CLINICAL STUDY PROTOCOL

Protocol Title:	A Phase II randomised, double blind, parallel group dose-ranging study of oral RV3-BB Rotavirus Vaccine administered at a titre of $1x10^7$, 3×10^6 or 1×10^6 as a 3 dose neonate schedule or administered at a titre of $1x10^7$as a 3 dose infant schedule.
Protocol Number:	MCRI-RV3-BB-004
Investigational Product:	RV3-BB Rotavirus Vaccine
Study Phase:	II
Indication:	Prevention of rotavirus gastroenteritis in neonates and infants
Sponsor:	Murdoch Children's Research Institute (MCRI) Royal Children's Hospital Flemington Road Parkville, Victoria 3052, Australia Tel: [redacted] Fax: [redacted]
Sponsor Protocol Approval:	[redacted]
Principal Investigator:	[redacted]
Version / Date:	4.0 / 11 January 2019

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INVESTIGATOR STATEMENT

The undersigned Investigator agrees:

1. To conduct the study in accordance with the study protocol, International Council on Harmonisation Good Clinical Practice and any national and local laws and regulations.
2. That alteration of the procedures described in the study protocol, other than to protect participant safety, rights, or welfare, is not allowed without prior written approval MCRI and ethics committee approval.
3. The study-specific data of the participants will be kept in the participants' files and documented in the case report form in a complete and accurate manner. All requested study-related records will be made available for direct access to MCRI representatives for monitoring or auditing the study.
4. To allow authorised qualified delegates of MCRI to perform regular visits to monitor the study data.
5. To dispose of used and unused Investigational Product and materials as instructed by MCRI.
6. To ensure that all persons at their site assisting with the clinical study are adequately informed and trained about the study protocol, the Investigational Product and their study-related duties and functions.

Name: _____

Signature: _____

Date: _____

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1 ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
BCG	Bacillus Calmette-Guérin (vaccine)
CPMP	Committee for Proprietary Medicinal Products
CRA	Clinical Research Associate
CRF	Case Report Form
D	Day
DMP	Data Management Plan
DSMB	Data Safety Monitoring Board
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunisation
FFU	Fluorescing cell focus units
g	Gram
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GIT	Gastro-intestinal tract (symptoms)
GLP	Good Laboratory Practice
cGMP	Current Good Manufacturing Practice
HBGA	Histo-blood group antigen
HEENM	Head, ears, eyes, nose and mouth
HepB	Hepatitis B
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IP	Investigational Product
IPV	Inactivated polio vaccine
ITT	Intention-to-Treat
kg	Kilogram
L	Litre
LLR	Lanzhou Lamb Rotavirus Vaccine
MCRI	Murdoch Children's Research Institute
MedDRA	Medical Dictionary for Regulatory Activities
mL	Millilitre
mITT	Modified Intention-to-treat
NIP	National Immunisation Programme
NIS	National Immunisation Schedule
OPV	Oral polio vaccine
PCR	Polymerase chain reaction
PI	Principal Investigator
PICF	Participant Information and Consent Form
PP	Per Protocol
RNA	Ribonucleic acid
RT-PCR	Reverse transcription and polymerase chain reaction
RV3	RV3 (serotype G3) strain of rotavirus
RV3-BB	Second generation RV3 rotavirus vaccine
SAE	Serious adverse event
SAGE	Strategic Advisory Group of Experts (on Immunization)

SAP	Statistical Analysis Plan
SC	Steering Committee
SD	Standard Deviation
SNA	Serum Neutralising Antibodies
SOP	Standard Operating Procedure
SRM	Study Reference Manual
U	Units
ULN	Upper limit of normal
USA	United States of America
WHO	World Health Organisation
WHO-DD	World Health Organisation - Drug Dictionary

2 PROTOCOL SYNOPSIS

Title:	A Phase II randomised, double blind, parallel group dose-ranging study of oral RV3-BB Rotavirus Vaccine administered at a titre of $1x10^7$, 3 x 10^6 or 1 x 10^6 as a 3 dose neonate schedule or administered at a titre of $1x10^7$as a 3 dose infant schedule.
Indication:	Prevention of rotavirus gastroenteritis in neonates and infants
Sites:	The study will be conducted at approximately four sites in Malawi.
Duration:	18 weeks per participant. The recruitment period is expected to be approximately 9 months, with total study duration of approximately 15 months.
Objectives:	Primary: • To assess a cumulative anti-rotavirus serum IgA response (defined as a ≥3 fold increase from baseline) 4 weeks after 3 doses of RV3-BB administered in a neonatal schedule at a vaccine titre of $1x10^7$, 3 x 10^6 or 1 x 10^6. Secondary: • To assess the cumulative anti-rotavirus serum IgA response (defined as a ≥3 fold increase from baseline) 4 weeks after 3 doses of RV3-BB administered in an infant schedule at a vaccine titre of 1 x 10^7. • To assess cumulative vaccine take and components of vaccine take after three doses of RV3-BB (titre of 1 x 10^7) administered as a neonatal schedule versus three doses of RV3-BB (titre of 1 x 10^7) administered as an infant schedule. • To assess cumulative vaccine take and the components of RV3-BB vaccine take after 3 doses of RV3-BB administered as a neonatal schedule at a vaccine titre of 1 x 10^7, 3 x 10^6 or 1 x 10^6 • To assess cumulative vaccine take and components of vaccine take after 2 doses of RV3-BB administered as a neonatal schedule at a vaccine titre of 1 x 10^7, 3 x 10^6 or 1 x 10^6 or 2 doses as an infant schedule at a vaccine titre of 1 x 10^7, • To assess cumulative vaccine take and components of vaccine take after the first dose of RV3-BB administered as a neonatal schedule at a vaccine titre of 1 x 10^7, 3 x 10^6 or 1 x 10^6 compared to placebo (1st dose of IP in the infant schedule). • To assess cumulative vaccine take and components of vaccine take after the first dose of RV3-BB at a vaccine titre of 1 x 10^7, 3 x 10^6 or 1 x 10^6 in the neonatal schedule compared with the first dose of RV3-BB at a vaccine titre of 1 x 10^7 in the infant schedule.

	• To describe the geometric mean titre of the anti-rotavirus serum IgA response after 3 doses of RV3-BB administered as a neonatal schedule or an infant schedule. • To describe the safety and tolerability of RV3-BB when administered as an infant or as a neonatal schedule. • To describe the occurrence of diarrhea episodes in participants, according to severity and detection of wild-type rotavirus. Exploratory: • To understand potential barriers to RV3-BB vaccine efficacy in the region: o To describe Histo-blood Group Antigens (Lewis and secretor) status of participants in association with anti-rotavirus sero-conversion and cumulative vaccine take after 3 doses RV3-BB administered in either the neonatal schedule (any titre) or infant schedule. o To assess maternal anti-rotavirus IgA and IgG levels in association with anti-rotavirus IgA seroconversion and cumulative vaccine take in participants following receipt of RV3-BB administered in either the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of 1×10^7. o To describe the gut microbiome in participants receiving RV3-BB administered in the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of 1×10^7. o To describe innate and adaptive immune responses in infants vaccinated with RV3-BB administered in the neonatal vaccine schedule (any titre) or in the infant schedule at a vaccine titre of 1×10^7.
Design	This is a Phase II, randomised, double blind, four arm parallel group study. Approximately 688 participants will be randomised
Methodology	The parents/guardians of potential study participants will be required to provide written informed consent prior to any study-specific procedures being performed. Antenatal recruitment: Pregnant women will be invited to provide preliminary consent for the study prior to labour and delivery, to provide them with adequate opportunity to consider the decision and to allow collection of a maternal blood sample and cord blood. Following delivery, parents/guardians who remain interested will be invited to provide written informed consent for their infant to participate in the study. Following the provision of consent and confirmation of eligibility for the trial, each participant will be randomly allocated in a blinded manner in a

	1:1:1:1 ratio to one of the following four treatment arms. Each study participant will receive four oral doses of IP, with each administration consisting of 1 mL RV3-BB Rotavirus Vaccine at one of three different dose strengths or a dose of 1mL of placebo. All participants will receive 4 doses in total, 3 of RV3-BB vaccine and one of placebo. The first dose will be prior to 5 days (144 hours) of age, the second at approximately 6 weeks of age, the third at approximately 10 weeks of age and the final dose at approximately 14 weeks of age, according to the following treatment arms; • High dose neonatal RV3-BB vaccine schedule ($1x10^7$titre dose). RV3-BB Vaccine for doses 1, 2 and 3 and placebo for dose 4. • Mid dose neonatal RV3-BB vaccine schedule ($3x10^6$titre dose). RV3-BB Vaccine for doses 1, 2 and 3 and placebo for dose 4. • Low dose neonatal RV3-BB vaccine schedule ($1x10^6$titre dose). RV3-BB Vaccine for doses 1, 2 and 3 and placebo for dose 4. • Infant RV3-BB vaccine schedule ($1x10^7$titre dose). Placebo for dose 1 and RV3-BB Vaccine for doses 2, 3 and 4. All participants will also receive a single dose of oral Rotarix®, at approximately 18 weeks of age. A maternal blood sample, up to approximately 1.0mL, will be collected after preliminary consent, any time during the second or third trimester of pregnancy or after delivery of the placenta. Cord blood, up to approximately 2.5mL, will be collected immediately following delivery of the placenta. Where cord blood is unavailable for collection a 1.0mL blood sample will be collected at Visit 1 prior to IP administration. A saliva sample will be collected on all randomised participants prior to the first administration of IP to determine histo-blood group antigen status (genotype) (Lewis and secretor). Saliva will also be collected at IP dosing visits prior to dosing to determine histo-blood group antigen to monitor changes in expression during the study. A baseline stool sample will be collected from pre-consented participants any time prior to the administration of the first dose of IP. A Stool sample will be collected from randomised participants on day 3, 4 or 5 following each dose of IP. Samples will also be collected for any cases of diarrhoea (defined as 3 or more stools within a 24 hour period that are looser than normal for that participant). Up to two samples will be collected during each diarrhoea episode. Stool and diarrhoea samples will be tested at the local study laboratory for excretion of rotavirus using PCR. Up to approximately 1-2.5 mL venous blood will be collected from randomised participants within the 2 days prior to each IP dose and at 18 weeks.

	Participants will be followed up for the duration of the study, until 18 weeks of age. Face to face visits will be conducted at least monthly. Adverse events, including diarrhoea episodes, will be recorded for the duration of the study.
Entry Criteria	Inclusion criteria: • Neonate is less than 6 days (≤144 hours) of age at the time of first dose. • Neonate is in good health as determined by clinical judgment, including a medical history and physical exam, which confirms the absence of a current or past disease state considered significant by the investigator. • Neonate birth weight 2500-4000g inclusive or 2250-4000g inclusive at Visit 1. • Neonate's parents/guardians expect to be available for the duration of the study, and agree to adhere to all protocol requirements. • Neonate's parents/guardians have provided written informed consent prior to study-related procedures being performed. Exclusion criteria: • Any medical, psychiatric, or social condition of a parent/guardian that in the opinion of the investigator would prevent the neonate's parents/guardians from giving proper informed consent or from complying with the study protocol. • Neonates with known or suspected major congenital malformations or genetically determined disease. • Neonates with intussusception. • Neonates with a known or suspected bleeding diathesis, or any condition that may be associated with a prolonged bleeding time. • Neonates who have ever received any blood products, including immunoglobulin, or for whom receipt of any blood product during the course of the study is anticipated. • Neonates in whom EPI vaccines or components are contraindicated. • Neonates who have received or who expect to receive during the study period, any rotavirus vaccine other than those which will be administered as part of this study. • Neonates who have ever received, or who are anticipated to receive during the study period, any investigational agent other than those which will be administered as part of this study • Neonates with a previous anaphylactic reaction to any drug, vaccine or vaccine component. • Neonates with a significant evolving neurological disorder.

	• Neonates whose parents/guardians are site team employees with direct involvement with the investigators, or who are working on the study. • Neonates who have been exposed to immunosuppressive courses of glucocorticosteroids, cytotoxic drugs or blood products through prenatal exposure and/or breast milk in the four weeks prior to randomisation should be discussed with the Medical Monitor and sponsor and a decision on eligibility made on a case by case basis. • Neonates with diarrhoea or vomiting in the 24 hours preceding randomisation. • Neonates with any moderate or severe illness, and/or who have a temperature of ≥37.5˚C axillary/oral or ≥38˚C rectal/tympanic within the 48 hours preceding randomisation.
IP, Dose and Mode of Administration	Each active dose of RV3-BB will be given orally as 1 mL of RV3-BB Rotavirus Vaccine. The doses are as follows: • 1 x 10^7 FFU/mL • 3 x 10^6 FFU/mL • 1 x 10^6 FFU/mL Each dose of placebo will be given orally as 1 mL of cell culture medium/10% sucrose. The 2^{nd}, 3^{rd} and 4^{th} doses of IP will be preceded by a 2 ml dose of antacid solution (Mylanta Original) to neutralise stomach acidity.
Endpoints	<u>Immunogenicity will be assessed through:</u> • Serum anti-rotavirus IgA response (defined as a ≥3 fold increase from baseline) at each serum collection time-point to 4 weeks after 3 doses of RV3-BB. • Vaccine take defined as at least a threefold increase in serum anti-rotavirus immunoglobulin A (IgA) from baseline to post IP dosing, or detectable RV3 shedding in stools (by ELISA or PCR) any day from day 3 to 5 following administration of IP. • Cumulative vaccine take defined as vaccine take observed at the current assessment time point or following any previous dose. • Anti-rotavirus IgA response (defined as at least a threefold increase from baseline to post IP dosing) at each serum collection time point. • Presence of RV3 shedding in stools (by ELISA or PCR) from day 3 to 7 following each dose of IP.

