-inferiority of EPI vaccines when co-administered with the BRV-PV as compared to their co-administration with placebo.

2.2 Assessment of Objectives

2.2.1 Efficacy Assessments

To identify and study all cases of gastroenteritis, cases of gastroenteritis episodes will be captured through facility- and home-based surveillance from the moment the first dose of vaccine or placebo is administered until 2 years of age. All Gastroenteritis episodes will be captured in the Gastroenteritis Surveillance CRF. Given the time interval between the first and 28 days post the third dose, a period over which many subjects may develop rotavirus gastroenteritis, without having received the benefit of full vaccination, gastroenteritis episodes between the first and 28 days post the third dose will not be counted in the primary analysis. All efficacy analyses will use all follow-up through 117 cases (primary analysis) and up to 2 years of age (end of follow up), as well as up to 1 year of age and 1-2 years.

Thus efficacy endpoints assessments will be performed based on following applicable definitions mentioned in protocol:

Gastroenteritis (GE): GE is defined as the passing of three or more watery or looser-than-normal stools within 24-hour period, with or without forceful vomiting. Every episode of GE will be evaluated clinically and the combined symptoms and signs summarized using the Vesikari Severity Score system. An episode with a Vesikari score of ≥11 will be considered as a severe GE case (see Section 4.4.6.3 for details on Vesikari score). Gastroenteritis episodes will be classified as two separate episodes if there is an interval of 5 or more consecutive, symptom-free days between the episodes.

Rotavirus Gastroenteritis (RVGE): A case of RVGE will be defined as the production of three or more watery or looser-than-normal stools within 24-hour period, with or without forceful vomiting, along with the detection of rotavirus in a stool specimens obtained within seven days after the end of symptoms. The RVGE cases will be ascertained by Laboratory and include all serotypes unless specified otherwise.

Severe Rotavirus Gastroenteritis (SRVGE): If Laboratory confirmed RVGE case is qualified as severe as per Vesikari score. If Vesikari score ≥ 11 as per data captured on the Gastroenteritis Surveillance CRF then RVGE is considered as SRVGE case.

Vaccine Efficacy is expressed as proportionate reduction in disease attack rate (AR) between placebo (ARP) group i.e. placebo group and vaccinated (ARV) groups i.e. rotavirus vaccine group will be calculated from hazard ratio (HR) of disease among the rotavirus vaccine (vaccinated) group using the following formula:

Vaccine Efficacy = (1- HR)*100, HR = ARV / ARP and is based on each subject follow-up time in years / Time to Event.

There are potentially two analysis time points for this study. The first analysis time point when 117 per protocol subjects with at least one SRVGE occurring from 28 days after the third vaccine/placebo dose. This will be the primary analysis time point for the primary efficacy endpoint for the study and will be referred to as the primary analysis period with the first 117 SRVGE cases. The secondary analysis time point is when all participating subjects reach two years of age and will be referred to as the entire study period up to 2 years of age. If the accrual of 117 per protocol subjects doesn't happen prior to all subjects reaching two years of age, there will be only one analysis time point.

2.2.1.1 Primary Efficacy Endpoints

Section	Planned Endpoint	Analysis Time Point Planned in the Protocol	Assessment Details about Primary Endpoint
2.2.1.1.1	SRVGE cases	Primary Analysis Time Point: 117 cases of SRVGE accrued or until all participating subjects reach two years of age	• Will be assessed in subjects who received complete correct vaccination regimen of three doses of vaccine or placebo included in the PP population -specified vaccination windows) as defined in section 4.1.6. Subjects that receive incorrect product, or receiving product out of the acceptable vaccination windows will not be included. • First episode of SRVGE occurring from 28 days after the 3rd dose of the study vaccine/placebo in a subject will count towards the primary endpoint for PP populations defined above. Second/third episodes of SRVGE will not count for the primary analysis. • This will be repeated for ITT as Secondary analysis which will include all the

2.2.1.2 Secondary Efficacy Endpoints

Section	Planned End Points as per Protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint
2.2.1.2.1	SRVGE cases matched with Serotypes included in vaccine	Primary Analysis Time Point : 117 cases of SRVGE accrued or until all participating subjects reach two years of age	• By individual serotype, vaccine-specific serotype, G1, 2, 3, 4 vs. others • Will be assessed in PP population and in ITT population. • All SRVGE episodes available in the analysis populations will be taken into consideration and first case of each G serotype will be counted for each subject to find efficacy with respect to each G serotype strain.
2.2.1.2.2	SRVGE cases matched with Serotypes included in vaccine	Up to 2-years of age (End of Study)	• Will be assessed in PP population and in ITT population. • All SRVGE episodes available in the analysis populations will be taken into consideration and first case of each G serotype will be counted for each subject to find efficacy with respect to each G serotype strain.
2.2.1.2.3	RVGE cases	Primary Analysis Time Point : 117 cases of SRVGE accrued or until all participating subjects reach two years of age	• Will be assessed in PP population and in ITT population. • First episode of RVGE available in the analysis populations will count towards this secondary endpoint.

Section	Planned End Points as per Protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint
2.2.1.2.4	SRVGE cases	Up to 1-year of age	• Will be assessed in PP population and in ITT population. • First episode of SRVGE available in the analysis populations will count towards this secondary endpoint.
2.2.1.2.5	SRVGE cases	From 1-year of age up to 2-years of age	• Will be assessed in PP population and in ITT population. • First episode of SRVGE available in the analysis populations will count towards this secondary endpoint.
2.2.1.2.6	SRVGE cases	Up to 2-years of age (End of Study)	• Will be assessed in PP population and in ITT population. • First episode of SRVGE available in the analysis populations will count towards this secondary endpoint. .
	RVGE cases with Vesikari ≥ 17		
	RVGE cases by 1 and 2 doses		

Section	Planned End Points as per Protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint
	RVGE cases by GMT		

Section	Planned End Points as per Protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint
2.2.1.2.9	GE cases / 100 children-year	Up to 2-years of age (End of Study)	• Will be assessed in PP population and in ITT population. • First episode of SRVGE available in the analysis population will count towards this secondary endpoint. • To be done for SRGE, RVGE and GE. • To be estimated as number of diarrhea days / child years. • Vaccine Impact for 2-year follow-up of subject age.
2.2.1.2.10	All Severe GE episodes (may or may not be RVGE episode)	Primary Analysis Time Point : 117 cases of SRVGE accrued or until all participating subjects reach two years of age	• Will be assessed in PP population and in ITT population. • All episode of severe GE available in the analysis population will count towards this secondary endpoint

Section	Planned End Points as per Protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint
2.2.1.2.11	All Severe GE episodes (may or may not be RVGE episode)	Primary Analysis Time Point and Up to 2-years of age (End of Study)	• Will be assessed in PP population and in ITT population. • All episode of severe GE, irrespective of rotavirus status, occurring post study vaccine/placebo administration will count towards this secondary endpoint The occurrence of all severe GE episodes will be considered as defined in ITT population.
2.2.1.2.12	All GE episodes (may or may not be RVGE episode)	Primary Analysis Time Point : 117 cases of SRVGE accrued or until all participating subjects reach two years of age	• Will be assessed in PP population and in ITT population. • All episode of GE irrespective of severity and rotavirus status, occurring available in the analysis population will count towards this secondary endpoint The occurrence of all severe GE episodes will be considered as defined in ITT population.
2.2.1.2.13	All GE episodes (may or may not be RVGE episode)	Up to 2-years of age (End of Study)	• Will be assessed in PP population and in ITT population. • All episodes of GE irrespective of severity available in the analysis population will count towards this secondary endpoint The occurrence of all severe GE episodes will be considered as defined in ITT population.

Section	Planned End Points as per Protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint

2.2.1.3 Immunogenicity Endpoints

A subset of up to 1320 subjects will be enrolled for the immunogenicity endpoints.

To determine and quantitative vaccine seroresponses, 3 mL of blood will be collected before administration of 1st dose and 28 days (±7 days) after 3rd dose administration. The sera will be tested in a validated anti-rotavirus IgA ELISA assay at Cincinnati Children's Hospital? The rate of seroresponse, i.e. Percentage (%) of subjects with ≥3-fold rise and ≥4-fold rise from baseline to 28 days post Dose 3, and Geometric Mean Titer (GMT) will be compared among vaccine and placebo recipients.

Section	Planned Endpoints as per protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint
2.2.1.3.1	Anti-rotavirus IgA ELISA assay: Seroresponse Rates in a subset of up to 1320 Infants: Percentage (%) of subjects with ≥3-fold increase from baseline at 28 days after 3rd dose in	To be performed at Primary Analysis Time Point: 117 cases of SRVGE accrued OR at Final	For subjects in immunogenicity cohorts sera will be obtained immediately before the first dose of vaccine (Baseline) and 28 $\pm$ 7 Days post 3rd dose of

Section	Planned Endpoints as per protocol	Analysis Time Point Planned in the Protocol	Assessment Details about Secondary Endpoint
2.2.1.3.2	Anti-rotavirus IgA ELISA assay: Seroresponse Rates in a subset of 200 Infants: Percentage (%) of subjects with ≥ 4-fold increase from baseline at 28 days after 3rd dose in rotavirus IgA titers	analysis i.e. at the end of 2-years age follow-up period – Based on completed testing of all samples collected from the immunogenicity cohort	vaccination, to test for rotavirus antibody in an ELISA IgG assay.
2.2.1.3.3	Geometric mean Titers (GMTs) in all subjects regardless of baseline value and by baseline sero-status (i.e. seropositive defined as value ≥20 IU/ml and seronegative defined as value <20 IU/ml)		
2.2.1.3.4	Percentage (%) of subjects with rotavirus IgA titers ≥ 20 IU/ml at 28 days after 3rd dose (among those with baseline status < 20 IU / ml)		

2.2.2 Safety Assessments

The safety will be monitored by recording AEs and SAEs experienced by subjects.

All AEs occurring through 28 days after 3rd vaccination and all SAEs will be reported and captured in CRF during study period.

2.2.2.1 Secondary Safety Endpoints

2.2.2.1.1 Immediate Post Vaccination Reactions (within 30 min Post Vaccination)

After each dosing, all participants will be kept at the clinic site for 30 minutes to check for any immediate AEs. At 30 minutes post-vaccination, vital signs will be measured and a targeted physical examination will be performed.

2.2.2.1.2 All Adverse Events (AEs)

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. AEs include all events, including but not limited to fever, diarrhea, vomiting, decreased appetite, decreased activity level, otitis media, and nasopharyngitis. All adverse events will be assessed using facility- and home-based surveillance in all participants and graded for severity from the time of first dose until 28 days post-Dose 3. Caregivers will be informed about the signs and symptoms of adverse events and will be asked to seek care at a local facility or inform a study member in the village in the event any adverse event is suspected and of concern. Study staff will record history of symptoms of the current illness through caregiver interview and conduct a physical examination to document physical signs and clinical condition.

The analysis of adverse events will be done using the ITT population, i.e. all infants who received at least one dose of the study vaccine or placebo, and include follow up from the time of enrollment until 28 days post-dose 3. The incidence of adverse events will be compared between groups with the two-sided asymptotic score test for the null hypothesis of identical incidence by group.

2.2.2.1.3 All Serious Adverse Events (SAEs)

All SAEs and follow-up information occurring between the time of randomization through second year of age will be analyzed. Serious adverse events are defined as follows:

Results in death

Is life threatening

Requires inpatient hospitalization* or prolongation of existing hospitalization

Results in persistent or significant disability**/incapacity, or

is medically important event / reaction that may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Hospitalization is an official admission to a hospital with overnight stay. Hospitalization or prolongation of a hospitalization constitutes a criterion for an AE to be serious**Disability is defined as a substantial disruption in a person's ability to conduct normal life functions. If there is any doubt about whether the information constitutes an SAE, the information is treated as an SAE. All SAEs and follow-up information occurring between the time of randomization and second year of age will be reported in expedited fashion independent of the relationship to study product.

SAEs will be coded using the MedDRA dictionary, Version 16.0. The exact version of the dictionary will be mentioned in the footnote of the respective Listing and/or Table.

Intussusception will qualify as an SAE for this study. Any SAE for which if MedDRA Preferred Term is coded as "Intussusception" will be considered to analyze this endpoint.

SAEs for all Infants will be analyzed at Primary Analysis Time Point i.e. when 117 cases of SRVGE will be accrued and at the end of the study.

2.2.2.1.4 Death

Any SAE for which "Outcome" is marked as "Fatal" will be considered to analyze this endpoint.

Deaths will be analyzed at Primary Analysis Time Point i.e. when 117 cases of SRVGE will be accrued and at the End of the study.

2.2.2.1.5 Hospitalization

Any SAE that is accompanied by hospitalization will be considered to analyze this endpoint. Hospitalizations will be analyzed at Primary Analysis Time Point i.e. when 117 cases of SRVGE will be accrued and at the End of the study.

2.2.2.1.6 Intussusception

Any SAE for which AE Preferred term as per MedDRA dictionary is reported as "Intussusception" will be considered, to analyze this endpoint.

Medical personnel at the sites will be trained on screening tools to promptly identify and assess any suspected cases of intussusception. They include bloody stools, continuous vomiting, abdominal distension and/or abdominal "lumps". Children presenting any suspected symptoms at the time of home

visits or presentation to a study facility will be referred for evaluation. Parents will be instructed by staff to keep close watch of the symptoms noted above and contact study staff if they are detected.

An Intussusception Adjudication Committee constituted by experts (pediatricians, pediatric surgeons) will be formed to review all suspected cases of intussusception and make a final determination on the diagnosis in each case.

The Intussusception cases that are confirmed by Intussusception Adjudication Committee will be reported as SAE with Preferred term as "Intussusception" in the CRF.

Intussusception will be analyzed at Primary Analysis Time Point i.e. when 117 cases of SRVGE will be accrued and at the end of the study.

2.2.3 Other Assessments

2.2.3.1 Vital Signs

Vital signs will be performed at every clinic visit from screening Vital Signs will be assessed twice at visits where study product is administered, with the first assessment before administration of Vaccine and the second assessment done 30 minutes post Vaccine administration. Vital Signs measurement will consist of following parameters:

- Axillary Temperature (^{0}Celsius)
- Heart Rate (Beats/min)
- Respiratory Rate (Breaths/min)

2.2.3.2 Physical Examination

Physical Examination will be will be assessed twice at every vaccination visits, first assessment will be done before administration of Vaccine and the second assessment will be done 30 minutes post Vaccine administration.

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General Physical Examination of the following parameters and body systems:

- Head and Neck
- Eye
- Ears, Nose and Throat
- Skin
- Musculoskeletal
- Central Nervous System

- Respiratory
- Cardiovascular
- Gastrointestinal
- Genitourinary

2.2.3.3 Concomitant Medications

Concomitant medications will be recorded throughout the study period. Concomitant medications will be coded using WHO- DD Version March 2013.

2.3 Study Design

The study is designed as a multisite, double-blinded, randomised, end-point driven placebo controlled trial with two parallel groups of subjects receiving either vaccine or placebo at a 1:1 allocation to evaluate efficacy, safety and immunogenicity of BRV-PV given to healthy subjects aged 6-8 weeks (42-56 days) at the time of first dose of vaccination. Participants will be enrolled in multiple sites across Maradi, Niger. Three doses of BRV-PV/Placebo to healthy subjects. BRV-PV will contain ≥ Log105.6 FFU/dose of each rotavirus serotype G1, G2, G3, G4 and G9.

A total of 7,770 participants will be enrolled in the study. Allocation of treatment to individual subjects will be based on a randomization schedule. The participants will receive three oral administrations as follows:

BRV-PV n = 3,885	Will receive lyophilized BRV-PV reconstituted with 2.5 ml of buffered diluent
Placebo n = 3,885	Will receive lyophilized placebo, reconstituted with 2.5 ml of buffered diluent

Infants will be screened at 6-8 weeks (42-56 days) of age if parent consent is available. Of those screened, eligible subjects will be enrolled and given the first administration of the test vaccine/placebo at 6-8 weeks (42-56 days) of age followed by two more doses at one month (and up to four additional weeks) intervals.

2.4 Sample Size and Power Considerations

The primary aim of the study is to assess the efficacy of the study vaccine against severe rotavirus gastroenteritis. The point estimate of the vaccine efficacy (VE), i.e., [1 – Hazzard Ratio of the vaccine

group relative to the placebo group] x 100%], and the associated 95% CI will be calculated using the Cox proportional hazards model.

Assuming a 2% attack rate of severe rotavirus gastroenteritis, a 50% true vaccine efficacy and a 20% participant non-assessibility (including withdrawal and loss to follow up), the study will enroll 3885 children per group (total n = 7770) to have at least 90% power to detect a vaccine efficacy with a lower 95% confidence interval bound greater than 0%. Under these assumptions, a sample size of 7,770 participants will result in 117 cases of severe rotavirus gastroenteritis (78 unvaccinated and 39 vaccinated) required to fulfill the primary study objective and trigger the primary analysis.

The trial is event driven, with the final analysis triggered by the occurrence of the 117nd case of primary endpoint (78 unvaccinated and 39 vaccinated)). If not all of the 117 needed cases are accrued by the time all subjects reach their second year of age, the analysis of efficacy will be conducted based on all available data.

2.5 Randomization

Eligible participant will be randomised to one of the two groups (1:1) to receive either SII BRVPV or Placebo. Randomization is defined as the process of assigning a participant to a study arm. Randomization will be carried out by assigning each participant a code used for blind allocation to vaccine or placebo.

2.6 Blinding

The study will be conducted in double-blind manner. The clinical site staff and Sponsor will be blinded to treatment assignments. Only DSMB, designated statistician, IP packaging, labeling, Storage & distribution personnel and CRO/third party vendor packaging personnel who are not involved in the trial, will be unblinded to treatment assignments.

The code for an individual participant should be broken only in case of medical emergency where the identity of the IP must be known in order to properly treat the study subject. The investigator must contact the sponsor for concurrence. If agreed, the IP assignment information will be provided through the CRO. All such cases will be fully documented by the investigator and written notification should be provided to sponsor

Since this is endpoint driven study the primary analysis will be performed once 117 SRVGE cases are accrued and if not then primary analysis will be done at the end of the study. If 117 SRVGE cases accrued prior to all subjects reach 2 years of age then the unblinded analysis will be performed. All study site

personnel involved in the study conduct will remain blinded at subject level until the completion of the study. Selected members of sponsor, SIIL, will be unblinded at group or subject level to prepare for the clinical study report and the regulatory submission dossiers.

3 General Analysis Requirements

3.1 Study Duration

Projected duration of accrual is about 18 months. Active surveillance for SRVGE will take place by weekly contacts with the participating subjects starting from the time of the first vaccination until subjects reach two years of age. The primary endpoint will be achieved when 117 cases of severe rotavirus gastroenteritis (SRVGE) are accrued, or until all participating subjects reach two years of age.

The conduct of the study trial from Screening Visit to End of Study Visit is as follows:

- Screening and Dose 1:
    - Written Informed Consent, Subject Demography, Vital Signs, Physical examination, evaluation of Inclusion/Exclusion Criteria, Medical History
    - Randomization
    - Study Vaccination
    - EPI Vaccination
    - Blood collection for immunogenicity in sub cohort
    - Safety assessments (Vital Signs, Physical examination, AEs, SAEs, and Concomitant Medications)
- Dose 2:
    - Study Vaccination
    - EPI Vaccination
    - Safety assessments (Vital Signs, Physical examination, AEs, SAEs, and Concomitant Medications)
- Dose 3:
    - Study Vaccination
    - EPI Vaccination
    - Safety assessments (Vital Signs, Physical examination, AEs, SAEs, and Concomitant Medications)
- Dose 3: Day 28day post Dose 3
    - Blood collection for immunogenicity

    - Safety assessments (Vital Signs, Physical examination, AEs, SAEs, and Concomitant Medications)
- 6month (visit 5) ,9 month (Visit 6), 12 month (Visit 7), 18 Month (Visit 9) and 24 month (Visit 10)
    - Medical History
    - Safety assessments (Vital Signs, SAEs, and Concomitant Medications)
    - Blood collection for immunogenicity (12 and 24 months)

3.2 Schedule of Study Visits, Visit windows and Procedures

Table 2: Time and Events Table

WEEK OF AGE		W0	W6	W10	W14	W18	W24	W36	W52	W64	W76	W88	W104
Study Visit	Pregnancy	Pre - randomization	Randomization and Dose 1	Dose 2	Dose 3	Dose 3 + 28 days	6 months	9 months	12 months	15 months	18 months	21 months	24 months
ALL PARTICIPANTS													
Home Visit*			→	→	→	→	→	→	→	→	→	→	→
Scheduled Facility Visit			X	X	X	X	X	X	X		X		X
Surveillance													
Gastroenteritis and SAE			X	X	X	X	X	X	X	X	X	X	X
AE**			X	X	X	X							
Laboratory Assessment													
Child stool***			X	X	X	X	X	X	X	X	X	X	X
IMMUNOGENCITY SUB-COHORT													
Home Visit*	→	→	→	→	→	→	→	→	→	→	→	→	→
Laboratory Assessment													
Child stool***			X	X	X	X	X	X	X	X	X	X	X
Child blood			X			X			X				X
Child urine			X	X	X	X	X	X	X	X	X	X	X
Maternal blood	X	X	X				X						
Maternal stool	X	X	X				X						
Maternal urine	X	X											
Breast milk		X	X				X						

* Home Visits scheduled for Pre-randomization and on a weekly basis until 2 years of age. In the immunogenicity sub-cohort, home visits will be conducted among all consenting women of reproductive age and continue until the child is 2 years of age.