	• Anti-rotavirus IgA titres defined as geometric mean titres at each serum collection time point. • Episodes of diarrhea, from the time of randomisation to 28 days following the last dose of IP. Episodes will be confirmed by Rotavirus antigen ELISA on samples. <u>Safety and tolerability</u> Safety and tolerability will be assessed through: • Unsolicited Adverse Events (AE) in the days 0-28 following each dose of IP. • Serious Adverse Events (SAE) from the first dose of IP until 28 days post last dose of IP. • Review of episodes of blood in stool. <u>Exploratory</u> Exploratory endpoints will be assessed through: • HBGA Lewis and secretor status will be determined by ELISA/PCR on saliva collected at Visits 1, 3, 5 and 7 and using whole blood at week 18 for non secretors. • Maternal serum IgA and IgG response will be determined on maternal serum sample collected pre study or at Visit 1. • Bacterial taxonomic and genetic functional profiling of stool microbiome will be conducted on maternal stool samples and participant stool samples collected post administration of IP and at birth (prior to first IP dose). • B and T cell function will be measured by flow cytometry and innate and adaptive responses measured by whole killed pathogen assay and Toll like receptor ligand assays from whole blood collected at birth (cord), Visit 3 and Visit 9.
Sample Size and Statistical Analyses	With the high titre RV3-BB neonatal vaccine schedule as the active control arm, the sample size is calculated to demonstrate the non-inferiority of the lower titre vaccine arms with respect to the proportion of participants who have a serum IgA response 4 weeks after 3 doses of vaccine. A non-inferiority margin of 20% for the difference between arms will be used. The primary analysis is based on the per protocol population defined as participants who receive all 3 doses and have no major protocol violations. Thirty percent of participants are expected to be excluded from the per protocol (PP) population due to death, study withdrawal, loss to follow-up, or study non-compliance. Among those in the PP population, a 50% response probability is assumed for the active controls. Based on a one-sided 0.025 level score test with 90% power under the alternative of no

	difference in response probabilities, 172 participants per arm are required for a total sample size of 688 participants. Non-inferiority of the lower titre vaccine will also be assessed in the intention to treat population (ITT). The cumulative probability of response by 18 weeks of age will be estimated by the Kaplan-Meier method for each vaccine arm. The difference between high and low titre arms in the cumulative probabilities and its 95% confidence interval will be calculated. If the upper bound of the confidence interval is below 20%, non-inferiority of the lower titre vaccine will be demonstrated.

3 INTRODUCTION

Investigators should be familiar with the RV3-BB Investigator's Brochure (IB).

3.1 Rotavirus disease

Rotavirus gastroenteritis results in more childhood deaths from diarrhoea than any other single agent (Vesikari, 1997). Globally, rotavirus causes approximately 215,000 deaths annually in children under five years of age. The majority (56%) of these deaths occur in Sub-Saharan Africa; with 121,000 rotavirus deaths from rotavirus diarrhoea in 2013 representing 38.9% of the global total for diarrheal deaths in this age group (Tate et al, 2016).

Children with severe rotavirus diarrhoea are at risk of dying from dehydration if they do not receive intravenous fluids urgently. In low-income countries, since many children do not readily have access to health care to administer this treatment, prevention of rotavirus diarrhoea is critically important. Primary rotavirus infection does not protect against re-infection by this ubiquitous pathogen, however, does provide protection against severe disease on re-infection (Bishop et al., 1983; Velazquez et al., 1996)

Vaccines are a key intervention for the prevention of rotavirus gastroenteritis morbidity and mortality, as the virus is largely transmitted from person to person, rather than contaminated food and water, so improvements in hygiene, sanitation and drinking water do not adequately prevent rotavirus (Glass et al., 1999; Tate et al., 2016).

3.2 The first licensed Rotavirus Vaccine

The first rotavirus vaccine licensed in the United States of America (USA) was a rhesus-human reassortant tetra-valent vaccine (Rotashield®). This was a live-attenuated vaccine given orally as a three dose regime starting in infancy and found to be highly efficacious for the prevention of severe diarrhoea and hospitalisation due to rotavirus infection (Bresee et al., 1999; Joensuu et al., 1997; Perez-Shael et al., 1997). In 1998 the American Academy of Pediatrics recommended universal immunisation of all USA infants at two, four and six months of age. However, nine months after licensure and after more than 500,000 of the 3.8-4 million USA birth cohort had received at least one dose of Rotashield®, the programme was suspended (Smith et al., 2003). This action was prompted by reports of intussusception in vaccine recipients (Bines, 2005). In October 1999, recommendations for the programme were withdrawn and the manufacturer voluntarily withdrew the vaccine (Bines, 2006).

3.3 Current Globally Licensed Rotavirus Vaccines

Since 2006, two live oral attenuated rotavirus vaccines, RotaTeq® and Rotarix®, have been globally licensed for the prevention of rotavirus gastroenteritis. Both RotaTeq® (pentavalent bovine-human reassortant vaccine: Merck/CSL) and Rotarix® (monovalent G1 human vaccine: GlaxoSmithKline) have been shown to be safe and efficacious in clinical trials of over 70,000 infants (Vesikari et al., 2006, Ruiz-Palacios et al., 2006). Since initial licensure, rotavirus vaccines have been licensed in over 100 countries and >100 million doses administered safely.

Compared to the high vaccine efficacy found in developed countries (95-100% against severe disease (Buttery and Kirkwood, 2007) rotavirus vaccine efficacy against severe rotavirus disease was found to be significantly lower in clinical trials conducted in developing countries: 49% in Malawi, 48% in Bangladesh and Vietnam and 58% in Nicaragua (Madhi et al., 2010; Zaman et al., 2010; Patel et al., 2009). The underlying reason for the suboptimal efficacy in high rotavirus disease burden countries is not yet completely understood but is suspected to be due to maternal and breast milk antibodies, characteristics of the gut microbiome, nutritional factors and genetic factors, including histo-blood group antigen (HBGA) status.

Despite the low efficacy of the licensed rotavirus vaccines in low-income countries, they have achieved a substantial reduction in severe rotavirus gastroenteritis. The phase III trial of Rotarix® conducted in both middle-income South Africa and low-income Malawi, demonstrated significant vaccine-attributable reduction in severe rotavirus episodes in the first year of life in both countries. The vaccine efficacy in Malawi of 49% was much lower than the efficacy of 77% for the same vaccine when studied in South Africa, however in Malawi the vaccine had a greater impact, preventing seven cases of severe rotavirus diarrhoea for every 100 Malawian infant-years, compared with four cases per 100 South African infant-years. The disease is transmitted year-round in Malawi and the country had a higher baseline rate of rotavirus (Madhi et al., 2010). Diarrhoea is a leading killer of children in Malawi, causing approximately 11 percent of deaths in children under five years of age before vaccine introduction (Liu et al., 2012), with rotavirus infection causing an estimated one-third of diarrheal disease hospitalizations of Malawian children under five (Cunliffe et al., 2010).

Similar effects of vaccination were demonstrated in a trial of Rotateq® in Ghana, Kenya and Mali, with an overall efficacy in the first year of life of 64% (Armah et al., 2010)

In 2009 the World Health Organisation (WHO) made the following recommendation: “Rotavirus vaccines should be included in all national immunization programmes and considered a priority, particularly in countries with high rotavirus gastroenteritis associated fatality rates, such as in south and south-eastern Asia and sub-Saharan Africa.” (Rotavirus Vaccines WHO Position Paper January 2013). This decision was based on the high

rotavirus disease burden in low-income countries and the fact that a significant number of children would be saved by a rotavirus vaccine program despite the lower vaccine efficacy observed.

Introduction of these vaccines into the routine immunisation schedule has proven effective in reducing both morbidity and mortality in early introducer countries. In Brazil, a 22–28% reduction in deaths and 21-25% reduction in hospital admissions due to rotavirus disease were found among children under two years of age (Patel et al., 2011) and in Mexican infants <11 months a 41% reduction in diarrhoea-related mortality was noted in 2008/9 after rotavirus vaccine introduction in 2007 (Richardson et al., 2010).

In October 2012, Malawi became the fourth Gavi-eligible country in Africa to introduce rotavirus vaccines into the national immunization programme. A 43% reduction in rotavirus diarrhoea hospitalisation in infants (Bar-Zeev et al., 2016) and a vaccine effectiveness of 64% (Bar-Zeev et al., 2015), have been reported following vaccine introduction in Malawi. Similarly, reductions in rotavirus diarrhoea hospitalisations in children <5y by 49% have also been observed in Ghana, after vaccine introduction in 2012; by 61-70% in Rwanda, after vaccine introduction in 2012; and by 32% in Togo, after vaccine introduction in 2014 (Armah et al., 2016; Ngabo et al., 2016; Tsolenyanu 2016).

3.4 Current locally licensed Rotavirus vaccines

In addition to the two current globally licensed vaccines, another three rotavirus vaccines are available in national markets only: Rotavac® in India; Lanzhou Lamb in China and Rotavin-M1 in Vietnam. These rotavirus vaccines have been developed by indigenous vaccine manufacturers with the primary aim to provide for their country's own National Immunisation Programs.

Rotavac® is manufactured by Bharat Biotech International Limited, licensed in India in 2014 and planned to be introduced through a phased approach into India's national immunization program, using an infant schedule, as the vaccine has not been tested in the neonatal age group. The vaccine demonstrated 56.4% efficacy in first year of life and 48.9% efficacy in the second year of life (Bhandari et al., 2014). Importantly, the projected price US$1 per dose is substantially cheaper than the current globally licensed vaccines, Rotarix® and Rotateq®. Rotavac® contains a G9P[11] human rotavirus strain.

Lanzhou Lamb Rotavirus Vaccine (LLR) is manufactured by Lanzhou Institute of Biological Products and licensed for use in China since 1998. The efficacy of LLR is yet to be established through a randomised clinical trial. LLR contains a G10P[12] lamb rotavirus strain

Rotavin-M1 vaccine is manufactured by the Center for Research and Production of Vaccines and licensed for use in Vietnam. Rotavin-M1 contains a G1P[8] human rotavirus

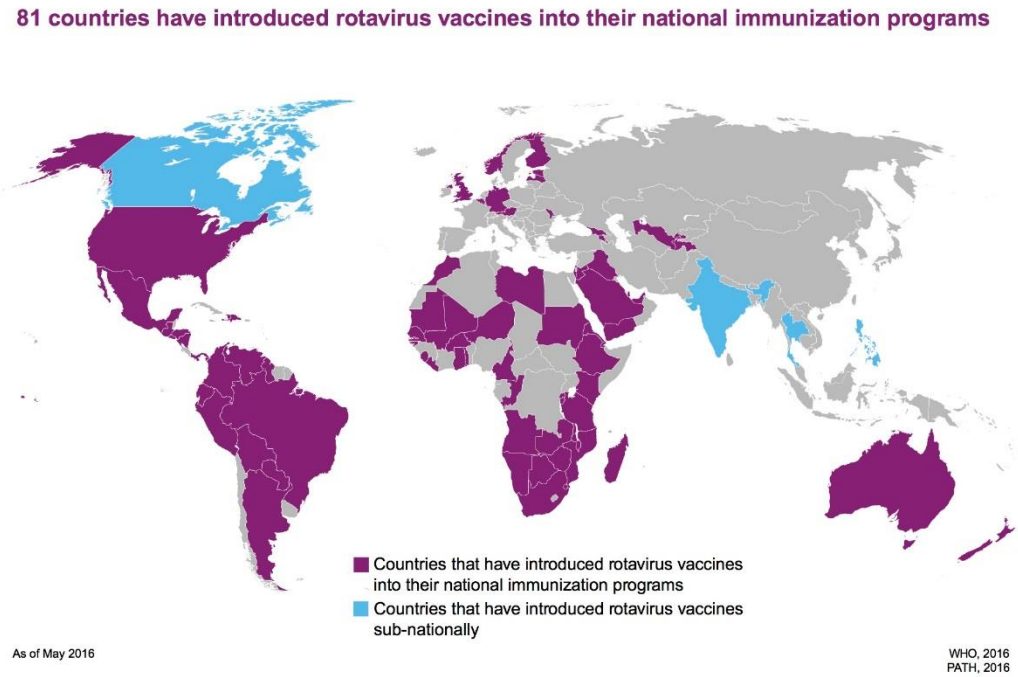
strain. The efficacy of Rotavin-M1 is yet to be established through randomised controlled trials, however immunogenicity has been demonstrated with up to 73% IgA seroconversion in infants (Dang et al., 2012).

3.5 Limitations of the current rotavirus vaccines

Despite being heralded as a major breakthrough in the prevention of rotavirus disease burden, the commercially available rotavirus vaccines have their limitations. In particular, the high costs of the current vaccines, despite tiered pricing agreements, are a major barrier to rotavirus vaccine implementation to children in developing countries.

Despite the WHO recommendation, by May 2016, only 41% countries had introduced rotavirus vaccines into their national immunization programmes, with the consequence that 94 million infants still do not have access to this vaccine. Unfortunately, countries with high child mortality in Africa and Asia have been particularly slow to introduce rotavirus vaccines (Figure 1) and therefore the promise of these vaccines for reduction in burden of disease has not fully been realised.

Figure 1. Rotavirus Vaccine Introductions.



http://rotacouncil.org/toolkit/rotavirus-burden-vaccine-introduction-map/

3.5.1 Challenges to Rotavirus Vaccine Implementation

Besides affordability and supply limitations, there are some implementation challenges associated with the current globally licensed rotavirus vaccines.

The volume of administration (Rotarix 1.5ml/dose; RotaTeq 2ml/dose) the volume required for storage and transport in the cold chain and the vaccine waste has been a major challenge for vaccine programs, particularly in remote, low resource settings. Rotavirus vaccines currently take more space in the cold chain than all other EPI vaccines combined. This has required additional resources for transportation in cool boxes and additional refrigerators to keep the vaccine at 2-8^{o}C.

Safety concerns continue despite evidence of a positive risk-benefit in all studies conducted to date. It is acknowledged that there is a four to five-fold increased risk of intussusception in the one to seven days following the first dose of the two currently licensed rotavirus vaccines (Rotarix® and RotaTeq®) (Carlin et al., 2013, Buttery et al., 2011, Patel et al., 2011). This increased risk equated to approximately one to two additional cases of intussusception among every 100,000 infants vaccinated in Australia (Therapeutic Goods Administration, 2011; do Carmo et al., 2011). WHO recommends that countries planning to introduce rotavirus vaccines into their NIPs enhance post-marketing surveillance activities in order to detect any increase in cases of intussusception that may occur after introduction. Vaccine providers are advised to inform all parents of the signs and symptoms of intussusception so that in the unlikely event of intussusception occurring post immunization, this can be identified and treated promptly.

Because the risk of intussusception is highest following the first dose of a rotavirus vaccine and is highest if the first dose is delivered late, the WHO SAGE meeting initially recommended that the first dose of a rotavirus vaccine be restricted to < 6 months of age to limit the risk of intussusception. This provided a major challenge for the implementation of rotavirus vaccines in developing countries where timely administration of the current Expanded Program on Immunisation vaccines is often not achieved and the exact age of infants may be unknown (Buttery & Graham, 2009). However, there are concerns that restrictions on the age of administration of the first and last dose of rotavirus vaccines has prevented vaccination of many vulnerable children in settings where the DTP doses are given late. In response to this concern the WHO have now relaxed age restrictions for rotavirus vaccination by allowing infants to receive rotavirus vaccines together with DTP regardless of the time of vaccination as long as this is after 6 weeks of age (WHO, 2013).

3.6 Rationale for the development of Rotavirus Vaccine RV3-BB

The RV3-BB live attenuated rotavirus vaccine has been developed from an unusual naturally occurring strain of rotavirus (RV3) found in healthy babies in obstetric hospital

nurseries in Melbourne between 1974 and 1983. Uniquely, it caused infection in babies, but did not cause disease. These babies were followed-up for three years and found to be protected against subsequent severe gastroenteritis, a natural form of immunisation (Bishop et al., 1983). This unique rotavirus strain is the basis of the RV3-BB rotavirus vaccine. The goal of the RV3-BB Rotavirus Vaccine Program is to develop an effective, affordable rotavirus vaccine aimed at prevention of rotavirus disease from birth. Administration of the RV3-BB vaccine at birth has a number of potential advantages since it may offer earlier protection for infants in developing countries than the currently licensed rotavirus vaccines, and it may offer opportunity to achieve the timely implementation of rotavirus vaccines.

The development strategy for RV3-BB involves the manufacture of the vaccine in collaboration with an emerging country vaccine manufacturer with the aim to provide a low-cost vaccine that is affordable for children in resource poor countries.

3.6.1 Advantages of the birth dose strategy

Rotavirus infection often occurs very early in life, particularly in developing countries. In Africa, 38% of hospitalisation due to rotavirus infection occurs in infants less than 6 months of age (Cunliffe, 1998). The rotavirus seropositivity rate among placebo recipients 1 month after the last dose had been given in a trial of Rotarix® was greater among Malawian infants than among South African infants (40.4% vs 16.7%) suggesting that Malawian infants have high exposure to wild-type rotavirus infection in the first 5 months of life (Cunliffe et al., 2012). The current vaccines administered from 6 weeks of age, with completion of a 2- or 3-dose course at 4–6 months may be too late to protect some infants. Neonatal vaccine administration offers the earliest possible protection.

Providing a rotavirus vaccine at birth has the potential to address sub-optimal efficacy of rotavirus vaccination in high burden countries and improve safety concerns. It also may increase the proportion of infants living in remote regions completing a full course of a rotavirus vaccine.

Birth dose administration may limit interference to vaccine take, as most newborns have a neutral gastric environment and minimal breast milk intake. The intestinal microbiota is not yet well established and intussusception is rare (Dennehy et al., 2007). Based on the unique structural and functional characteristics of neonatal rotavirus strains, it is proposed that RV3-BB is an ideal vaccine for a birth dose strategy. Natural infection with RV3 is associated with replication without symptoms but provides heterotypic protection against severe rotavirus disease for the first 3 years of life (Bishop et al., 1983). A birth dose of a rotavirus vaccine may also be safer as the natural intussusception risk is lowest in the neonatal period.

Neonatal rotavirus strains replicate well in the immature newborn intestine despite the presence of maternal and breast milk antibodies. They generally cause asymptomatic infections, but induce a protective immune response against rotavirus on re-infection (Bishop et al., 1983). The RV3-BB rotavirus vaccine based on a neonatal rotavirus strain may be the ideal candidate for a birth dose vaccine strategy.

3.6.2 Potential advantage of the P[6] genotype of RV3

The RV3-BB vaccine is the only rotavirus vaccine with a P[6]VP4 gene which may be advantageous in settings, including African countries, with a high proportion of Lewis and secretor – negative children. In US and Australia, the predominant circulating rotavirus strain are a P[8] strains. In contrast in Africa, a higher proportion of P[6] strains are observed. This could explain the lower efficacy to RotaTeq® and Rotarix®, vaccines that both have a P[8] genotype. In addition, the frequency of Lewis phenotypes varies among populations, with the Le (a-b+) most common overall, identified in 75% of Europeans and Le (a-b-) phenotype more common in African (19%) populations. In vitro studies have demonstrated that the common human rotaviruses recognize the human HBGA in a P genotype specific manner. Specifically, P[8] rotavirus strains only infected Lewis-negative and secretor-negative children, whereas rotavirus strains with a P[6] VP4 genotype infected both Lewis-positive and- negative children. This may also, at least in part, explain why there is reduced vaccine efficacy of Rotarix® and Rotateq® in populations with a high percentage of Lewis-negative individuals (Nordgren et al., 2014).

3.7 Development of RV3

RV3 is a naturally attenuated strain of rotavirus isolated from newborn infants. It is representative of a rotavirus strain that caused widespread endemic infections in newborn babies cared for in obstetrical hospital nurseries in Melbourne from 1974-1983. Asymptomatic neonatal infection was shown to be 100% protective against severe disease and 75% protective against moderate disease in the first three years of life (Bishop et al., 1983). Despite the strain being endemic in neonatal nurseries, it has never been detected in faeces from children admitted to hospital with severe gastroenteritis, despite active surveillance. The natural attenuation of this strain is also supported by the observation that RV3 disease did not occur in the siblings of infants, even if the infants were still excreting at home following discharge from hospital.

As a vaccine candidate, RV3-BB is a live attenuated vaccine being developed for administration at birth so as to prevent severe rotavirus disease in neonates and infants.

3.7.1 Immunity Conferred by RV3 Infection

RV3 is a G3P2A[6] strain containing neutralising epitopes on VP7 that react with monoclonal antibodies specific for G1, G3, G4 and G9 strains (Lazdins et al., 1995). The

strain therefore has the potential to confer "heterotypic" protection against these four serotypes, based upon *in vitro* measures of protection. In a three year infant cohort study following Melbourne infants infected as newborns, complete protection against severe rotavirus gastroenteritis was conferred by neonatal RV3 infection, observed during a period of four years when serotype G2P[4] infections comprised >80% of rotaviruses excreted by children admitted to hospital (Bishop, Unicomb & Barnes, 1991). In addition, there is circumstantial evidence that neonatal infection with RV3 conferred widespread protection against severe rotavirus diarrhoea in the community (Bishop & Barnes, 1997). The total number of Melbourne children under five years old admitted with severe diarrhoea was reduced by ~50% after 1975 when neonatal RV3 rotavirus infection was first recognised to be established in nurseries.

Humoral immune responses were measured in the Melbourne cohort of children naturally infected with RV3 at birth and followed during their first three years of life. Three months after neonatal infection with the RV3 strain, neutralising antibody to G1, G3 and G4 viruses persisted in 50-75% of sera. Asymptomatic re-infection with G1, G2 or G4 rotaviruses induced strong heterotypic responses (Ruth Bishop, unpublished). While neutralising antibody levels are not the primary serologic correlate of protection, the results support heterotypic priming of the humoral immune response by neonatal infection with a single rotavirus strain.

3.8 Development of RV3 Rotavirus Vaccine (RV3-BB)

The RV3-BB Rotavirus Vaccine has been successfully adapted to growth in the qualified Vero cell line ATTC TL-CCL-81.4. The research seed stock was prepared in a dedicated room using dedicated equipment under Good Laboratory Practice (GLP) conditions at the MCRI and transferred to Meridian Life Sciences, USA, for preparation of a master seed lot and subsequent manufacture using current Good Manufacturing Practices (cGMP).

In a preclinical oral toxicity study, conducted under GLP conditions, in juvenile rats, RV3-BB was well tolerated throughout the duration of the study with no clinical signs considered to be treatment related. Details of this study are provided in the IB.

3.8.1 Phase I clinical study of RV3-BB

This single-centre, double-blind, randomised placebo-controlled study evaluated the safety and tolerability of a single 1mL oral dose of RV3-BB in a total of 60 participants across three age groups (Protocol MCRI-RV3-BB-001). The study was completed in 2011 in Melbourne, Australia.

A single dose of vaccine or placebo was administered initially to 20 adult males aged 18-50 years (Cohort 1), progressing to 20 children aged 3-8 years (Cohort 2), then to 20 infants aged 6-8 weeks (Cohort 3). Each cohort included 10 vaccine and 10 placebo recipients.

The results demonstrate that the single dose of vaccine was well tolerated in adults, children and infants (Danchin et al., 2013). Evaluation of vital signs revealed no clinically significant changes in any participant. There were no clinically significant abnormalities in haematology. Clinically significant increases in serum ALT above the upper limit of normal (ULN) were reported at the Day 28 time point for 5 participants in cohort 3 (4/10 vaccine, 1/10 placebo). All ALT abnormalities had resolved on follow up with no evidence of long-term consequences. No AEs were considered to have a definite or probable relationship to the study vaccine. One SAE was reported in Cohort 3; this was an episode of pneumonia in the vaccine group that was assessed by an independent physician as unrelated to the IP.

Vaccine response was a secondary objective in this trial, although it was expected that there would be limited immune response following a single dose of vaccine. In Cohort 3 a serum immune response (>3-fold increase in SNA and/or IgA from baseline) was identified in 5/9 infants receiving RV3-BB (56%) compared to 2/7 placebo recipients (28%). A vaccine response, defined as evidence of RV3-BB -BB rotavirus replication detected in stool between day 3-6 and/or response of serum IgA or SNA, was observed in cohort 3 in 8/9 infants receiving RV3-BB (89%) and 2/7 placebo recipients (28%). The data from the Phase I trial of RV3-BB are promising in that excretion consistent with viral replication occurred in the majority of infants after a single dose of RV3-BB.