** Surveillance for adverse events from the time of Dose 1 until 28 days post-Dose 3.

*** Stool collected from all participants for any case of gastroenteritis identified at facility or home within a recall period of 7 days until 2 years of age. In the immunogenicity sub-cohort, stool samples will be collected independent of gastroenteritis status at the time of each Dose, 28 days post-Dose 3, 6 months of age and every 3 months thereafter until 2 years of age.

3.3 Study Cohorts

Participants will be 6-8 weeks old healthy subjects. Potential volunteer families may be contacted from before the babies are born through the noted targeted age. Final eligibility determination will depend on the results of the medical history, clinical examination, fulfillment of the inclusion and absence of any of the exclusion criteria, appropriate understanding of the study and completion of the consent process. All subjects targeted for enrollment will need to have parents that can comprehend the purpose of the study and provide written informed consent. In addition, the families should be resident in the area without plans to leave the study site during the course of the study. Sufficient number of healthy subjects will be screened with parental consent to enroll seven thousand seven hundred seventy participants in the study.

3.3.1 Inclusion Criteria

Fulfillment of all of the following criteria is required to accept the subject in the study:

(1) aged 6-8 weeks at the time of inclusion

(2) able to swallow and no history of vomiting within 24 hours

(3) resident in Madarounfa Health District and within the catchment area of the health facilities

(4) intending to remain in the study area for 2 years

(5) parent/guardian providing written informed consent

3.3.2 Exclusion Criteria

Any of the following will exclude the subject from the study:

(1) known history of congenital abdominal disorders, intussusception, or abdominal surgery

(2) receipt of intramuscular, oral, or intravenous corticosteroid treatment within 2 weeks

(3) receipt or planned administration of a blood transfusion or blood products, including immunoglobulins

(4) any known immunodeficiency condition

(5) any serious medical condition

(6) any other condition in which, in the judgment of the Site Principal Investigator, would interfere with or serves as a contraindication to protocol adherence or the parent/guardian's ability to give informed consent.

Presence of vomiting in the previous 24 hours or on the day of enrollment, immediate hospitalization, and inability to swallow IP are temporary exclusions.

After informed consent has been obtained and the child is identified as meeting inclusion and exclusion criteria for enrollment, the child will be enrolled in the study and assigned a randomization number.

3.3.3 Withdrawal / Discontinuation Criteria

The participants may be withdrawn from the study for any of the following situations:

If parent of subject wishes to withdraw consent

If families move away from the study site permanently. However the data collected up to the last contact will be part of the analysis.

If it is felt in the Principal Investigator's (PI's) opinion that further participation in the study may be detrimental to the interests of the participant

Subject lost to follow-up

In all such cases, the participant will be withdrawn from the study and the reason for withdrawal will be documented in an appropriate case report form. Subjects who move away from the study site or are lost to follow-up and later present to the site and are willing to continue participation, will continue to be followed through age two.

All subjects, who withdraw early from the study for any reason, will be encouraged to complete the end of study assessments as well as their scheduled EPI vaccines. Study subjects whose participation in the study is terminated by the investigator will remain eligible for care at the site until two years of age.

Vaccinations may be discontinued, but follow-up continued, for any of the following situations:

If subject suffers from immediate hypersensitivity reaction following vaccination

If subject suffers from significant inter-current illness If there is protocol violation

If the participant receives a licensed rotavirus vaccine

If it is felt in the Principal Investigator's (PI's) opinion that it is not in the subject's best interest to continue vaccinations

3.4 Treatment Assignment and Study arms

Based on a central computer-generated randomization schedule, subjects will be randomised to one of the two study arms:

- BRV-PV
    - Live Attenuated Pentavalent (G1-G2-G3-G4-G9) Human X Bovine Reassortant Rotavirus Vaccine (BRV-PV), at a dosage of ≥ Log105.6 fluorescent focus units (FFU)/Serotype/Dose in 2.5 ml of buffered diluent
- Placebo

- Lyophilized minimal essential medium (MEM) + excipients reconstituted in 2.5 ml of buffered diluents

4 Statistical Methods

4.1 Analysis Populations

4.1.1 Intent-To-Treat (ITT) Analysis Population

The Intent-To-Treat (ITT) analysis population will consist of all randomised subjects who have taken at least one dose of study vaccine/placebo, independently of whether they receive the full appropriate regimen of vaccine/placebo or receiving the incorrect IP.

In ITT population subjects will be analyzed as per the randomised vaccine group and not as per the actual vaccine received at Dose 1.

4.1.2 Per Protocol (PP) Analysis Population

Per-Protocol analysis population is the subset of the ITT subjects with no major (important) protocol deviations which will impact primary / secondary efficacy analysis and subjects who have received all three doses of study vaccine/placebo and all three doses received are as per the randomised vaccine group.

The PP analysis population will be the primary cohort for the primary and secondary efficacy analyses.

4.1.3 Immunogenicity Analysis Cohort

Immunogenicity analysis population is a subset of approximately 1320 participants in the PP population for whom permission is obtained to assess immune response to the vaccine. To meet the desired target of 1320 evaluable paired pre- and post-vaccination blood samples, sufficient number of subjects will be enrolled in this subset. The CRO will assure equal numbers of vaccine and placebo recipients are enrolled in the immunogenicity cohort. This analysis population includes the subjects with valid measurement(s) required for the corresponding endpoint.

4.1.4 Safety Population

Safety population will consist of all randomised subjects who have received at least one dose of study vaccine/placebo, with or without EPI vaccines, and have some safety data available.

This population will be used for AEs, SAEs, hospitalizations, deaths, and Intussusceptions through Two Years of Age. Once a subject is determined to be in the safety population, the subject will be included in all safety analyses regardless of the availability of individual endpoint data.

In this analysis population subjects will be analysed as per the actual vaccine received at Dose 1. If subject receives mixed dosing then the safety data for dose wise analysis will be analysed on the basis of actual vaccine received for particular dose and for overall (all doses combined) the subject will be analysed as per the actual vaccine received at Dose 1. Listing of mixed dosed subjects will be

provided and any significant findings (e.g. IAE, SAE, Death or Intussusception and Related AE) then that will be discussed individually in the clinical study report.

4.1.5 Protocol Deviations

Protocol deviations will be tracked by the study team throughout the conduct of the study.

- Blind review of Protocol Deviation Listing will be performed by Sponsor personnel and statistician prior to freezing the database and will be finalized and approved to ensure all important deviations, and deviations which may lead to exclusion from the analysis, are captured and categorized on the protocol deviations dataset.
- Protocol Deviation dataset (Final Protocol Deviation Listing) will be the basis for the summaries and listings of protocol deviations.

- All Important protocol deviations (including deviations related to study inclusion/exclusion criteria, conduct of the trial, patient management or patient assessment) will be summarised and listed.
- Important deviations which result in exclusion from the analysis population will also be summarised and listed. (Please refer Table 3 below in the section "Protocol Deviation Management and Definitions for Per Protocol and Immunogenicity population".
- Based on vaccine/placebo administration window period the PP population is as follows:
  - **PP (based on the protocol defined vaccination window) Population:** This population follows window period defined in the protocol for each dose administration of vaccine / placebo as follows:

    Dose 1: 6-8 weeks of age i.e. 42-56 Days (both inclusive) of age at the time of first dose vaccination

    Dose 2: 4 weeks from Dose 1 (with a window of –1 to +4 weeks) implies 28 days from Dose 1 (with a window of 21 days to 56 days from Dose 1) implies 21 ≤ Dose 2 date – Dose 1 Date ≤ 56 days at the time of second dose vaccination.

    Dose 3: 4 weeks from Dose 2 (with a window of –1 to +4 weeks) implies 28 days from Dose 2 (with a window of 21 days to 56 days from Dose 2) implies 21 ≤ Dose 3 date – Dose 2 Date ≤ 56 days at the time of Third dose vaccination.

    Vaccine Efficacy evaluated using this population will be considered as primary analysis.

4.1.5.1.1 Protocol Deviation Management and Definitions for Per Protocol and Immunogenicity Population

Protocol deviations which impact upon the Per Protocol (PP) and Immunogenicity analyses can be full, partial, or time point-specific. Subjects will be excluded from the Per Protocol analysis Population if they have a protocol deviation defined as a Full exclusion in the relevant column in Table 3. Similarly, subjects will be excluded from the Immunogenicity cohort if they have a protocol deviation defined as a Full exclusion in the relevant column in Table 3.

Subjects identified as partial protocol deviation will be included in the PP population but will have their data excluded from PP analyses from the time of deviation onwards. Subjects with time-point specific protocol deviations will be included in the PP Population but only those data affected by the deviation will be excluded from PP analyses. Similar rules apply to the Immunogenicity analysis.

4.2 Timing of Analysis

No interim analysis is planned.

There are potentially two analysis time points for this study.

- The first analysis time point when at least 117 per protocol subjects with at least one SRVGE occurring from 28 days after the third vaccine/placebo dose. This will be the primary analysis time point for the study and will be referred to as the "Primary analysis period with 117 SRVGE cases" (at least 117 cases).
- The second analysis time point is when all participating subjects reach two years of age and will be referred to as the entire study period up to 2 years of age.

If the accrual of 117 per protocol subjects doesn't happen prior to or very close to all subjects reaching two years of age, there will be only one analysis time point. The exact timing of the primary analysis including the data cutoff will be defined as the date of resolution of the 117th case. Methods for handling missing data

- In general, missing data for efficacy and immunogenicity were considered missing at random and no imputation will be done.

Handling of Screen Failures / Ineligible subjects

Subjects failing to meet inclusion criteria and those who meet exclusion criteria at screening visit will not be randomised to either of the two study arms. A listing of screen-failure subjects will be provided with the following reasons of screen failure:

- Subject not fulfilling inclusion criterion / criteria only
- Subject fulfilling the exclusion criterion / criteria only
- Subject not fulfilling inclusion criterion / criteria and fulfilling the exclusion criterion / criteria

4.3 Statistical Analysis

All statistical analysis relating to the study will be performed on statistical software, SAS version 9.2 or later.The quantitative variables will be summarised as Number of subjects, Missing data [N (Missing)], Mean, Standard Deviation [Mean (SD)], Median, Quartiles (Q1, Q3), Range, Minimum and Maximum) [Range (Min, Max)], by study arms. The qualitative variables will be summarised as Number of subjects (n) and percentage (%) by study arms. All data used in summaries will be listed.

4.3.1 Derived Data

Baseline Values:

Baseline value of any parameter is defined as the latest non-missing value of the parameter on or before the date of administration of dose 1 of study vaccine/placebo

Date of birth:

For Date of Birth (DOB) if day and/or month are missing then the day/month will not be imputed and Date of Birth will be treated as missing for age calculation. If exact DOB is missing but the age is provided by parents then the provided age will be used.

Assessment window:

In general, visit-specific evaluations will be taken as nominal visit value without any consideration of window days around the visit day.

4.3.2 Multiplicity Consideration

For the primary endpoint, no adjustment for multiplicity is required since only a single confidence interval will be constructed.

Multiple confidence intervals will be constructed to assess the secondary efficacy, safety, and immunogenicity endpoints. No adjustment for multiple comparisons is planned.

4.3.3 Subject Disposition

The number of screened subjects will be provided without reference to study arms. The Number and percentage of subjects who were screen failures, along with the reason of screen failure will be provided. The number of subjects randomised to the study, along with number and percentage of subjects randomised to each study arms, will be provided.

Randomised subjects are those subjects who have been randomised to one of the study arms but may or may not have taken at least one dose of vaccine/placebo. Thus randomised subjects will include dropouts before first dose administration of study vaccine /placebo.

The analysis cohorts will be summarised by study arms for all randomised subjects who have taken at least one dose of vaccine/placebo.

A subject is called a completer for primary analysis period if the subject did not prematurely discontinue the study up to the time of primary analysis period.

A subject is called a completer for final analysis if he/she has completed the final study visits, i.e. did not prematurely discontinue up to 2 years of age. Premature discontinuation from the study will be summarised by study arms along with reasons for discontinuation, using all randomised subjects. In this study, the subjects for whom further doses of study vaccine/placebo are not given due to safety / other reason but if subject's parents are ready to continue in the study then that subject will be followed till end of the study to collect safety data and will be categorized as "Discontinued further vaccination but followed-up for safety". This data will be filtered from "End of The Study" CRF.

The summary of important protocol deviations (PD) provided in Table 3 will be provided by study arms using the ITT population. All protocol deviations captured during the study conduct will be provided as listing.

The number and percent of subjects in each analysis population will be provided by study arms.

4.3.4 Baseline Characteristics

Demography (age, sex, and weight at birth), Health status, Physical examination, Vital signs,Medical history, as well as household and parental characteristics, will be assessed at the screening visit. Length, Weight, Physical examination and Vital sign will be done at all visits when the clinical visit is scheduled i.e. Visit 1, Visit 2 (Week 14 visit), Visit 3 (Week 18 visit), Visit 4 (Week 18 visit, after 28 days post-Dose3), Visit 5 (6 months age visit),Visit 6 (9 months age visit), Visit 7 (12 months age visit), Visit 8 (18 months age visit), Visit 9 (24 months age visit i.e. End of the study visit).

Baseline characteristics like age, sex, and weight at birth, physical examination, vital signs, and medical history will be summarised by study arms.

4.3.4.1 Demographics

This analysis will be presented for ITT, PP, and Immunogenicity cohort.

Demographic data will include Date of Birth, Gender and Weight at birth. The type of variable, and the corresponding analysis, which will be provided by study arms, is presented in the table given below:

Variable	Type of Variable	Type of Analysis	Unit of Measurements	Formulae for Derived Variable
Age (Derived variable)	Quantitative	Summary Statistics by study arms	days	Age (days) = [Date of Screening Visit – Date of Birth] +1 Age (days) = Date of first SRVGE episode – Date of birth.

Variable	Type of Variable	Type of Analysis	Unit of Measurements	Formulae for Derived Variable
				If Date of Birth is missing but age (days) is provided in CRF then that age will be imputed.
Gender	Qualitative (Male/ Female)	Frequency and Percentage by study arms	Not applicable	--
Length at Baseline	Quantitative	Summary Statistics by study arms	Cms.	--
Weight at birth	Quantitative	Summary Statistics by study arms	Kgs.	--
Weight at Baseline	Quantitative	Summary Statistics by study arms	Kgs.	--

4.3.4.2 Medical History

This will include immunization history, any ongoing diarrhea or other illness. Also it will include past medical history, surgical history, previous hospitalizations, history of any allergy to food, drugs, and current medication history,.

Subjects will be assessed at screening visit for any conditions under medical history and pre-existing conditions.

Medical History will be those medical events which would have occurred during past and stopped before administration of dose 1 of study vaccine/placebo. Pre-existing conditions are those medical events which are ongoing at the time of administration of dose 1 of study vaccine/placebo. If the stop date is missing or partial such that the stop date of the event could not be determined unambiguously with respect to date of administration of dose 1 of study vaccine/placebo, the event will be considered as Pre-existing condition.

Medical history and pre-existing conditions will be coded using the MedDRA dictionary, Version 16.0.The exact version of the dictionary will be mentioned in the footnote of the respective Listing and/or Table. Medical history and pre-existing conditions of all the subjects will be summarised by SOC (System Organ Class) and preferred term and study arms. For this analysis both the PP and ITT analysis population will be used.

4.3.4.3 Medications/Therapies pre- treatment

Medications will be coded as per the WHO Drug Dictionary. The WHO Drug Name (Preferred Term) will be taken as the 5th Level Term Chemical Substance and the ATC Class is the 1st Level ATC. The version of the WHO DD used will be 'Version March - 2013'. The exact version will be mentioned in the footnote of the respective Listing and/or Table.

Prior medications/therapies pre-treatment will be classified as follows:

- Prior medications/therapies stopped before administration of first dose of study vaccine / placebo.

Medications/Therapies that were stopped before the start of the vaccination are referred to as Prior medications/therapies stopped before vaccination.

- Prior medications/therapies ongoing at the time of administration of first dose of study vaccine / placebo

Medications/Therapies that were started before the administration of first dose of study vaccine / placebo and were continuing at the time of administration of first dose of study vaccine / placebo are referred to as Prior medications/therapies ongoing at the time of administration of dose 1 of study vaccine/placebo. If the stop date is missing or partial such that the stop date of the medication could not be determined unambiguously with respect to date of administration of dose 1 of study vaccine/placebo, the medication will be considered as Prior medications/therapies ongoing at the time of administration of first dose of study vaccine / placebo.

Prior medications/therapies for each of the above classifications will be summarized by ATC class and WHO Drug Name (Preferred Term) by study arms. For this analysis, PP and ITT analysis population will be used.

4.3.5 Efficacy Analysis

For efficacy analysis

- For Per Protocol analysis population SRVGE / RVGE / Serotype SRVGE cases will be considered if SRVGE / RVGE / Serotype SRVGE first case occurs from 28 days after the 3rd dose [4 Weeks post Dose 3] of study vaccine/placebo [Start date of GE – Date of 3rd dose of study vaccine/placebo + 28] among subjects receiving the full vaccine/placebo regimen
- For ITT analysis population the first SRVGE / RVGE / Serotype SRVGE case occurring from 28 days after the 3rd dose of study vaccine/placebo [Start date of GE – Date of 3rd dose of study vaccine/placebo + 28)] will be considered irrespective of number of doses administered.
- For Dose – response population the first SRVGE / RVGE / Serotype SRVGE case occurring from 28 days after last dose of study vaccine/placebo received [Start date of GE – Date of last

dose study vaccine/placebo + 28)] will be considered irrespective of number of doses administered.

4.3.5.1 Primary Efficacy Analysis

Statistical Methods used for Primary and Secondary Analysis
Statistical Method used for Binomial Proportion for each treatment group: XXX
Statistic Represented for following Analysis: n = Total Number of subjects with at least one case N = Total number of subjects in each study groups in respective cohort F = Total length of follow-up across all subjects in person years Incidence Rate per Person Year Follow-up = n/F Incidence Rate per 100 Person Year Follow-up = (n/F)*100 n/N (Proportion) along with two-sided 95% CI XXX Follow up for each subject (days) (ITT analysis population) = Minimum [Date of first case of respective endpoint from 1st Dose, Date of premature study discontinuation, Date of study completion depending on subject status] – [Date of Dose 1 vaccine administration] Follow up for each subject (days) (PP analysis population) = Minimum [Date of first case of respective endpoint from 28 days post 3rd Dose, Date of premature study discontinuation, Date of study completion depending on subject status] – [Date of Dose 3 vaccine administration +28 (i.e. Week 18 Date)]
Statistical Method used for Vaccine Efficacy: Cox Proportional Hazard Model

Table 4: Efficacy Analysis Endpoints

Efficacy Analysis	**Population**	**No. of Doses**	**Correct Dosing**	**Start of F/U for Endpoint ***	**GE Episode**	**Episode Considered for Analysis**	**Timing of Analysis**
Primary Efficacy	PP (based on protocol specified vaccination window)	3	Required	28 days post/3	SRVGE	1st only	n=117**
Secondary	PP	3	Required	28 days post/3	RVGE (any severity)	1st only	n=117

Efficacy Analysis	Population	No. of Doses	Correct Dosing	Start of F/U for Endpoint *	GE Episode	Episode Considered for Analysis	Timing of Analysis
Secondary	PP	3	Required	28 days post/3	RVGE (Vesikari ≥ 17)	1st only	n=117
Secondary	PP	3	Required	28 days post/3	RVGE (Vesikari ≥ 17)	1st only	through 24 months
Secondary	PP	3	Required	28 days post/3	SRVGE	1st only	through 12 months #
Secondary	PP	3	Required	28 days post/3	SRVGE	1st only	through 24 months ##
Secondary	PP	3	Required	28 days post/3	SRVGE	1st only	12-24 months
Serotype Specific	PP	3	Required	28 days post/3	SRVGE	1st only	n=117
Serotype Specific (at 23 months)	PP	3	Required	28 days post/3	SRVGE	1st only	through 24 months
Rotavirus Hospitalization (at n=117)	PP	3	Required	28 days post/3		1st only	n=117
All cause Hospitalization (at n=117)	PP	3	Required	28 days post/3		1st only	n=117
Hospitalization (through 23 months)	PP	3	Required	28 days post/3		1st only	through 24 months
Incidence (episode per 100 child years)	PP	3	Required	28 days post/3	SRVGE		through 24 months
Incidence (GE days per 100 child years)	PP	3	Required	28 days post/3			through 24 months
All Severe GE episodes (regardless of rotavirus status)	PP	3	Required	28 days post/3	SGE	any	n=117
All Severe GE episodes (regardless of rotavirus status)	PP	3	Required	28 days post/3	SGE	any	through 24 months

Efficacy Analysis	Population	No. of Doses	Correct Dosing	Start of F/U for Endpoint *	GE Episode	Episode Considered for Analysis	Timing of Analysis
All GE episodes (regardless of rotavirus status)	PP	3	Required	28 days post/3	GE	any	n=117
All GE episodes (regardless of rotavirus status)	PP	3	Required	28 days post/3	GE	any	through 24 months

Per Protocol analysis populations will be considered as primary datasets for testing primary endpoint.