3.8.2 Phase IIa immunogenicity study of RV3-BB

A phase IIa double-blind, randomised, placebo controlled study (Protocol MCRI-RV3-BB-002) in 95 participants was completed in New Zealand in 2014. This was a study of the immunogenicity, safety, tolerability and reactogenicity of three doses of oral RV3-BB Rotavirus Vaccine (8.3×10^6 FFU/mL), with the first dose of vaccine administered either at birth (0-5 days of age) or in infancy (8 weeks of age). The results suggest that RV3-BB vaccine administered as three 1 mL oral doses was immunogenic and well tolerated (Bines et al., 2015). For both the Neonatal and Infant Vaccine schedules, solicited and unsolicited AE were similar across treatment groups and there was no evidence to suggest a relationship between the frequency of the events and treatment assignment. There were no fatal SAE.

Immunogenicity was the primary outcome and was measured by vaccine take after any of the three doses of RV3-BB vaccine. Vaccine take was defined as ≥3 fold increase in serum anti-rotavirus IgA or SNA (at 28 days post dose) compared with baseline, and/or RV3-BB excretion in stool (measured by RT-PCR on days 3-7 post dose). Over 90% of infants receiving RV3-BB as infant or neonate schedule had evidence of vaccine take compared with 13% (neonatal:placebo comparison) and 25% (infant:placebo comparison).

For more information refer to the Investigators Brochure.

3.8.3 Phase IIb efficacy study of RV3-BB

A phase IIb double-blind, randomised, placebo controlled study (Protocol MCRI-RV3-BB-003) of 1649 participants was completed in Indonesia in 2016. Participants received 3 doses of RV3-BB vaccine as a neonate or an infant schedule, or placebo, and were followed to 18 months of age. This study tested the hypotheses that RV3-BB Rotavirus Vaccine induces protective efficacy when given as either an infant or as a neonatal schedule, in a developing country setting.

Two sub-studies were included: Sub-study A assessed immunogenicity of RV3-BB when co-administered with the routine childhood vaccines, including inactivated polio vaccine (IPV), and Sub-study B assessed immunogenicity of oral polio virus (OPV) when co-administered with RV3-BB. Limited biochemistry parameters were also assessed in a subset of 90 participants in each Sub-study.

The primary outcome of the study was to assess the efficacy of three scheduled oral doses of RV3-BB vaccine in neonates and in infants against severe rotavirus gastroenteritis, up to 18 months of age, compared with placebo. This was measured using episodes of severe rotavirus gastroenteritis (defined as a modified Vesikari score ≥11 and rotavirus antigen detected in stool by ELISA) from 2 weeks after 4 doses of IP product to 18 months of age.

Severe rotavirus gastroenteritis in the period from 2 weeks after the fourth dose of IP to 18 months was identified in 28 of 504 participants in the placebo group (5.6%) as compared to 21 of 1009 participants in the combined vaccine group (2.1%), resulting in a vaccine efficacy of RV3-BB against the primary outcome of severe rotavirus gastroenteritis of 63% (95% confidence interval [CI], 34 to 80; $p<0.001$). When assessed with respect to each administration schedule, three doses of RV3-BB administered in the neonatal schedule was associated with a vaccine efficacy against severe rotavirus gastroenteritis from 2 weeks post dose three to 18 months of age of 75% (95% CI 44 to 91; $p<0.001$) and against any severity rotavirus gastroenteritis of 63% (95% CI 36 to 81; $p<0.001$). Three doses of RV3-BB vaccine administered in the infant schedule was associated with a vaccine efficacy of 51% (95% CI 7 to 76; $p=0.027$) severe rotavirus gastroenteritis from 2 weeks post dose four to 18 months of age. RV3-BB vaccine demonstrated efficacy across the spectrum of severity of rotavirus gastroenteritis. Of note, only 2 episodes of very severe rotavirus gastroenteritis (Vesikari score ≥ 15) occurred in participants in the neonatal vaccine schedule group compared to 14 in the placebo group suggesting that the vaccine may particularly offer protection from the severest form of the disease. Of the 49 episodes of severe rotavirus gastroenteritis, 46 were due to the strain G3 P[8] (neonatal vaccine group 7/7 G3 P[8]; infant vaccine group 13/14 G3 P[8] and 1 G3 P untypable; placebo group 26/28 G3 P[8]; 2 P[8] untypable).

Cumulative vaccine take following three doses of vaccine was identified in 78/83 (94%) of participants in the neonatal vaccine schedule and 83/84 (99%) of participants in the infant vaccine schedule in the PP population. Although cumulative vaccine take was identified in 42% of the neonatal placebo schedule and 47% of the infant placebo schedule, there was a

significant difference in proportions between vaccine and placebo groups (neonatal vaccine schedule versus neonatal placebo schedule 0.52 [0.39, 0.64]: p<0.001; infant vaccine schedule versus infants placebo schedule 0.52 [0.40, 0.63]: p<0.001). After two doses in the infant vaccine schedule, cumulative vaccine take was identified in 73/84 (87%) compared to the infant placebo schedule in 22/79 (28%) (difference in proportions 0.59 [0.45, 0.71]: p<0.001).

For both the Neonatal and Infant Vaccine schedules, SAE and non-serious solicited and unsolicited AE were similar between each vaccine schedule group compared with placebo and there was no evidence to suggest a relationship between the frequency of the events and treatment assignment.

General disorders and administration site conditions were the most frequently reported solicited AEs; events included decreased activity, fever and irritable. Gastrointestinal disorders were also reported including diarrhoea, loose stools and vomiting. These events occur with similar frequency across all study groups (neonatal vaccine 16.7% vs neonatal placebo group 16.9%; infant vaccine group 15.6% vs infant placebo group 18.4%).

There were 11 fatal events reported throughout the study period; ten events assigned as unrelated by the Investigator and one (neonatal aspiration; placebo group) was assigned as unlikely related to IP. Six fatal events occurred in participants in the placebo group and five occurred in participants in the vaccine group.

A single case of intussusception was reported in a participant in the infant vaccine schedule group. This SAE was reported 114 days after the last dose of IP and was assessed as not related to IP. The participant was successfully treated with surgery and recovered without sequelae.

In summary, this Phase IIb study has demonstrated that administration of the RV3-BB vaccine (8.3 x 106 FFU/mL) was well tolerated, immunogenic and efficacious in infants when given as an infant or neonatal schedule. The data from this Phase IIb trial of the RV3-BB vaccine support continued development of the vaccine.

For more information refer to the Investigators Brochure.

3.9 Dose and study rationale for current study

This dose-ranging study of the RV3-BB vaccine in Malawi - a low income, high rotavirus disease burden region in Africa - aims to address an important “missing piece” in the development of the RV3-BB vaccine.

This study will provide important information about the optimal titre of the RV3-BB vaccine required to produce an immune response needed to protect against severe rotavirus disease. This data will inform vaccine development and ensure that the vaccine is produced

efficiently and at the lowest possible cost by influencing the manufacturing costs and capacity. This study will also inform the ultimate dosage schedule of the RV3-BB vaccine.

This project will address 3 key elements that are central to the development of the RV3-BB vaccine:

1. *Can a lower titre of the RV3-BB vaccine (administered in a 3 doses schedule) produce an immune response that predicts protection from severe rotavirus disease?*

This information is critical to inform vaccine development. The titre of the RV3-BB vaccine used in the Phase I, IIa and IIb trials has been ~$8.3x10^6$. Reducing the titre of the final commercial vaccine would improve efficiencies in the manufacturing process as well as reduce cost of goods (the variable costs associated with each dose of vaccine) – impacting on final vaccine cost and vaccine manufacturing capacity.

Even in highly competitive markets or tiered pricing arrangements, vaccine prices cannot fall below the floor which is set by manufacturing costs. Reducing the manufacturing costs significantly therefore has great potential to boost affordability of RV3-BB rotavirus vaccine, which will facilitate greater access to protection for more children globally, while also assisting an RV3-BB vaccine manufacturer to develop a sustainable business model for the vaccine.

2. *Is a neonatal administration schedule of the RV3-BB vaccine (first dose 0-5 days of birth) associated with a similar immune response to an infant schedule of the RV3-BB vaccine (first dose delivered at 6 weeks) in infants in Africa?*

There are a number of potential advantages of a birth dose strategy for a rotavirus vaccine, particularly in high burden regions where there are often challenges to ensuring complete vaccine coverage of a 3 dose infant vaccine schedule.

The administration of vaccines under an infant schedule, with completion of a 2- or 3-dose course at 4–6 months may be too late to protect some infants. Neonatal vaccine administration may offer the earliest possible protection.

The intrinsic characteristics of the RV3-BB vaccine may make it an ideal vaccine to deliver in a birth dose administration schedule. However, some countries may still prefer an infant dose schedule. Understanding how RV3-BB performs in a birth dose schedule or an infant schedule will assist the clinical development required for the regulatory submission and define the ideal schedule/s for the RV3-BB vaccine.

3. *Does RV3-BB rotavirus vaccine with a P[6]VP4 gene offer specific advantages in infants in Africa?*

The RV3-BB vaccine is the only rotavirus vaccine with a P[6]VP4 gene which may be advantageous in settings, including African countries with a high proportion of Lewis and secretor – negative children

With the high burden of rotavirus disease in low-income countries like Malawi, even modest gains in efficacy through alternative vaccine such as RV3-BB could have significant public health impact.

4 STUDY OBJECTIVES

4.1 Primary Objective

The primary objective is to assess the cumulative anti-rotavirus serum IgA response (defined as a ≥3 fold increase from baseline) 4 weeks after 3 doses of RV3-BB administered in a neonatal schedule at a vaccine titre of $1x10^7$, 3×10^6 or 1×10^6.

4.2 Secondary Objectives

The secondary objectives of the study are;

- To assess the cumulative anti-rotavirus serum IgA response (defined as a ≥3 fold increase from baseline) 4 weeks after 3 doses of RV3-BB administered in an infant schedule at a vaccine titre of 1×10^7.
- To assess cumulative vaccine take and components of vaccine take after 3 doses of RV3-BB (titre of 1×10^7) administered in a neonatal schedule versus three doses of RV3-BB in an infant schedule at a vaccine titre of 1×10^7.
- To assess cumulative vaccine take and the components of RV3-BB vaccine take after 3 doses of RV3-BB administered as a neonatal schedule at a vaccine titre of 1×10^7, 3×10^6 or 1×10^6.

  To assess cumulative vaccine take and the components of vaccine take after 2 doses of RV3-BB administered as a neonatal schedule at a vaccine titre of 1×10^7, 3×10^6 or 1×10^6 or 2 doses in the infant schedule at a vaccine titre of 1×10^7.
- To assess cumulative vaccine take and components of vaccine take after the first dose of RV3-BB administered as a neonatal schedule at a vaccine titre of 1×10^7, 3×10^6 or 1×10^6 compared to placebo (1^{st} dose of IP in the infant schedule).
- To assess cumulative vaccine take and components of vaccine take after the first dose of RV3-BB at a vaccine titre of 1×10^7, 3×10^6 or 1×10^6 in the neonatal schedule compared with the first dose of RV3-BB at a vaccine titre of 1×10^7 in the infant schedule.
- To describe the geometric mean titre of the anti-rotavirus serum IgA response after 3 doses of RV3-BB administered as a neonatal or an infant schedule
- To describe the safety and tolerability of RV3-BB when administered as an infant or as a neonatal schedule.
- To describe the occurrence of diarrhea episodes in participants, according to severity and detection of wild-type rotavirus.

4.3 Exploratory Objectives

The basis of the exploratory objectives is to further understand potential barriers to rotavirus vaccine efficacy in the region. The exploratory objectives are;

- To describe Histo-blood Group Antigens (Lewis and secretor) status of participants in association with anti-rotavirus sero-conversion and cumulative vaccine take after 3 doses RV3-BB administered in either the neonatal schedule (any titre) or infant schedule.
- To assess maternal anti-rotavirus IgA and IgG levels in association with anti-rotavirus IgA seroconversion and cumulative vaccine take in participants following receipt of RV3-BB administered in either the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of 1×10^7.
- To describe the gut microbiome in participants receiving RV3-BB administered in the neonatal schedule (at any vaccine titre) or infant schedule at a vaccine titre of 1×10^7.
- To describe innate and adaptive immune responses in infants vaccinated with RV3-BB administered in a neonatal vaccine schedule (any titre) or in the infant schedule at a vaccine titre of 1×10^7.

5 TRIAL PLAN

5.1 Study Design

This is a Phase II, randomised, double-blind, placebo-controlled, four-arm parallel group study of two different dosing schedules of oral RV3-BB, and placebo.

Approximately 688 participants will be randomised to the study; 172 to each treatment arm.

Pregnant women will be invited to provide preliminary consent for the study prior to labour and delivery. A maternal blood sample and a stool sample will be collected. Following delivery, those who remain interested will be invited to provide written informed consent for their baby to participate in the study.

Following confirmation of eligibility for the trial post-birth, participants will be randomised in a 1:1:1:1 ratio to one of four treatment arms; high-dose, mid-dose or low-dose neonatal schedule, or a high-dose infant schedule. Each study participant will receive four oral doses of IP, with each administration consisting of 1 mL RV3-BB Rotavirus Vaccine or 1mL of placebo. All participants will receive a total of 3 doses of RV3-BB vaccine, one dose of placebo, and one dose of Rotarix®.

A baseline stool sample will be collected prior to the administration of the first dose of IP. A stool sample will also be collected on either Day 3, 4 or 5 following each dose of IP. Samples will also be collected for any cases of diarrhoea (defined as 3 or more stools that are looser than normal for that participant).

A saliva sample will be collected at birth and prior to IP doses 2, 3 and 4.

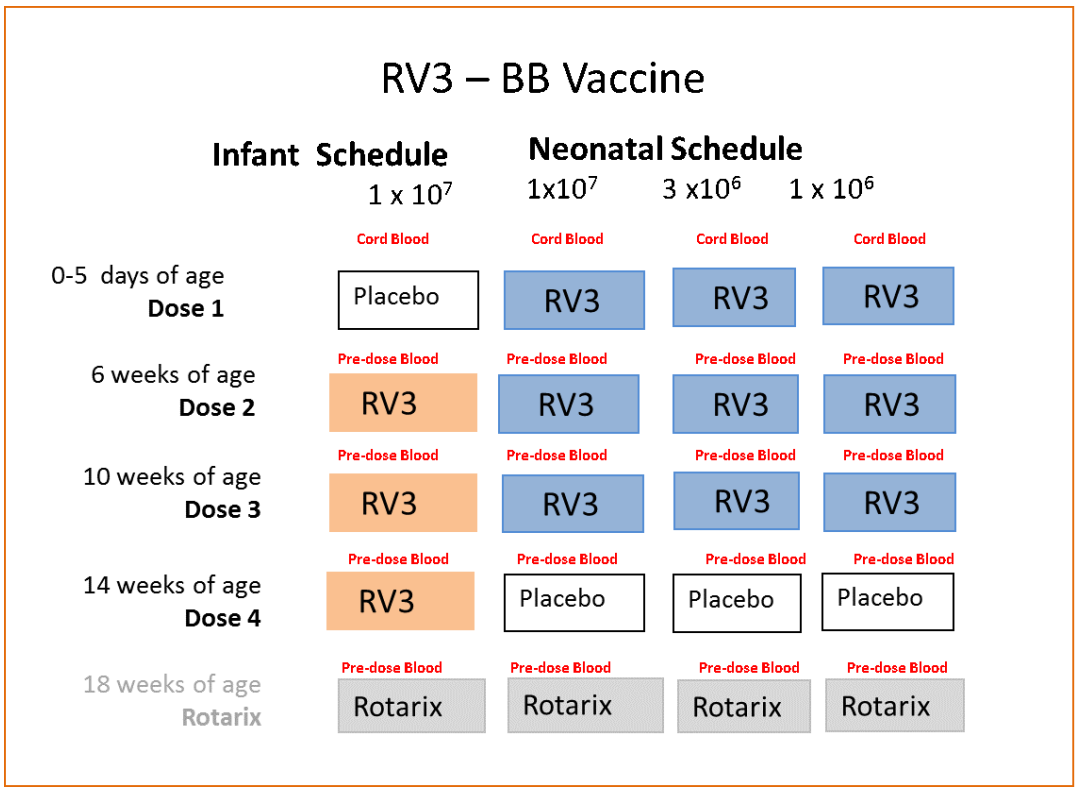
Cord blood will be collected at birth, and blood samples taken prior to IP doses 1 (if cord blood not collected) 2, 3 and 4, and prior to the Rotarix® dose.

Participants will be followed until 18 weeks of age. Face to face visits will be conducted at least monthly. Adverse events, including blood-in-stool occurrences, and diarrhoea episodes will be recorded for the duration of the study.

The recruitment period is estimated at 9 months. The study period for each participant will be 18 weeks, and the total duration of the study approximately 15 months.

An independent Data Safety Monitoring Board (DSMB) will review safety data at agreed recruitment and progression milestones throughout the study, as agreed and documented in the DSMB Charter. The DSMB will recommend whether the study continues unchanged, continues with amendments, or is stopped. Recruitment will continue during the DSMB review periods.

Figure 2. Study Design



5.2 Study Population

5.2.1 Number of Participants and Study Sites

Approximately 688 babies will be randomised across approximately four sites in Malawi. Study visits will be conducted at the sites or at participant homes.

5.2.2 Trial Selection Record

Investigators must keep a record of those who were considered for the study but were not enrolled. This information is necessary to establish that the patient population was selected without bias.

Only babies who meet all of the inclusion and none of the exclusion criteria will be eligible to participate in the study. No protocol waivers for eligibility will be allowed.

5.2.3 Inclusion Criteria

- Neonate is less than 6 days (≤144 hours) of age at the time of first dose.
- Neonate is in good health as determined by clinical judgment, including a medical history and physical exam, which confirms the absence of a current or past disease state considered significant by the investigator.
- Neonate birth weight 2500-4000g inclusive or 2250-4000g inclusive at Visit 1.
- Neonate's parents/guardians expect to be available for the duration of the study, and agree to adhere to all protocol requirements.
- Neonate's parents/guardians have provided written informed consent prior to study-related procedures being performed.

5.2.4 Exclusion Criteria

- Any medical, psychiatric, or social condition of a parent/guardian that in the opinion of the investigator would prevent the neonate's parents/guardians from giving proper informed consent or from complying with the study protocol.
- Neonates with known or suspected major congenital malformations or genetically determined disease.
- Neonates with intussusception.
- Neonates with a known or suspected bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
- Neonates who have ever received any blood products, including immunoglobulin, or for whom receipt of any blood product during the course of the study is anticipated.
- Neonates in whom EPI vaccines or components are contraindicated.
- Neonates who have received or who expect to receive during the study period, any rotavirus vaccine other than those which will be administered as part of this study.
- Neonates who have ever received, or who are anticipated to receive during the study period, any investigational agent other than those which will be administered as part of this study.

- Neonates with a previous anaphylactic reaction to any drug, vaccine or vaccine component.
- Neonates with a significant evolving neurological disorder.
- Neonates whose parents/guardians are site team employees with direct involvement with the investigators, or who are working on the study.
- Neonates who have been exposed to immunosuppressive courses of glucocorticosteroids, cytotoxic drugs or blood products through prenatal exposure and/or breast milk in the four weeks prior to randomisation should be discussed with the Medical Monitor and sponsor and a decision on eligibility made on a case by case basis.
- Neonates with diarrhoea or vomiting in the 24 hours preceding randomisation.
- Neonates with any moderate or severe illness, and/or who have a temperature of ≥37.5˚C axillary/oral or ≥38˚C rectal/tympanic within the 48 hours preceding randomisation.

5.3 Screening failures

A screening failure is a participant for whom informed consent is obtained and documented in writing from the legally authorised representative, but who is deemed ineligible for the trial and is therefore not randomised. Reasons for screening failure include conditions detected on history or during the screening examination that deem the participant ineligible.

Records will be kept of screening failures, including reasons.

5.4 Concomitant medications and vaccines

The following medications are contraindicated for use at any time during the study period:

- Immunosuppressive therapy, including immunosuppressive courses of systemic corticosteroids
- Immunoglobulins (antibodies) or other blood products;
- Other investigational products
- Other rotavirus vaccines;
- Aspirin or aspirin-containing medications
- Medications that may affect the liver, for example, chloramphenicol including eye drops. A list of other drugs will be provided in the Study Reference Manual (SRM).

If a contraindicated medication is administered, future IP dosing of the participant will be decided by MCRI (or designee) on a case-by-case basis.

All participants will receive EPI vaccines during the study at scheduled time points. Standard vaccines approved for use in Malawi will be administered by study staff during the study but will be provided via usual channels, not provided by the study. The exceptions are polio vaccine, which will be provided by the study as IPV rather than the usual OPV, and Rotarix® which will only be administered at Visit 9.

6 TRIAL PROCEDURES

Table 1: Schedule of Procedures

Visit	Pre-study	1	2	3	4	5	6	7	8	9[9]
Timing	Second trimester to birth	Day 1	Day 8	Week 6	Week 7	Week 10	Week 11	Week 14	Week 15	Week 18
Window		Birth to 144 hrs old	7±1 days post V1	+7 days	7±1 days post V3	±7 days and at least 21 days post V3	7±1 days post V5	±7 days and at least 21 days post V5	7±1 days post V7	±7 days and at least 21 days post V7
Preliminary consent	X									
Cord blood collection	X									
Maternal blood sample	X									
Maternal stool sample	X									
Study consent		X								
Eligibility confirmation		X								
Demographics and medical history		X								
Physical exam		X								
Vital signs[1]		X	X	X	X	X	X	X	X	X
Stool sample	X[5]	X[6]		X[6]		X[6]		X[6]		
Saliva sample		X		X		X		X		
Randomisation		X								
IP administration		X		X		X		X		
Antacid administration[2]				X		X		X		
EPI vaccine administration[9]		X		X		X		X		X
IPV administration				X		X		X		
Rotarix® administration										X[7]
Pre dose blood sample		X[10]		X		X		X		X
Issue/review diarrhoea log[3]		X		X		X		X		X
Issue/review study reminder Card[3]		X		X		X		X		X
Issue stool sample kits[3]		X		X		X		X		
Feeding review[4]		X	X	X	X	X	X	X	X	X
AE recording		X	X	X	X	X	X	X	X	X

Con med recording		X	X	X	X	X	X	X	X	X
Follow up contacts			Weekly contact (clinic, home or telephone) throughout the study							

1. Temperature, pulse rate, respiratory rate. At dosing visits vital signs will be measured within one hour pre dose and 15 and 30 mins post dose.
2. 2mL of Mylanta Original® will be administered within the 10 minutes prior to the IP dose.
3. Logs, reminder card and kits will be issued and reviewed as required.
4. Record if baby receiving breast milk, formula, or other.
5. A pre dose stool sample will be collected at any time from birth before the first dose of IP.
6. A stool sample will be collected 3 – 5 days post IP dose.
7. If the participant withdraws prior to receiving all IP doses they will be given a Rotarix® dose one month following their last dose of IP.
8. Day 1 pre dose sample will be cord blood.
9. EPI vaccines will be provided via usual supplier (not study) but may be administered by study staff at appropriate time points according to standard schedule. The exceptions are OPV and other rotavirus vaccines, which will not be given (see Section 5.4).
10. A 1.0mL blood sample will be collected if cord blood is not able to be collected.

6.1 Recruitment

Sites will have information about the study on display and pregnant women will be identified at the time of antenatal care. During the second or third trimester, pregnant women will be approached by a member of the study team who will describe the study to them, provide written information and, if they are interested, arrange to discuss the study in more detail at a later time. Expectant women will be encouraged to discuss the study with their partner, usual doctor, midwife, and family members.

6.1.1 Informed Consent

The investigator or delegate must obtain the written informed consent according to local laws and requirements before any study-related procedures are performed, including any study specific screening procedures.

A two-stage consent process will be followed:

- Pregnant women who are interested in participating will be invited to provide preliminary consent during the second or third trimester and prior to going into labour. This consent is to allow a pre-birth maternal blood sample (taken any time from consent until Visit 1), collection of cord blood at the birth, and collection of their baby's stool (taken from birth up to Visit 1). Consent for a stool sample from the pregnant woman will also be requested.
- Post birth, the parents/guardians will be invited to provide consent for their baby to participate in the study.

6.2 Birth Procedures

An approximate 2.5 mL cord blood sample will be taken immediately following delivery, after the umbilical cord has been cut. A baseline stool sample will also be collected from the baby. Inability to collect a baseline stool sample does not exclude the baby from further trial participation.

If not previously obtained, a maternal blood sample (approximately 1mL) will also be collected. Inability to collect a maternal blood sample does not exclude the baby from further trial participation.

If not previously obtained, a maternal stool sample will also be collected. Inability to collect a maternal stool sample does not exclude the baby from further trial participation

6.3 Visit 1 Procedures (0-5 days of age)

After consent for the baby to participate in the study is obtained, the following will be conducted/recorded at any time up to and including 5 days (144 hours) of age;

- Demographic data including date of birth, gender, ethnicity, gestational age and birth weight, current weight and height/length.
- Medical history.
- Feeding status (breast milk and/or formula).