4.3.5.2 Secondary Efficacy Analysis

The secondary endpoints related to Gastroenteritis (GE) are defined in table above Table 4. The same statistical method as the primary efficacy endpoint will be used for all secondary endpoints that are based on the first episode per subject. All SGE and GE episodes endpoints regardless of rotavirus status based on counting multiple episodes per subject were analyzed using Andersen-Gill method.

4 4.4.4.2.1 Immunogenicity

The Rate of Rotavirus IgA Seroresponses will be compared between vaccine and Placebo Recipients in a subset of Subjects.

A subset of subjects will be selected across the study sites to examine the immunogenicity of the vaccine being tested in the study. Sufficient subjects will be enrolled in this subset to ensure the accrual of 1320 with evaluable paired sera.

Sera will be obtained before vaccination and four weeks after the third dose and tested for rotavirus antibody in an enzyme-linked immunosorbent assay (ELISA) IgA assay. The sera will also be used to test for poliovirus antibodies in neutralization assays.

The following immunogenicity endpoints for the immune responses to rotavirus and polio antibodies will be analyzed:

Rotavirus antibody in an enzyme-linked immunosorbent assay (ELISA) IgA assay:

- The number and percentage (%) of subjects with ≥3-fold increase in anti-rotavirus IgA titers from baseline to post-vaccination
- The number and percentage (%) of subjects with ≥4-fold increase in anti-rotavirus IgA titers from baseline to post-vaccination

- The number and percentage (%) of subjects whose rotavirus IgA titers are ≥ 20 IU/ml post-vaccination.
- Anti-rotavirus IgA GMT at pre and post-vaccination in all subjects regardless of baseline value and by baseline sero-status (i.e. seropositive defined as value ≥20 and seronegative defined as value <20)

The geometric mean titer and the corresponding two-sided 95% CI for rotavirus IgA and polio Neutralization assays pre and post vaccination will be summarized by study arm and compared between the study arms. Two-sided 95% CIs for GMTs and the GMT ratios between the study arms will be constructed based on t-distribution. Two-sided 95% CIs for the GMTs and the ratios of GMTs between study arms will beconstructed using log normal distribution. The log10 titers will be used to construct a CI using t-distribution for the mean and the mean difference. The mean and mean difference; and the corresponding CI limits were then exponentiated to obtain the GMT and the GMT ratio, respectively; and the corresponding CI

4.3.6 Safety Analysis

The analysis population for all safety analysis will be Safety analysis population.

All safety analysis will be summarised by study arm over time.

Any AE will be summarized as n (%) E, by study groups.

where n is the number of subjects who experienced that particular AE post each vaccination and overall

% Percentages w.r.t. number of subjects who are at risk post that particular vaccination and overall study

E Total number of episodes allowing for multiple episodes per subject for particular AE experienced post each vaccination and overall study.

In case of more than one AE with same preferred term (PT), if Stop date of the AE that occurred first is same as start date of AE when it appears second time (i.e. AE with Same PT term) then it will be treated as same AE and will be considered only once with maximum severity grading and final outcome and worst relatedness. If AEs are not contiguous then the second AE will be considered as "New" AE.

Severity grading of adverse events

Reactions (Protocol specified terms)	Severity grade	Observation
Diarrhea (>3 loose stools/day)	1 (Mild)	At least 3 looser-than normal stools without dehydration
	2 (Moderate)	diarrhea with some dehydration (per IMNCI definition)

Reactions (Protocol specified terms)	Severity grade	Observation
	3 (Severe)	diarrhea with severe dehydration (per IMNCI definition)
	4 (Life-Threatening)	diarrhea with hypovolemic shock
Vomiting	1 (Mild)	Transient or intermittent vomiting with no or minimal interference with oral intake
	2 (Moderate)	Frequent episodes of vomiting with no or mild dehydration
	3 (Severe)	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)
	4 (Life-Threatening)	Life-threatening consequences (e.g., hypotensive shock)
Fever (Axillary temperature of ≥ 37.5°C)	1 (Mild)	≥ 37.5°C to < 38.5°C
	2 (Moderate)	≥ 38.5°C to < 39.5 °C
	3 (Severe)	≥ 39.5°C to < 40.5 °C
	4 (Life-Threatening)	≥ 40.5°C
Decreased appetite (Anorexia)	1 (Mild)	Loss of appetite without decreased oral intake
	2 (Moderate)	Loss of appetite associated with decreased oral intake without significant weight loss
	3 (Severe)	Loss of appetite associated with significant weight loss
	4 (Life-Threatening)	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
Decreased activity level	1 (Mild)	Slightly subdued, but responds normally to stimuli
	2 (Moderate)	Subdued and does not respond as readily as normal to stimuli
	3 (Severe)	Lethargic
	4 (Life-Threatening)	Obtunded
Irritability	1 (Mild)	Crying more than usual but easily consoled
	2 (Moderate)	Crying more than usual and somewhat difficult to console

Reactions (Protocol specified terms)	Severity grade	Observation
	3 (Severe)	Continuous crying that is inconsolable
	4 (Life-Threatening)	

- Duration(Days): Resolution Date of AE – Start Date of AE + 1
- Onset (Days) from Dose j: Start Date of AE – Vaccine administration Date of dose j + 1

4.3.6.1.1 Immediate Post-vaccination Reactions (within 30 min post vaccination)

Immediate Adverse Events (IAEs) at each dose are defined as adverse events occurring within 30 minutes post each Rotavirus (BRV-PV) / Placebo Vaccination.

IAEs will be coded using the MedDRA dictionary, Version 16.0 or later (except for Immediate Solicited Reactions). This exact version of the dictionary will be mentioned in the footnote of the respective Listing and/or Table.

The IAEs will be coded using the categories of Preferred Term (PT) and System Order Class (SOC) from MedDRA. In case the preferred term for any Event is not available then Preferred Term will be replaced by Lower Level Term (LLT), and if the LLT is also not available in such cases the CRF Reported Term for the IAEs will be considered as Preferred Term in the tables.

The IAEs will be summarized as n (%), by BRV-PV and Placebo group for each vaccine dose and for overall study (Only for all 3 doses combined), as follows:

- IAEs by System Organ Class (SOC) and Preferred term (PT).
- IAEs by Severity and Relatedness
- All IAEs by outcome.
- IAEs of Grade 3 and Grade 4 severity by SOC and Preferred Term. (Only for all 3 doses combined)
- Related IAES by SOC and PT (Only for all 3 doses combined)
- Fatal IAEs by SOC and PT. In this case “Outcome” field in the CRF marked as “Fatal” will be filtered. (Only for all 3 doses combined)
- IAEs leading to discontinuations of further vaccine doses by SOC and PT. In this case “Action taken for further dose” field in the CRF marked as “withdrawn” will be filtered. (Only for all 3 doses combined)

4.3.6.1.2 Adverses Events (AEs)

The AEs - diarrhea, vomiting, fever, decreased appetite, ear pain, skin irritation, acute respiratory infection, decreased activity level, irritability will be observed from the time of the first dose to 28 days post-Dose 3.

Analysis of AE will include Immediate AEs and SAEs. GE data will be analyzed separately.

The AEs will be summarized as n(%)E, by BRV-PV and Placebo group for each vaccine dose and for safety population , as follows:

- AEs as per Protocol Specified Term in descending order of % w.r.t Rotavirus (BRV-PV) vaccine group.
- AEs by Protocol Specified Term and by severity. For this analysis the AEs with maximum intensity will be considered for each subject. AEs are considered to be related.
- AEs by outcome.
- AEs of Grade 3 and Grade 4 severity by SOC and Preferred Term. (Only for all 3 doses combined)
- Fatal AEs In this case “Outcome” field in the CRF marked as “Fatal” will be filtered. (Only for all 3 doses combined)
- AEs leading to discontinuations of further vaccine Doses. In this case “Action taken for further dose” field in the CRF marked as “withdrawn” will be filtered (Only for all 3 doses combined).

Severity for AE (excluding GE data)

Events	Severity grade	Observation
Events	1 (Mild)	Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
	2 (Moderate)	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
	3 (Severe)	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible
	4 (Life Threatening)	Any adverse experience that places the child, in the view of the investigator, at immediate risk of death from the reaction as it occurred. (The investigator should not grade a reaction as life-threatening if had it occurred in a more severe form then it might have caused death.)

- Relationship to Investigational Product

Related

Not related

- Duration(Days): Resolution Date of AE – Start Date of AE + 1
- Onset (Days) from Dose j: Start Date of AE – Vaccine administration Date of dose j + 1
- Outcome
    - Recovered
    - Recovered with sequelae
    - Ongoing till the end of study
    - Lost to follow-up/Unknown
    - Fatal

The adverse event term will be coded using the categories of preferred term (PT) and System Order Class (SOC) from MedDRA. In case the preferred term for any Event is not available then Preferred Term will be replaced by Lower Level Term (LLT), and if the LLT is also not available in such cases the CRF Reported Term for the adverse event will be considered as Preferred Term in the tables.

4.3.6.2 GE Episodes

GE analysis will be presented separately. The analysis will be same as AE, only severity will be based on adapted Vesikari scoring and not the severity judged by investigator.