- All medications, supplements, traditional/complementary/herbal products and immunisations administered to the baby since birth.
- All medications, supplements, traditional/complementary/herbal products and immunisations administered to the mother from 4 weeks pre-delivery.
- Physical examination (see section 8.1).
- Vital signs (see section 8.3).
- Collection of a pre dose stool sample from the baby (inability to collect a stool sample does not exclude the baby from further trial participation).
- Collection of a saliva sample from the baby.
- Collection of a 1.0mL pre dose blood sample from the baby (when cord blood is not collected)

Confirmation of eligibility must be completed within the 24 hours prior to administration of the first dose of IP. If the administration of the dose is delayed, assessments must be repeated.

Eligible participants will be randomised and the following procedures performed pre dose;

- Vital signs (see section 8.3) within the 60 minutes pre dose.

IP will be administered. A dose of 1 ml will be given orally from a syringe (see section 10.9).

The following procedures will be performed post dose;

- Administer any scheduled EPI vaccines other than OPV and rotavirus vaccines according to local guidelines (see section 9.2).
- Vital signs (see section 8.3) will be measured at 15 minutes and 30 minutes post dose (±5 minutes).
- Record any AE.
- Issue the parents/guardians with the Study reminder card and provide instructions for its completion (see section 8.3).
- Issue the parents/guardians with the Diarrhoea Log and provide instructions for its completion (see section 8.4) and for notifying the site of any episodes of diarrhoea.
- Issue kits and containers for stool and diarrhoea samples, and instructions for collection and storage of samples (see section 8.4).
- Explain the definition of diarrhoea (three or more stools in a 24 hour period that are looser than normal for that child).
- Issue thermometer and instructions for measuring temperature (see section 8.3) and for notifying the study staff if the participant develops a temperature ≥37.5°C.
- Remind the parents/guardians of potential AEs and to contact the study staff immediately if any illness or hospitalisation occurs during the study period.

Study staff will contact the parents/guardians (clinic or home visit) the day following dosing, and on day 3, 4 or 5 following dosing, to review AEs and medications, and collect stool and diarrhoea samples.

6.4 Visit 2 (7 ±1 days after Visit 1)

The following will be performed/recorded:

- Vital signs (see section 8.3).
- Review AEs.
- Review concomitant medications (participant, and mother if breastfeeding).
- Review feeding status.
- Review Diarrhoea Log and study reminder card.

Throughout the remainder of the study, study staff will contact the parents/guardians at least weekly via telephone, home visit, or clinic visit, to review AEs, medications, and diarrhoea episodes. If the baby has a diarrhoea episode a sample will be collected.

6.5 Visit 3 (Week 6 +7 days)

The following will be performed/recorded at the trial site no earlier than 6 weeks of age:

- Review AEs.
- Review concomitant medication.
- Review feeding status.
- Review Diarrhoea Log and study reminder card.
- Collect a pre dose blood sample.
- Collect a saliva sample.
- Vital signs (see section 8.3) within the 60 minutes pre dose.
- Administer 2mL of Mylanta Original® antacid formulation within the 10 minutes pre dose.
- Administer the IP dose.

The following procedures will be performed post dose;

- Administer IPV and any scheduled EPI vaccines other than OPV and rotavirus vaccines according to local guidelines (see section 9.2).
- Vital signs (see section 8.3) will be measured at 15 minutes and 30 minutes post dose (±5 minutes).
- Record any AEs.
- Issue kits and containers for stool and diarrhoea samples, and instructions for collection and storage of samples (see section 8.4).

Study staff will contact the parents/guardians (clinic or home visit) the day following dosing, and once on day 3, 4 or 5 following dosing, to review AEs and medications, and collect stool and diarrhoea samples.

6.6 Visit 4 Procedure (7 ±1 days after Visit 3)

The following will be performed/recorded:

- Vital signs (see section 8.3).
- Review AEs.
- Review concomitant medications (participant, and mother if breastfeeding).

- Review feeding status.

6.7 Visit 5 (Week 10 ±7 days)

The following will be performed/recorded at the trial site, no sooner than 21 days after the previous dose of IP (Visit 3):

- Review AEs.
- Review concomitant medication.
- Review feeding status.
- Review Diarrhoea Log and study reminder card.
- Collect a pre dose blood sample.
- Collect a saliva sample.
- Vital signs (see section 8.3) within the 60 minutes pre dose.
- Administer 2mL of Mylanta Original® antacid formulation within the 10 minutes pre dose.
- Administer the IP dose.

The following procedures will be performed post dose;

- Administer IPV and any scheduled EPI vaccines other than OPV and rotavirus vaccines according to local guidelines (see section 9.2).
- Vital signs (see section 8.3) will be measured at 15 minutes and 30 minutes post dose (±5 minutes).
- Record any AEs.
- Issue kits and containers for stool and diarrhoea samples, and instructions for collection and storage of samples (see section 8.4).

Study staff will contact the parents/guardians (clinic or home visit) the day following dosing, and once on day 3, 4 or 5 following dosing, to review AEs and medications, and collect stool and diarrhoea samples.

6.8 Visit 6 (7 ±1 days after Visit 5)

The following will be performed/recorded:

- Vital signs (see section 8.3).
- Review AEs.
- Review concomitant medications (participant, and mother if breastfeeding).
- Review feeding status.

6.9 Visit 7 (Week 14 ±7 days)

The following will be performed/recorded at the trial site, no sooner than 21 days after the previous dose of IP (Visit 5):

- Review AEs.
- Review concomitant medication.
- Review feeding status.

- Review Diarrhoea Log and study reminder card.
- Collect a pre dose blood sample
- Collect a saliva sample
- Vital signs (see section 8.3) within the 60 minutes pre dose.
- Administer 2mL of Mylanta Original® antacid formulation within the 10 minutes pre dose.
- Administer the IP dose.

The following procedures will be performed post dose;

- Administer IPV and any scheduled EPI vaccines other than OPV and rotavirus vaccines according to local guidelines (see section 9.2).
- Vital signs (see section 8.3) will be measured at 15 minutes and 30 minutes post dose (±5 minutes).
- Record any AE.
- Issue kits and containers for stool and diarrhoea samples, and instructions for collection and storage of samples (see section 8.4).

Study staff will contact the parents/guardians (clinic or home visit) the day following dosing, and once on day 3, 4 or 5 following dosing, to review AEs and medications, and collect stool and diarrhoea samples.

6.10 Visit 8 (7 ±1 days after Visit 7)

The following will be performed/recorded:

- Vital signs (see section 8.3).
- Review AEs.
- Review concomitant medications (participant, and mother if breastfeeding).
- Review feeding status.

6.11 Visit 9 (Week 18 ±7 days)

The following will be performed/recorded at the trial site, no sooner than 21 days after the previous dose of IP (Visit 7):

- Review AEs.
- Review concomitant medication.
- Review feeding status.
- Review Diarrhoea Log.
- Collect a pre dose blood sample.
- Vital signs (see section 8.3) within the 60 minutes pre dose.
- Administer Rotarix®.
- Administer any scheduled EPI vaccines according to local guidelines (see section 9.2).

Study staff will contact the parents/guardians (clinic or home visit) the day following dosing.

6.12 Discontinuation of Participants

6.12.1 Criteria for discontinuation

The participant may be discontinued from the trial at any time at the discretion of the investigator(s).

Specific reasons for withholding further doses or discontinuing a participant in this trial may include:

- Withdrawal of informed consent by parents/guardians.
- Participant lost to follow-up.
- Protocol non-compliance.
- Investigator considers that it is not in the interest of the participant to continue.
- MCRI terminates the study for administrative, financial or other reasons.

If a participant is discontinued from dosing, unless they withdraw consent they should continue to have blood samples collected at the scheduled time points and continue to collect diarrhoea samples and complete the Diarrhoea Log. Investigators are encouraged to discuss possible treatment discontinuations and withdrawals with the Medical Monitor, if appropriate, prior to ceasing treatment or withdrawing the infant. The decision to replace withdrawn subjects will be made on a case by case basis. Participants withdrawn due to AEs will not be replaced.

All withdrawn participants should be seen and assessed approximately 28 days after their last dose of IP, primarily for the purposes of monitoring participant safety. Wherever possible, AEs should be followed up until resolution. Parents/guardians who withdraw consent should be asked whether they agree to this follow up.

Participants who withdraw from study or IP will continue to receive IPV until the course is complete. They will receive Rotarix® at standard scheduled time points according to the National Immunisation Program.

7 TRIAL ENDPOINTS

Immunogenicity will be assessed through:

- Serum anti-rotavirus IgA response (defined as a ≥3 fold increase from baseline) at each serum collection time-point to 4 weeks after 3 doses of RV3-BB.
- Serum anti-rotavirus IgA levels at each serum collection time point.
- Vaccine take defined as at least a threefold increase in serum anti-rotavirus immunoglobulin A (IgA) from baseline to post IP dosing, or detectable RV3 shedding in stools (by ELISA or PCR) any day from day three to day five following administration of IP.
- Cumulative vaccine take defined as vaccine take observed at the current assessment time point or following any previous dose.
- Anti-rotavirus IgA response (defined as at least a threefold increase from baseline to post IP dosing) at each serum collection time point.
- Anti-rotavirus IgA titres defined as geometric mean titres at each serum collection time point.
- Presence of RV3 shedding in stools (by ELISA or PCR) from day 3 to day 5 following each dose of IP.

Safety and tolerability will be assessed through:

- Adverse Events (AE) in the 28 days following each dose of IP.
- Serious Adverse Events (SAE) during the study.
- Review of episodes of blood in stool.

The exploratory objectives will be assessed through:

- HBGA Lewis and secretor status will be determined by ELISA/PCR on Saliva collected at Visits 1, 3, 5 and 7 and whole blood collected at Visit 9 for non secretor participants.
- Maternal serum IgA and IgG responses will be determined by ELISA on Maternal Serum sample collected pre study or at Visit 1.
- Bacterial taxonomic and genetic functional profiling of stool microbiome will be conducted by 16s rRNA or shotgun sequencing on maternal stool and stool samples collected post administration of IP and at birth (prior to first IP dose).
- B and T cell function will be measured by Flow cytometry and innate and adaptive responses measured by whole killed pathogen assay and Toll like receptor ligand assays from whole blood collected at birth (cord), Visit 3 and Visit 9.

8 TRIAL MEASUREMENTS AND OUTCOMES

8.1 Eligibility assessments

A full physical examination will be performed at study entry by a clinician prior to randomisation at Visit 1, according to common medical practice. The examination will include assessment of general appearance, HEENM (head, ears, eyes, nose and mouth),

skin, cardiovascular system, musculoskeletal system, respiratory system, gastrointestinal system and nervous system. Length (height) and weight will be measured. New findings or clinically significant changes noted in any physical examinations conducted post dosing will be recorded as AEs.

8.2 Immunogenicity

Faecal samples will be collected from participants following each dose of IP. One sample will be collected during the day 3 – 5 post dose period. These samples will be tested at the study central laboratories for shedding of rotavirus.

Serum samples will also be tested at the central laboratories, for anti-rotavirus IgA.

See Section 8.6 for testing at each laboratory.

Faecal and serum samples collected for this study may be stored for up to 4 years post-study, and if consent has been obtained from the parents/guardians, may be used for additional testing outside of this protocol. Stored samples will be labelled with the participants study number. Any additional testing would be performed under a protocol approved by an ethics committee, and would maintain strict confidentiality.

8.3 Safety

Safety will be assessed by recording of AEs, vital signs, and occurrence of blood in stool throughout the study.

- Parents/guardians will be questioned and monitored throughout the study with regard to any AEs their baby may have experienced. See Section 11 for further details on recording AEs.
- Vital signs will be measured at each site visit and will include pulse rate, respiratory rate and temperature.
- Parents/guardians will be issued with thermometers and will be instructed on how to take the participant's axillary temperature, which should be measured and recorded if the baby has a diarrhoea episode.
- Parents/guardians will be instructed to immediately contact study staff if the participant has any episode of blood in stool during the study. A doctor will assess the participant for each case of blood in stool. An abdominal ultrasound may be conducted, including if intussusception is suspected. Blood in stool and any ultrasound findings will be recorded as AEs.

8.4 Diarrhoea capture

Incidence of diarrhoea will be assessed by recording and grading of diarrhoea episodes during the study. For this study the definition of diarrhoea is three or more stools in a 24 hour period that are looser than normal for that child.

A Diarrhoea Log will be completed by the parents/guardians throughout the study. For each diarrhoea episode the date, participant's temperature, time of first and last loose stool of the diarrhoea episode, number of loose stools in each 24-hour period, time of first and

last vomit of any vomiting associated with the diarrhoea episode, and number of vomits in each 24-hour period will be recorded.

Parents/guardians will be instructed to contact the study staff to notify them of each episode of diarrhoea. The study staff will record the participant's symptoms, including degree of dehydration, treatment, duration of illness and outcome.

Two faecal samples should be collected per diarrhoea episode, from separate stools. Faecal samples will be tested at the study central laboratories for presence and strain of rotavirus. Instructions for sample collection, transport, processing and storage are contained in the SRM.

The details of the diarrhoea episode will be scored in the study database using a modified version of the Vesikari clinical score for gastroenteritis in infants (Appendix 1).

Episodes of diarrhoea will also be recorded as AE.

Analysis of separate faecal samples by stool microscopy and stool culture for identification of an underlying causative agent besides rotavirus may be done in the hospital or MLW Laboratory, Blantyre for cases where a participant is admitted to hospital with diarrhoea (or for other cases if clinically indicated or if requested by the investigator and/or Medical Monitor).

See Section 8.6 for testing at each laboratory.

8.5 Exploratory

A saliva sample will be collected from participants at Visit 1. The sample will be tested at the laboratory to determine the Lewis and secretor histo-blood group antigen status. A saliva sample will be collected at all IP dosing visits, to determine the phenotypic expression of the Lewis and secretor histo-blood group antigen status of participants.

The maternal serum sample will be tested for serum IgA as per Section 8.2, and for anti-rotavirus IgG.

Bacterial taxonomic and genetic functional profiling of stool microbiome will be conducted on a maternal stool sample and stool samples collected post administration of IP and at birth (prior to first IP dose) by 16S and shotgun sequencing. Assessment of T cell and B cell function will be completed by flow cytometric assays on whole blood (cord blood, Week 6 and Week 18). Innate and adaptive immunity will be assessed by *in vitro* reactivity in toll-like receptor ligand assays and whole killed pathogen assays by measurement of cytokines in supernatants collected from whole blood cultures with TLR agonists and whole killed pathogens.

See Section 8.6 for testing at each laboratory.

8.6 Central laboratories

Central laboratories for this study, and the tests they are performing, are;

- MLW Laboratory, Blantyre, Malawi:
    - Immunogenicity endpoints: shedding of rotavirus in post dose stool samples.

  - Diarrhoea endpoint: presence and strain of rotavirus in diarrhoea samples.
  - Exploratory endpoints: Lewis and secretor histo-blood group antigen status and phenotypic expression from saliva sample and whole blood.
  - T and B cell function from cord and blood samples, and innate and adaptive immunity from cord and blood samples.
- Enteric Viruses Research Laboratory, MCRI, Melbourne, Australia
  - Immunogenicity endpoints: sequence analysis of faecal samples for shedding, and serum sample (including maternal) testing for immunogenicity.
  - Exploratory endpoints: IgA and IgG in maternal serum sample, maternal and stool microbiome testing and cytomegalovirus and Epstein-Barr virus testing of serum for the assessment of the immune development exploratory endpoint.

Instructions for sample collection, transport, processing and storage are contained in the SRM.

9 CONCOMITANT MEDICATIONS AND VACCINES

9.1 Concomitant medications

All prescription and non-prescription medication including traditional, herbal and complementary medicines given to the participant from birth until end of study will be recorded.

In addition, prescription and non-prescription medication including traditional, herbal and complementary medicines taken by the mother will be recorded from 4 weeks prior to delivery, during delivery, and post-delivery until end of study (or until cessation of breastfeeding).

Concomitant medications records will include the drug name, total daily dose, route of administration, start and stop date of administration, and indication (for participants only).

9.2 Routine Concurrent Vaccines

During the study period, all trial participants should continue to receive standard EPI immunisations in accordance with the routine schedule and local recommendations, with the following exceptions;

- All study participants will be administered IPV by study staff at weeks 6, 10 and 14, rather than OPV, as a transition from OPV to IPV in Malawi is planned during the period of the trial
- Participants will not receive the standard Rotarix® schedule of vaccines during the study. As RV3-BB vaccine efficacy is not yet established, all participants will be administered a single dose of Rotarix® approximately one month after their final dose of IP.

Additional licensed vaccines may be administered as appropriate for the participant.

Commercial name, batch number, and date of administration will be recorded for all vaccinations.

10 INVESTIGATIONAL PRODUCT

The RV3-BB Rotavirus Vaccine and placebo will be manufactured, packaged and labelled by Meridian Life Sciences, USA.

Table 2: Identity of IP

Name:	RV3-BB Rotavirus Vaccine	Placebo
Formulation:	Each dose is a cell free solution containing attenuated rotavirus particles • High dose at a concentration of 1.0 x 10^7 FFU/ml) in 10% sucrose/cell culture medium. • Mid dose at a concentration of 3.0 x 10^6 FFU/ml) in 10% sucrose/cell culture medium. • Low dose at a titre of 1 x 10^6 FFU/ml) in 10% sucrose/cell culture medium	Sterile solution of cell culture medium and 10% sucrose
Route of administration:	Oral	Oral
Dosage:	1 mL	1 mL
Dosage form:	Single dose sterile aqueous solution for oral administration	Single dose sterile aqueous solution for oral administration

10.1 Special Warnings and Precautions for use of IP

RV3-BB Rotavirus Vaccine

Please see the Investigator Brochure for detail.

Based on data preclinical animal testing, the two clinical trials of the first generation RV3 Rotavirus Vaccine, and data from the RV3-BB clinical trials conducted to date (Danchin et al, 2013, Bines et al, 2015), side effects from RV3-BB candidate could include gastrointestinal events (for example: vomiting, diarrhoea/loose bowel motions and abdominal discomfort).

Although no previous recipient of either the first or second generation RV3 Rotavirus Vaccine experienced an anaphylactic reaction there is a low risk of a generalised anaphylactic reaction to the IP.

As for any study in neonates, particularly in low-income settings, the Investigator should be aware of the following:

- Jaundice is not uncommon in the neonatal period and is usually self-limited often occurring in association with establishment of breast feeding. Jaundice was observed in infants in both the vaccine and placebo groups in the Phase IIa and IIb clinical trials of the RV3-BB vaccine

- Sepsis is an important cause of death and illness in the neonatal period, particularly in low income settings. Neonatal Sepsis occurred in infants in both the neonatal and infant vaccine schedule in the Phase IIa and IIb study.

In the phase IIb study, one participant developed intussusception, although this was 114 days after the final dose of IP. As intussusception is a known rare side-effect of other rotavirus vaccines, the cautious approach is to assume it may be a class effect and parents/guardians will be advised of potential symptoms of intussusception.

Placebo

The Placebo contains only cell culture medium and 10% sucrose. There are no known warnings or risks associated with the use of the Placebo.

10.2 Labelling and Packaging

IP will be supplied as 1 mL single doses in prefilled plastic cryovials, which will be packed and stored in bulk outer boxes. Each box of IP will contain up to 81 vials of either RV3-BB or placebo.

The IP will be labelled according to local legal requirements and GMP guidelines.

Syringe labels

IP will be thawed and drawn into polypropylene syringes for dispensing and administration. The syringe will be labelled by the pharmacist indicating protocol number, randomisation number, date and time of thawing, and time by which IP must be administered (expiry time).

10.3 Storage requirements

Vaccine and placebo vials will be stored at –70°C ±10°C in a temperature-monitored and alarmed freezer. The IP will be stored in a secure and access-restricted location, isolated from any potential sources of contamination.

Once thawed, IP must not be refrozen but may be stored at 2-10°C for a maximum of 6 hours until time of administration.

Following removal from 2-10°C storage, the IP should be administered as soon as possible, within a maximum of 20 minutes at room temperature.

10.4 IP accountability

All material supplied is for use only in this clinical study and should not be used for any other purpose.

The PI or designee is responsible for IP accountability, reconciliation and record maintenance. The PI or designated site staff must maintain IP accountability records throughout the course of the study including records of the amount of IP received, the identification of the participant for whom it was dispensed, and the date(s) and quantity of the IP dispensed.

The IP and records must be available for inspection by a study monitor during the study. IP supplies, including unused, partially used or empty vials and syringes, must be retained and will either be returned by the site to MCRI or designee, or destroyed on site if written approval to do so is given by MCRI and appropriate facilities and procedures are available. Records shall be maintained by the PI of any disposition of the IP. These records must show the identification and quantity of IP disposed of, the method of destruction, and the person who disposed of the IP.

10.5 Doses and treatment regimens

Approximately 688 participants will be prospectively randomised to one of the following four treatment groups in a 1:1:1:1 ratio.

- High dose neonatal RV3-BB vaccine schedule ($1x10^7$titre dose). RV3-BB Vaccine for doses 1, 2 and 3 and placebo for dose 4
- Mid dose neonatal RV3-BB vaccine schedule ($3x10^6$titre dose). RV3-BB Vaccine for doses 1, 2 and 3 and placebo for dose 4.
- Low dose neonatal RV3-BB vaccine schedule ($1x10^6$titre dose). RV3-BB Vaccine for doses 1, 2 and 3 and placebo for dose 4.
- High dose Infant RV3-BB vaccine schedule ($1x10^7$titre dose). Placebo for dose 1 and RV3-BB Vaccine for doses 2, 3 and 4

Participants will also receive a dose of commercially available Rotarix® at 18 weeks of age.

10.6 Method for Assigning Participants to Treatment Group

Participant eligibility will be established before randomisation. Eligible participants will be assigned to a treatment arm according to the randomisation schedule.

At each site, a unique Screening Number will be allocated to each pregnant woman who provides preliminary informed consent, so that potential participants can be identified without making assumptions about their subsequent eligibility for the main trial. From birth, the baby will have the same Screening Number as their mother.

Randomisation will be stratified by birth multiple (single vs multiple), to minimise imbalance across the treatment arms. Multiple births will be randomised to the same treatment arm to avoid potential contamination across treatment arms.

Eligible participants will be randomised at V1 following confirmation of eligibility. The pharmacist will assign the next available Randomisation Number and prepare the IP dose.

The Randomisation Number will be used to identify the participant throughout the study period and on all study-related documentation.

If study consent is not given, or a participant fails screening, the Screening Number will not be reused. If a participant is discontinued after randomisation, the Randomisation Number will not be reallocated.

10.7 Blinding and procedures for breaking the blind

This is a double-blind study; only the pharmacists dispensing the IP and the unblinded CRA will know the treatment allocation of the participants. The site staff, parents/guardians of participants, sponsor, and monitoring personnel will be blind to treatment arm allocations.

To maintain the blind, the identity of the IP in each dispensed syringe will not be indicated on the label.

The laboratory personnel completing assays on routine stool samples will have potential deductive knowledge of the participants' treatment allocation as a result of conducting assays. For this reason, communication will be restricted between the laboratory personnel and blinded study personnel, until after final database lock.

Only in a medical emergency, when the investigator determines that adequate medical care cannot be provided without knowing the treatment assignment, the blind may be broken. In this instance the Investigator may contact the Pharmacy to obtain the treatment identity. If possible, such emergencies should be discussed with the Medical Monitor before breaking the blind. Details for contacting the Medical Monitor will be provided in the SRM.

If the blind has been broken, the investigator must document the date and the reason the blind was broken in the participant's notes and CRF.

The study monitor should be notified if the blind is broken. MCRI or designee will decide on a case-by-case basis whether unblinded infants will receive further doses of IP.

10.8 Preparation and Dispensing of IP

The pharmacist will prepare and dispense the appropriate dose of IP, according to the participant's allocated treatment schedule.

Dose preparation and transport to sites is described in the Pharmacy Manual and SRM.

10.9 IP administration and treatment compliance

Prior to administration of the second, third and fourth doses of IP, participants will be given a 2ml oral dose of an antacid solution (Mylanta® Original), using a syringe without a needle. The antacid solution is administered to help neutralise stomach acid to ensure vaccine stability when administered to infants. The neonatal stomach is less acidic, so the antacid solution is not required for the first dose of IP.

The investigator or designee will administer the IP to the participant within 10 minutes after administering the antacid (where applicable). The participant should be held in a supine position, and the entire contents of the syringe administered orally to the inside of the cheek in small aliquots, taking care that the participant does not spit the IP out.

The participant must remain under supervision at the clinic for at least 30 minutes after administration of the IP.

A participant will be considered compliant with study treatment if they received the scheduled administration of all doses of the IP, within the protocol specified time windows.

10.9.1 Incomplete administration of IP

Participants who do not ingest the full 1 mL dose of IP (e.g. due to spitting out the IP or vomiting within the 30 minutes following administration) will not receive any further IP at that visit. The participant will however, continue to receive subsequent doses of the IP as per protocol.

Any incomplete administration of IP will be recorded in the participant notes and the CRF.

10.9.2 Criteria for temporary delay of IP dose

Administration of any dose of IP can be delayed at the discretion of the investigator or Medical Monitor.

For the second, third and fourth doses, if a participant has a fever (paediatrics: ≥37.5°C axillary/oral or ≥38.0°C rectal/tympanic) and/or acute illness that contraindicates vaccination, dosing will be delayed until the participant is afebrile. If the dose then falls outside of the protocol specified window it will be recorded as a protocol deviation.

11 ADVERSE EVENTS

The definitions of AEs and SAEs are given below. It is extremely important that all staff involved in the trial are familiar with the content of this section. The PI is responsible for ensuring this.