GE is defined as the passing of three or more watery or looser-than-normal stools within 24-hour period with or without vomiting and the GE Resolution Date will be the date of last ≥ 3 looser than normal stools before five consecutive diarrhea free days (≤ 2 looser than normal stools). **Diarrhea, Vomiting, Temperature**

- **Diarrhea:** Episodes with maximum of between 1 and 3 stools per day are given a score of 1, 4 or 5 stools per day a score of 2, 6 or more stools per day a score of 3.
- **Vomiting**: Episodes with a maximum of 1 vomit per day are given a score of 1, 2 to 4 vomiting episodes per day a score of 2, 5 or more vomiting episodes per day a score of 3.
- **Temperature:** Axillary Temperatures are collected in the database which will be converted to the rectal equivalent*. Episodes with maximum temperature below 37.1°C rectal equivalent are given 0 points; 37.1-38.4°C 1 point; 38.5-38.9°C 2 points; ≥39.0°C 3 points.

Rectal Equivalent Conversion:

1) Convert the temperature to Fahrenheit

- $T_F = (9/5*T_c)+32$

2. Add 2 degrees for Axillary (1 degree for oral or otic)

3. Covert back to Celsius

- $T_c = (5/9)*(T_F -32)$

Duration of Diarrhea and Vomiting Episodes

- **Duration of Diarrhea**: Number of days the subject had ≥3 Loser than Normal stool for the particular Episode between the start and Stop Date of GE Event
- **Duration of Vomiting:** Number of days the subject had Vomiting for the particular episode between the start and Stop Date of GE Event

Dehydration:

This measure is a composite score and includes four different parameters; sunken eyes, general condition, level of thirst, and skin turgor.

The assessment is based on WHO evaluation criteria to categorize dehydration in order to direct treatment. The criteria for assessing dehydration are as follows.

Severe dehydration (combination of two of the following signs):

- Lethargic or unconscious
- Sunken eyes
- Not able to drink or drinking poorly
- Skin pinch goes back very slowly

Some dehydration (combination of two of the following signs):

- Restless, irritable
- Sunken eyes
- Drinks eagerly, thirsty
- Skin pinch goes back slowly

No dehydration:

- Not enough signs to classify as some or severe dehydration

The process for determining the category of dehydration is as follows:

1. If a subject meets the criteria for severe dehydration, then the participant is classified as "severe" i.e. Score 3 (≥ 6 %) for the purposes of Vesikari scoring.
2. If the subject does not meet the severe dehydration classification, then subject is assessed for some dehydration and classified as "moderate" i.e. Score 2 (1 – 5%) if he/she meets the criteria defined.

3. If the subject does not meet the severe or some dehydration classification, then subject is considered to have no dehydration i.e. Score 0 (N/A).

Number of Plan B events (Moderate)	**Number of Plan C events** (Severe)	**Dehydration Category**	**Dehydration Category Score**
Any	≥ 2	>=6%	3
≥ 1	1 (not sunken eyes)	1-5%	2
≥ 2	1 (Sunken eyes)	1-5%	2
≥ 2	0	1-5%	2
1 (Sunken eyes)	1(Sunken eyes)	N/A	0
1	0	N/A	0
0	1	N/A	0
0	0	N/A	0

Treatment

If the subject is hospitalized for at least 24 hours then the score of 2 will be considered, else in case subject either receives Intravenous therapy or Oral Rehydration therapy without hospitalization then Score 1 will be considered.

The following endpoints will be assessed for GE.

- Severity and Relationship
    - Severity for GE data:

Vesikari Score	**Severity grade**
<7	Mild
7 -10	Moderate
≥11	Severe

    - Relationship to Investigational Product

      Related

      Not related
- Onset (Days) from Dose j: Start Date of GE – Vaccine administration Date of dose j + 1
- If Serious: Outcome
    - Recovered

- Recovered with sequelae
- Lost to follow-up/Unknown
- Fatal

4.3.6.2.1 Occurrence of GE Post 28 Days after Third Vaccination

All GEs for which onset day is post 28 days after 3rd vaccination will be filtered from GE module of CRF. For Dose wise analysis of GE, (Onset (Days) of GE from Dose j should be > 28). For this analysis PP and ITT will be used.

For GE analysis, "Infections and infestations" will be considered as SOC and "Gastroenteritis" as Preferred Term.

The GEs will be summarized as n(%)E unless specified, by BRV-PV and Placebo group for each vaccine dose and overall study for Safety population, as follows:

- GEs by severity and relatedness. If for any GE severity by Vesikari score is missing will be treated as missing.
- Fatal GEs. In this case "Outcome" field in the CRF marked as "Fatal" will be filtered.

4.3.6.3 Deaths, Serious Adverse Events and Significant Adverse Events

4.3.6.3.1 Deaths, Serious Adverse Events

For this analysis Safety population will be used.

SAEs will be captured throughout the study.

The endpoints assessed for SAE are

- Duration(Days): Resolution Date of SAE – Start Date of SAE + 1
- Onset of SAE (Days) from Dose j: Start Date of SAE – Vaccine administration Date of dose j + 1
- Classification of SAE by SAE Code:
  - 1 - Results in death
  - 2 - Is life threatening
  - 3 - Results in persistent or significant disability/incapacity
  - 4 - Requires in-patient hospitalization or prolongation of existing hospitalization
  - 5 - Is a congenital anomaly/birth defect in the offspring of a study subject
  - 6 - Is an important medical event that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above should be considered serious.
- Relationship to Investigational Product (Responses as mentioned above for AE)
- Death as Outcome

SAEs will be coded using MedDRA dictionary. All SAEs will be classified on the basis of Preferred Term and System Organ Class (SOC). In case the preferred term for any Event is not available then Preferred Term will be replaced by Lower Level Term (LLT), and if the LLT is also not available in such cases the CRF reported term (if available) for the serious adverse event will be considered as Preferred Term in the tables. For GE "Infections and infestations" will be considered as SOC and "Gastroenteritis" as Preferred Term.

The SAEs will be summarized as n(%)E unless specified, by BRV-PV and Placebo group for each vaccine dose and for overall study, as follows:

- SAEs by SOC and by preferred term.
- SAEs by Preferred term in descending order of % w.r.t Rotavirus (BRV-PV) vaccine group.
- SAEs by severity Related SAEs. For this analysis out of all SAEs experienced by subjects, SAEs with Relatedness marked as "Related" will be considered for each subject. In case if relatedness is missing then will be considered as "Related".
- SAEs leading to discontinuations of further vaccine Doses.
- SAEs by outcome.
- Number and percentage of subjects who died (fatal SAE) will be tabulated for each study group.
- Separate listings of fatal SAEs (with outcome=Death), and non-fatal SAEs will be provided.

For Death (Fatal SAEs) tables will be presented as follows:

  - These will be presented as overall that is up to 28 days post last vaccination for all doses combined (this will consider subjects who received at least one dose of vaccination) and for each dose.
  - These will be presented as overall that is > 28 days post last vaccination up to age 2-year of the age of subject for all doses combined (this will consider subjects who received at least one dose of vaccination).
  - These will be presented as overall that is up to age 2-year of the age of subject for all doses combined (this will consider subjects who received at least one dose of vaccination).

4.3.6.3.2 Intussusception

The intussusception cases, "Confirmed by Intussusception Adjudication Committee" will be presented. For this analysis Safety population will be used.

- Number and percentage of subjects experiencing SAEs - Intussusception, will be tabulated for each study group.
- Intussusception by Brighton Collaboration criteria (Diagnostic criteria 1,2,3) as determined by the Intussusception Adjudication Committee.
- Intussusception by severity and Relatedness. For this analysis the SAEs with maximum intensity will be considered for each subject.
- Related Intussusceptions cases.

  For this analysis out of all Intussusceptions events, the events with Relatedness marked as "Related" will be considered for each subject. Intussusceptions leading to discontinuations of further vaccine Doses.
- Intussusceptions by outcome
- Duration of Intussusceptions event from last dose

For intussusception tables will be presented as follows:

These will be presented as overall Listing of intussusceptions will also be provided separately.

The safety analysis (planned / additional) related to all types of AEs for which tables will be prepared and the filtration that will be done for analysis is explained in the table below; The occurrence of at least one AE (any category) will be compared between BRV-PV and Placebo group using Fisher's Exact test.

Type of AEs	**Cohort / Population**	**SAEs included in Analysis**	**Time Point for Data to be included in Analysis**
IAEs	Safety Population	Included (planned as per Protocol)	Within 30 minutes post each vaccination. IAE data will be taken from IAE CRF (Form 8) and confirmed with CRF for administration of doses (Forms 7AB). Date of onset of IAE is same as Date of Vaccination (Dose 1 / Dose 2 / Dose 3)

Type of AEs	Cohort / Population	SAEs included in Analysis	Time Point for Data to be included in Analysis
AEs (any severity) up to 28 days post last vaccination	Safety population	Included (planned as per Protocol)	Captured from Dose 1 vaccination (post) up to 28 days post Dose 3 Vaccination. Data captured on AE CRF. AE Start Date and Time ≥ Dose 1 Date and Time and AE Start Date and Time – Date and Time of Last dose of vaccination +1 should be ≤ 28
GE up to 28 Days after Third Vaccination	Safety population	Included (planned as per Protocol)	Captured from Dose 1 vaccination (post) up to 28 days post Dose 3 Vaccination. Data captured on GE module of CRF.GE Start Date ≥ Dose 1 Date and GE Start Date and Time – Date and Time of Last dose of vaccination +1 should be ≤ 28
Death and Intussusception within 28 days post last vaccination	Safety population	Included (planned as per Protocol)	Captured from Dose 1 vaccination (post) up to 28 days post Dose 3 Vaccination. Death (Fatal SAEs) and

Type of AEs	Cohort / Population	SAEs included in Analysis	Time Point for Data to be included in Analysis
			intussusception cases, "Confirmed by Intussusception Adjudication Committee" will be used AE Start Date and Time ≥ Dose 1 Date and Time and AE Start Date and Time – Date and Time of Last dose of vaccination +1 should be ≤ 28
Death and Intussusception > 28 Days post last vaccination up to 2 yrs. of age i.e. Up to End of the Study	Safety population	Included (planned as per Protocol)	Captured from > 28 Days post last Vaccination up to end of the Study Death (Fatal SAEs) and intussusception cases, "Confirmed by Intussusception Adjudication Committee" will be used AE Start Date and Time – Date and Time of Last dose of vaccination +1 should be > 28 and Severity ≥ 3

Type of AEs	Cohort / Population	SAEs included in Analysis	Time Point for Data to be included in Analysis
Serious Adverse Event (any severity) From Post Dose 1 up to 2 yrs. of age i.e. Up to End of the Study This will also be presented separately for Death and Intussusception	Safety population	Included (planned as per Protocol)	Captured post Dose 1 up to end of the Study Data captured on Reactogenicity, GE and Unsolicited AE module of CRF. AE Start Date and Time ≥ Dose 1 Date and Time

4.3.6.4 Vital Signs

The vital signs captured in CRF are Weight, Length, Axillary Temperature, Heart Rate and Respiratory Rate.

Listing of vital signs will be provided.

All the Vital signs will be tabulated and analyzed by study arms. All quantitative variables will be summarised as N, Mean, SD, Q1, Median, Q3 and Range (Max - Min) by study arm and visit wise.

Qualitative data i.e. Axillary Temperature, Heart Rate and Respiratory Rate: Vital signs will be categorized as “Normal (N)”, “Abnormal- Not Clinically Significant (NCS)”, “Abnormal- Clinically Significant (CS)”, “Not Done” or “Missing” and summarised as n (%) by study arm and visit wise.

Change in vital signs at each vaccine administration visit will be calculated w.r.t vital signs prior to administration of Rotavirus / Placebo vaccine for that respective dose.

4.3.6.5 Physical Examinations

Physical examination includes assessment of Head and Neck, Eye, Ears Nose and Throat, Skin, Musculoskeletal, Central Nervous System, Respiratory, Cardiovascular, Gastrointestinal and Genitourinary is captured in CRF for each clinical visit.

Listing of physical examinations will be provided.

All these examinations are qualitative results and will be classified as “Normal (N)”, “Abnormal- Not Clinically Significant (NCS)”, “Abnormal- Clinically Significant (CS)”, “Not Done” or “Missing” and will be summarized as N (%) by study groups and visit wise.

4.3.6.6 Clinical Laboratory Evaluation

Not Applicable

4.3.6.7 Concomitant Medications

Medications/therapies will be coded as per the WHO DD. The WHO Drug Name (Preferred Term) will be taken as the 5th Level Chemical Substance Term and the ATC Class is the 1st Level ATC term. The Version of the WHO DD used will be Version March 2013 or later. The exact version will be mentioned in the footnote of the respective Listing.

Medications/Therapies that were started on or after the start of the study treatment are referred to as Concomitant Medications. If the start date of the medication/therapy is missing or ambiguous such that the status of concomitancy cannot be determined, but the stop date is after the start of study treatment, the medication/therapy will be considered as Concomitant Medication. If above conditions cannot be determined unambiguously, the medication/therapy will be considered Concomitant Medication.

Details of cconcomitant medications including each medication such as, dose, frequency, route, etc. will be contained in the data listing and will not be summarized / tabulated.

4.3.7 Quality of Life Analysis

Not Applicable

4.3.8 Pharmacokinetics and Pharmacodynamic Analysis

Not applicable

5 Evaluation of Treatment Compliance and Exposure

5.1 Exposure to Study Drug

5.1.1 Extent of Exposure

The number of subjects who received number of doses of BRV-PV/Placebo and childhood vaccines will be summarized as frequency and percentage.

Even tables will be presented for PP (Protocol) to summarize number of doses received by the per protocol analysis population as per defined windows.

5.2 Subgroup / Exploratory Analysis

5.3 Effect modification of all endpoints will be explored by child sex, maternal IgA, birth weight, gestational age, breastfeeding status, concurrent diarrhea, and malnutrition status. Statistical Analytical Issues

Since Confidence Interval for Difference in Proportion using Agresti and Caffo method is not available in SAS 9.2 and also in NCSS software, Hence Wilson score method available in SAS will be used for calculating the Confidence Interval for Difference in proportion.

6 Interim Analysis and Safety Monitoring Analysis

6.1 Interim Analysis

No formal Interim Analyses are planned. However, at the time of the primary analysis based on the accrual of 117 per protocol SRVGE cases, follow-up of some safety and secondary efficacy endpoints will be completed. These preliminary interim analyses will be updated once the study is completed. The final conclusion of these analyses will be based on the complete data.

6.2 Safety Monitoring Analysis

DSMB will be appointed for review of safety data.

The DSMB will be authorized to unblind the treatment allocation if required during the trial.

The DSMB may unblind under the following conditions:

- An unanticipated serious adverse event that has been judged to be related to the BRV-PV by either the principal investigator/designee.
- A serious adverse event that has been judged as related to the BRV-PV and is of a severity or frequency higher than anticipated.
- An anticipated or other SAE where the management of the adverse event may depend on the intervention received.
- Other conditions where the DSMB may feel the need to unblind.

For each DSMB review meeting blinded data analysis will be provided as per approved mock displays approved for DSMB report.

The additional analysis for safety monitoring may be provided to DSMB members if required by DSMB members.

7 Statistical Tables to be generated

Following are the minimum tables presented in Statistical analysis

14.1 DEMOGRAPHIC AND BASELINE DATA SUMMARY

Table 14.1.1 Summary of Subject Disposition - Screened Subjects

Table 14.1.2 Summary of Subject Disposition by Study Groups - Randomized Subjects

Table 14.1.3 Summary of Premature Discontinuation by Study Groups - ITT Population

Table 14.1.4 Summary of Major Protocol Deviations by Study Groups - ITT Population

Table 14.1.5 Summary of Demographics and Baseline Characteristics by Study Groups - ITT Population

Table 14.1.7 Summary of Demographics and Baseline Characteristics by Study Groups - Immunogenicity Cohort

Table 14.1.8 Summary of Baseline Vital Signs by Study Groups – ITT Population

Table 14.1.9 Summary of Baseline Physical Examinations by Study Groups – ITT Population

Table 14.1.10 Summary of Baseline Characteristics - Medical History by Study groups – ITT Population

Table 14.1.11 Summary of Baseline Characteristics - Pre-Existing Conditions by Study Groups – ITT Population

14.2 EFFICACY TABLES

Table. Distribution of SRVGE / RVGE / Serotype SRVGE cases over calendar time, overall and by strain and severity. Table 14.2.1 Overview of Gastroenteritis (GE) Reported by Subjects and Episodes at the Time of Primary Analysis when First 117 SRVGE Cases Accrued

Table 14.2.2 Overview of Gastroenteritis (GE) Reported by Subjects and Episodes up to Two Years of Age (≤ 24 Months – End of Study)

Table 14.2.3 Efficacy of BRV-PV Vaccine against Severe RVGE (Primary Endpoint - SRVGE) at the Time of Primary Analysis when First 117 SRVGE Cases Accrued – PP Populations

Table 14.2.4 Efficacy of BRV-PV Vaccine against Severe RVGE (Primary Endpoint - SRVGE) at the Time of Primary Analysis when First 117 SRVGE Cases Accrued – ITT Population

Table 14.2.5 Efficacy of BRV-PV Vaccine against Severe RVGE (Primary Endpoint - SRVGE) up to One Year (≤ 12 Months) of Age

Table 14.2.6 Efficacy of BRV-PV Vaccine against Severe RVGE (Primary Endpoint - SRVGE) from 1-year (> 12 Months) of age up to 2-years

(≤ 24 Months) of age

Table 14.2.7 Efficacy of BRV-PV Vaccine against Severe RVGE (Primary Endpoint - SRVGE) up to Two Years of Age (≤ 24 Months – End of Study)

Table 14.2.8 Efficacy of BRV-PV Vaccine against RVGE of any Severity (Secondary Endpoint) at the Time when First 117 SRVGE Cases Accrued

Table 14.2.9 Efficacy of BRV-PV Vaccine against Severe RVGE Cases matched with G Serotypes (Secondary Endpoint) at the Time when First 117 SRVGE Cases Accrued

Table 14.2.10 Efficacy of BRV-PV Vaccine against Severe RVGE Cases matched with G Serotypes (Secondary Endpoint) up to Two Years of Age (≤ 24 Months – End of Study)

Table 14.2.11 Summary of Efficacy of BRV-PV vaccine Against SRVGE by Genotype (Secondary Endpoint) at the Time when First 117 SRVGE Cases Accrued

Table 14.2.12 Summary of Efficacy of BRV-PV vaccine Against SRVGE by Genotype (Secondary Endpoint) up to Two Years of Age (≤ 24 Months – End of Study)

Table 14.2.13 Efficacy of BRV-PV Vaccine against Hospitalization Cases due to SRVGE (Secondary Endpoint) at the Time when First 117 SRVGE Cases Accrued

14.3.1 Immediate Adverse Events (Immediate Vaccine Reactions)

14.3.3.2 Grade 3 or Higher or Serious Unsolicited Adverse Events Up to 28 Days Post Last Dose – Safety Population

Table 14.3.3.2.16 Summary of Related Grade 3 or Higher or Serious Unsolicited Adverse Events up to 28 Days Post Last Dose by MedDRA Coding (SOC and PT) - Safety Population

Table 14.3.3.2.17 Incidence of Grade 3 or Higher or Serious Unsolicited Adverse Events up to 28 Days Post Last Dose by Outcome and Study Groups: Dose Wise - Safety Population

Table 14.3.3.2.18 Summary of Fatal Grade 3 or Higher or Serious Unsolicited Adverse Events up to 28 Days Post Last Dose by MedDRA Coding (SOC and PT) – Safety Population

Table 14.3.3.2.19 Grade 3 or Higher or Serious Unsolicited Adverse Events up to 28 Days Post Last Dose Leading to Discontinuation by MedDRA Coding (SOC and PT) - Safety Population

Table 14.3.3.2.20 Count of Unsolicited Adverse Events up to 28 Days Post Last Dose – Overall – Safety Population

14.3.3.3 Grade 3 or Higher or Serious Unsolicited Adverse Events after 28 Days Post Last Dose up to 2 Years of Age (End of study) – Safety Population

Table 14.3.3.3.1 Overview of Grade 3 or Higher or Serious Unsolicited Adverse Events after 28 Days Post Last Dose up to 2 Years of Age (End of study) – Safety Population

Table 14.3.3.3.2 Overview of Grade 3 or Higher or Serious Unsolicited Adverse Events after 28 Days Post Dose 3 up to 2 Years of Age (End of study) – Safety Population

Table 14.3.3.3.3 Grade 3 or Higher or Serious Unsolicited Adverse Event by MedDRA Coding (SOC and PT) after 28 Days Post Last Dose up to 2 Years of Age (End of study) – Safety Population

Table 14.3.3.3.4 Grade 3 or Higher or Serious Unsolicited Adverse Event by MedDRA Coding (SOC and PT) after 28 Days Post Dose 3 up to 2 Years of Age (End of study)– Safety Population

Table 14.3.3.3.5 Grade 3 or Higher or Serious Unsolicited Adverse Event by MedDRA Coding (PT) after 28 Days Post Last Dose up to 2 Years of Age (End of study) – Safety Population

Table 14.3.3.3.6 Grade 3 or Higher or Serious Unsolicited Adverse Event by MedDRA Coding (PT) after 28 Days Post Dose 3 up to 2 Years of Age (End of study) – Safety Population

Table 14.3.3.3.7 Incidence of Grade 3 or Higher or Serious Unsolicited Adverse Events after 28 Days Post Last Dose by Severity, Relatedness and Study Groups: Dose Wise - Safety Population

Table 14.3.3.2.8 Summary of Severity Grade 3 and 4 Unsolicited Adverse Events Post Last Dose by MedDRA Coding (SOC and PT) – Safety Population

Table 14.3.3.2.9 Summary of Related Grade 3 or Higher or Serious Unsolicited Adverse Events after 28 Days Post Last Dose by MedDRA Coding (SOC and PT) - Safety Population

Table 14.3.3.3.10 Incidence of Grade 3 or Higher or Serious Unsolicited Adverse Events after 28 Days Post Last Dose by Outcome and Study Groups: Dose Wise - Safety Population

Table 14.3.3.3.11 Summary of Fatal Grade 3 or Higher or Serious Unsolicited Adverse Events after 28 Days Post Last Dose by MedDRA Coding (SOC and PT) – Safety Population

14.3.3.4 Grade 3 or Higher or Serious Unsolicited Adverse Events Post Dose 1 – Safety Population

14.3.4 Gastroenteritis Events (GE)

14.3.4.1 Gastroenteritis Events Up to 28 Days Post Last Dose - Safety Population

Table 14.3.5.6 Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of Study) by MedDRA Coding (SOC and PT) – Safety Population

Table 14.3.5.7 Serious Adverse Event by MedDRA Coding (SOC and PT): After Dose 1 and Prior to Dose 2 – Safety Population

Table 14.3.5.8 Serious Adverse Event by MedDRA Coding (SOC and PT): after Dose 2 and Prior to Dose 3 – Safety Population

Table 14.3.5.9 Serious Adverse Event by MedDRA Coding (SOC and PT): Up to 28 Days after Dose 3 – Safety Population

Table 14.3.5.10 Serious Adverse Event by MedDRA Coding (SOC and PT): After 28 Days Post Dose 3 up to 2 Years of Age – Safety Population

Table 14.3.5.11 Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of Study) by MedDRA Coding (PT) – Safety Population

Table 14.3.5.12 Serious Adverse Event by MedDRA Coding (PT): after Dose 1 and Prior to Dose 2 – Safety Population

Table 14.3.5.13 Serious Adverse Event by MedDRA Coding (PT): after Dose 2 and Prior to Dose 3 – Safety Population

Table 14.3.5.14 Serious Adverse Event by MedDRA Coding (PT): Up to 28 Days after Dose 3 – Safety Population

Table 14.3.5.15 Serious Adverse Event by MedDRA Coding (SOC and PT): After 28 Days Post Dose 3 up to 2 Years of Age – Safety Population

Table 14.3.5.16 Incidence of Serious Adverse Events Post Dose 1 up to 2 Years of Age by Severity, Relatedness and Study Groups: Dose Wise - Safety Population

Table 14.3.5.17 Summary of Severity Grade 3 and 4 - Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of study) by MedDRA Coding (SOC and PT) - Safety Population

Table 14.3.5.18 Summary of Related Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of study)by MedDRA Coding (SOC and PT) - Safety Population

Table 14.3.5.19 Incidence of Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of study) by Outcome and Study Groups: Dose Wise - Safety Population

Table 14.3.5.20 Incidence of Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of study) by Seriousness Criteria and Study Groups - Safety Population

Table 14.3.5.21 Summary of Fatal Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of study) by MedDRA Coding (SOC and PT) - Safety Population

Table 14.3.5.22 Summary of Serious Adverse Events Post Dose 1 up to 2 Years of Age (End of study) Leading to Discontinuations, by MedDRA Coding (SOC and PT) - Safety Population

14.3.6 Other Safety Information

Table 14.3.6.7 Summary of Vaccine Schedule Duration in Days followed by BRV-PV/Placebo administration - ITT Population

8 Statistical Listings to be generated

14.3.2 Listing of Deaths, Other Serious and Significant Adverse Events

Listing 14.3.2.1 Listing of Fatal SAEs

Listing 14.3.2.2 Listing of SAE - Intussusceptions

Listing 14.3.2.3 Listing of Stool Sample Collected for GE Episode

Listing 14.3.2.4 Listing of Non-Fatal SAEs

16.2 Subject Data Listings

16.2.1 Subjects Disposition

Listing 16.2.1.1 Subjects who are Screen-Failure

Listing 16.2.1.2 Subjects who discontinued during the Study after Randomization

16.2.2 Protocol Deviations

Listing 16.2.2.1 Important Major Protocol Deviations During the Study

Listing 16.2.2.2 Minor Protocol Deviations During the Study

16.2.3 Subjects Excluded from ITT, Reactogenicity, PP, Immunogenicity or Safety Analysis Populations

Listing 16.2.3.1 Disposition of Subjects - All Randomized Subjects

Listing 16.2.3.2 Exclusion of Data from Per Protocol Population - All Randomized Subjects

Listing 16.2.3.3 Exclusion of Data from Immunogenicity Cohort - All Randomized Subjects

16.2.4 Demographics and Baseline Characteristics

Listing 16.2.4.1 Demographic Data

Listing 16.2.4.2 Baseline Characteristics – Health Status

Listing 16.2.4.3 Baseline Characteristics – Medical History and Pre-existing Conditions

Listing 16.2.4.4 Baseline Characteristics – Prior Medications - I of II

Listing 16.2.4.4 Baseline Characteristics – Prior Medications - II of II

16.2.5 Compliance and Study Drug Exposure Data

Listing 16.2.5.1 Study Drug Dose Exposure – Vaccine Administration Details - I of II

Listing 16.2.5.1 Study Drug Dose Exposure – Vaccine Administration Details - II of II

16.2.6 Individual Serology and Immunogenicity Response Data

Listing 16.2.6.1 Gastroenteritis (GE) cases - I of II

Listing 16.2.6.1 Gastroenteritis (GE) cases - II of II

Listing 16.2.6.2 Immunogenicity Endpoint - Poliovirus antibody Titers in Neutralization Assays (Polio Antibodies)

Listing 16.2.6.3 Immunogenicity Endpoint – Rotavirus Antibodies in ELISA IgA Assays

16.2.7 Adverse Events Listings

Listing 16.2.7.1 Immediate Adverse Events (IAEs) Listings – I of II

Listing 16.2.7.1 Immediate Adverse Events (IAEs) Listings – II of II

Listing 16.2.7.2 Solicited Adverse Events Listings for Reactogenicity Cohort – I of II

Listing 16.2.7.2 Solicited Adverse Events Listings for Reactogenicity Cohort – II of II

Listing 16.2.7.3 Unsolicited Adverse Events Listings– I of II

Listing 16.2.7.3 Unsolicited Adverse Events Listings– II of II

Listing 16.2.7.4 Listing of Gastro Enteritis Episodes

16.4 Individual Subject Data Listings

Listing 16.4.1 Subject Eligibility Criteria

Listing 16.4.2 Universal Immunization Program and Other Vaccines

Listing 16.4.3 Listing of Vital Signs

Listing 16.4.4 Listing of Physical examination

Listing 16.4.5 Concomitant Medications - I of II

Listing 16.4.5 Concomitant Medications - II of II

Listing 16.4.6 Study Completion Status

Listing 16.4.7 Final Randomization List

9 Statistical Graphs to be generated

Figure 17.1: Study Flowchart - <<For Primary Analysis / End of the Study>>

Figure 17.2 Study Flowchart: Analysis populations - <<For Primary Analysis / End of the Study>>

14.2.2 Primary and Secondary Efficacy Analysis Related Figures

Figure 14.2.2.1 Kaplan-Meier Survival Curves for SRVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – PP Cohort

Figure 14.2.2.2 Kaplan-Meier Survival Curves for SRVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – ITT Cohort

Figure 14.2.2.3 Kaplan-Meier Survival Curves for SRVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo –m ITT Cohort.

Figure 14.2.2.4 Kaplan-Meier Survival Curves for RVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – PP Cohort

Figure 14.2.2.5 Kaplan-Meier Survival Curves for RVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – ITT Cohort

Figure 14.2.2.6 Kaplan-Meier Survival Curves for RVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – mITT Cohort

Figure 14.2.2.7 Kaplan-Meier Survival Curves for G- serotype SRVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – PP Cohort

Figure 14.2.2.8 Kaplan-Meier Survival Curves for G- serotype SRVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – PP Cohort

Figure 14.2.2.9 Kaplan-Meier Survival Curves for G- serotype SRVGE cases experienced by Subject Post 28 Days after Third Dose of BRV-PV / Placebo – PP Cohort

Figure 14.2.2.10 Forest Plot for Hazard Ratio on Log Scale for all Types of GE

14.2.3 Immunogenicity Analysis Related Figures

Figure 14.2.3.1 The Geometric Mean Titers (GMTs) for IgA antibody titers at Baseline and 28 days post 3rd dose of BRV-PV/ Placebo - Immunogenicity Cohort

Figure 14.2.3.2 Three-fold Responders for IgA antibody titers at 28 days post 3rd dose of BRV-PV / Placebo - Immunogenicity Cohort

Figure 14.2.3.3 Bar Chart showing Four-fold Responders for IgA antibody titers at 28 days post 3rd dose of BRV-PV / Placebo - Immunogenicity Cohort

Figure 14.2.3.4 Bar Chart showing percentage of subjects showing IgA antibody titers ≥ 20 IU/mL- Immunogenicity Cohort

14.2.3 Safety Figures

Figure 14.2.3.1 Unsolicited Severe AEs >3% by Preferred term and Study Groups - Percentage Bar Charts

Figure 14.2.3.2 SAEs >3% by Preferred term and Study Groups - Percentage Bar Charts

Figure 14.2.3.3 SAEs - Deaths by Study Groups - Percentage Bar Charts

Figure 14.2.3.4 SAEs - Intussusception by Study Groups - Percentage Bar Charts

Summary of Changes

There have been no changes to the SAP