11.1 Adverse event definitions

An AE is any untoward medical occurrence in a participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unexpected sign, symptom, or disease temporally associated with the use of an IP, whether or not related to the IP.

Pre-existing diseases are not considered AEs unless there is a change in frequency or severity. This change has to be reflected in the reported term. Lack of efficacy is not considered an AE, per se.

Parents/guardians must be instructed to inform the Investigator about all participant AEs and these must be documented in the participant records and Case Report Form (CRF) together with their intensity;

- Mild (Grade 1) AEs do not impact normal daily routine.
- Moderate (Grade 2) AEs impact the normal daily routine
- Severe (Grade 3) are those AEs which make normal daily routine impossible.
- Life Threatening (Grade 4)
- Fatal (Grade 5)

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilised for rating the intensity of an event, and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 11.2.

The Investigator must assign causality to each adverse event in relation to RV3-BB based on the following scale:

- Not related: AE for which there is evidence of another explanation, e.g. the adverse event is obviously explained by a concurrent disease(s), is in accordance with the known effect of a concomitant medication, or has occurred prior to first administration of IP.
- Unlikely related: AE with a time to IP administration that makes a relationship improbable (but not impossible), and disease or other drugs provide plausible explanations.
- Possibly related: AE with a reasonable time relationship to IP administration, but which could also be explained by disease or other drugs.
- Probably related: AE with reasonable time relationship to IP administration that is unlikely to be attributed to disease or other drugs.
- Definitely related: AE with plausible time relationship to IP administration which cannot be explained by disease or other drugs, and event is definitive pharmacologically

or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon).

All AEs must be documented by the Investigator, regardless of causality.

Expected AEs are defined as all AEs stated as such in the IB. If an AE has not been previously reported as expected (including type, degree, or frequency) in the IB, it is an unexpected adverse event. MCRI or designee is responsible for determining the expectedness of an AE.

If an AE leads to premature discontinuation of the study, the appropriate pages of the CRF must be completed.

11.2 Serious Adverse Events

An AE shall be defined as serious if it:

- results in death;
- is life-threatening;
    - Life threatening in the definition of serious refers to an event in which the participant 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.
- requires inpatient hospitalisation or prolongation of an existing hospitalisation;
    - Hospitalisation is defined as in-patient admission or care regardless of duration.
    - Out-patient treatment in an emergency room is not in itself an SAE, although the reasons for it may be (e.g. bronchospasm, laryngeal oedema). Elective surgery, hospitalisation for social reasons (with no causal AE), or hospital admissions and/or surgical operations planned before or during this study are not considered AEs if the illness or disease existed before the participant was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.
- results in a persistent or significant disability or incapacity;
- is an important medical event
    - This includes events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed above.

11.2.1 SAE exceptions

Conditions identified as a congenital anomaly or birth defect, regardless of when the condition is identified, will not be recorded as SAEs unless the condition progresses/worsens or is fatal. These conditions should be recorded as medical history, even if they are identified/diagnosed after randomisation.

11.3 Recording of AEs

AEs will be captured from the time of first dose of IP until the final study visit. Parents/guardians will be asked at each visit whether their baby has experienced any AEs.

SAEs that are at least possibly or definitely related to IP occurring to a study participant after the AE reporting period will be reported to the sponsor if the Investigator becomes aware of them.

It is preferable that AEs are reported as diagnoses if one is able to be made, rather than individual signs and symptoms. The AE description, start and stop dates, severity, causality and outcome must be recorded, as well as any actions taken.

Unless a diagnosis is made, or signs and symptoms are present, an abnormal laboratory value or vital sign should only be reported as AEs if they cause the participant to discontinue from the trial, the investigator feels they are clinically significant, or they meet a criterion for a SAE.

11.4 Management and follow-up of AEs and SAEs

Medical follow-up (such as history, physical examination, laboratory testing and/or treatment) may be necessary if a participant experiences an AE.

All AEs and SAEs recorded for this study, regardless of relationship to IP, should be followed until resolution or stabilisation.

Participants experiencing SAEs should be followed clinically until their health has returned to baseline status or until all parameters have returned to normal, are stabilised, or have otherwise been explained.

11.5 Reporting of Serious Adverse Events

Investigators and other site personnel must report SAEs to MCRI's representative within 24 hours of becoming aware of the SAE, regardless of causality.

STUDY CONTACTS AND PROCESSES FOR REPORTING SERIOUS ADVERSE EVENTS ARE DESCRIBED IN THE STUDY REFERENCE MANUAL.

Follow-up information on SAEs must also be reported by the investigational site within the same time frame. If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided within 24 hours.

All SAEs will be recorded in the participant records and the CRF. MCRI or designee is responsible for informing the regulatory authorities of the SAE as appropriate.

The investigator must notify their Independent Ethics Committee (IEC) of any SAEs within the time period specified by the IEC. MCRI or designee is responsible for informing other relevant IECs.

12 STUDY OVERSIGHT

12.1 Medical Monitors

The Medical Monitor is an appropriately qualified, designated physician who will be available to advise on trial related medical questions or problems, without other direct involvement with trial participants. The Medical Monitor will be available to respond to questions from the investigators about study eligibility, abnormal assessments, participant follow-up, assistance with AE and SAE assessment, concomitant medications and criteria for delay of IP. The contact details for the Medical Monitor are available in the SRM.

12.2 Steering Committee

The Steering Committee (SC) is a MCRI group and may include non-MCRI personnel as invited. The SC will provide oversight of the trial to review recruitment rates, site progress, and protocol compliance. The SC's responsibilities will be documented and will include input into protocol amendments, contact with study investigators to promote recruitment and high quality data, review, recommendation and implementation of major protocol changes, and implementation of DSMB recommendations.

12.3 Data Safety Monitoring Board

An independent DSMB will be established prior to recruitment start, with appropriate charter that defines its roles and responsibilities. The DSMB will monitor accruing trial safety results at intervals throughout the study. The main purpose of the DSMB will be to protect the interests of the participants included in the trial.

The charter will specify the intervals for formal DSMB meetings.

The DSMB will convey to MCRI their recommendations as to whether the trial may continue as planned or if the trial should be modified or stopped. The final decision on whether the study should be modified or stopped will be the responsibility of MCRI. Any decision to modify or stop will be communicated to investigators and regulatory agencies by MCRI (or their representatives).

13 STATISTICS AND DATA EVALUATION

The statistical analysis principles described below will be supplemented by a comprehensive statistical analysis plan (SAP) which will be finalised before the database is locked. Any changes to the statistical plans will be described and justified in the final report.

13.1 Sample Size Assumptions

With the high titre RV3-BB neonatal vaccine schedule as the active control arm, the sample size is calculated to demonstrate the non-inferiority of the lower titre vaccine arms with respect to the proportion of participants who have a serum IgA response 4 weeks after 3 doses of vaccine. A non-inferiority margin of 20% for the difference between arms will be used. The primary analysis is based on the per protocol population defined as participants who receive all 3 doses and have no major protocol violations. Thirty percent of participants

are expected to be excluded from the PP population due to death, study withdrawal, loss to follow-up, or study non-compliance. Among those in the PP population, a 50% response probability is assumed for the active controls. Based on a one-sided 0.025 level score test with 90% power under the alternative of no difference in response probabilities, 172 participants per arm are required for a total sample size of 688 participants.

Non-inferiority of the lower titre vaccine will also be assessed in the intention to treat population. The cumulative probability of response by 18 weeks of age will be estimated by the Kaplan-Meier method for each vaccine arm. The difference between high and low titre arms in the cumulative probabilities and its 95% confidence interval will be calculated. If the upper bound of the confidence interval is below 20%, non-inferiority of the lower titre vaccine will be demonstrated.

13.2 Analysis Sets

The following sets will be used for the statistical analyses:

Full analysis set (FAS): All participants randomised into the study. Participants will be analysed according to treatment to which they were randomised. Immunogenicity analyses performed in the FAS are considered supportive.

Safety set: All randomised participants who received at least one dose of IP. Participants will be analysed according to the treatment received.

Intention-to-treat (ITT) set: All randomised participants who received at least one dose of IP and have an evaluable baseline and post-baseline blood sample. Participants will be analysed according to the treatment to which they were randomised. Supportive immunogenicity analysis will be performed in the ITT set.

Per-protocol (PP) set: All participants from the ITT population who completed the study in compliance with the protocol and who reported no major violation of the study protocol. Participants will be analysed according to the treatment to which they were randomised. The final decision to exclude a participant from the PP set will be taken during a blinded data review meeting before database lock. Primary analyses are performed on the PP set.

13.3 Data Analysis Considerations

All immunogenicity and safety data will be listed and summarised using descriptive statistics by treatment group and nominal time. The descriptive summary for the categorical variables will include counts and percentages. The descriptive summary for the continuous variables will include means, medians, standard deviations and minimum and maximum values. Where possible, data from participants who withdraw prematurely from the study will be included in any analysis. Further details on the handling of withdrawals and/or missing data will be specified in the SAP.

All statements of statistical significance will be based on a two-sided test at the 5% level of significance, unless stated otherwise. Further details will be specified in the SAP.

The baseline value for each clinic assessment will be the last pre dose value obtained.

Full details of the statistical analyses will be presented in the SAP. Any deviations from the planned analyses detailed in the protocol will be documented in the SAP and final study report. If the study is prematurely discontinued, all available data will be listed and a review will be carried out to determine which statistical analyses are considered appropriate.

13.3.1 Demographics and Baseline Data

Demographic and baseline characteristics will be listed and summarised by treatment arm.

13.3.2 Immunogenicity Analyses

13.3.2.1 Definition of baseline IgA for the calculation of vaccine take

Due to the timing of the blood tests, baselines for serum anti-rotavirus IgA are defined differently for participants in the neonatal vaccine schedule and the infant vaccine schedule. Baseline serum anti-rotavirus IgA in the neonatal vaccine schedule is defined as the measurement from the cord blood. In the infant vaccine schedule baseline is defined as the measurement from analysis of serum collected prior to the second dose, although the measurement from the cord blood may be used if there are no data on IgA following the first dose of IP.

Further details of the analysis of secondary endpoints will be described in the SAP. Details about multiplicity and Type I error for secondary endpoints will be included in the SAP.

13.4 Safety Data

13.4.1 Extent of exposure

The number of participants exposed to study treatment will be summarised by treatment received.

13.4.2 Adverse events

AE data will be listed individually and incidence of AEs summarised by system organ class and preferred terms within a system organ class for each treatment group. When calculating the incidence of AEs, each AE, based on preferred terminology defined by Medical Dictionary for Regulatory Activities (MedDRA; Version 13.1, or later), will be counted only once for a given participant. A summary of the number and percent of participants with the following treatment emergent AEs will be displayed by treatment groups:

- All AEs
- IP related AEs
- Severe AEs
- SAEs
- AEs leading to permanent discontinuation of IP.

13.4.3 Other safety measures

Continuous variables will be summarised along with the change from baseline at each time point by treatment group. Other variables will be summarised as appropriate to the data.

14 DATA MANAGEMENT

Data collection and entry into the CRF will be completed by authorised study site personnel designated by the Investigator. Appropriate training and security measures will be completed with the Investigator and all authorised study site personnel prior to the study being initiated and any data being entered into the system for any study participants.

All data must be entered in English. The CRFs should always reflect the latest observations on the participants participating in the trial; therefore, the CRFs are to be completed as soon as possible after the participant's visit. The Investigator must verify that all data entries in the CRFs are accurate and correct. If some assessments are not done, or if certain information is not available or not applicable or unknown, this should be indicated in the CRF. The Investigator will be required to sign off on the clinical data.

The study monitor/Clinical Research Associate (CRA) will review the CRFs and evaluate them for completeness and consistency, and compare them to the source documents to ensure that there are no discrepancies between critical data. All entries, corrections and alterations are to be made by the responsible Investigator or designee. The CRA cannot enter data into the CRFs. Once data in the CRF have been entered, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who performed the change, together with time and date of the change, will be logged. Roles and rights of the site personnel responsible for entering the clinical data into the CRF will be determined in advance. If additional corrections are needed, the responsible CRA or Data Manager will raise a query. The appropriate investigational staff will answer queries. This will be audit trailed, as described above.

The CRF is essentially a data entry form and should not constitute the original, or source document. Source documents are all documents used by the Investigator or hospital that relate to the participant's medical history, that verify the existence of the participant, the inclusion and exclusion criteria and all records covering the participant's participation in the study. They may include, but are not limited to, laboratory reports, ECG results, imaging reports, pharmacy dispensing records, hospital records, participant files, etc. For this study, Study Reminder Card and Diarrhoea Logs will be considered source documents.

The Investigator is responsible for maintaining source documents. These will be made available for inspection by the CRA at each monitoring visit.

A CRF will be completed for all participants who are screened.

15 TRIAL MANAGEMENT

15.1 Contacts

Details of study sites, investigators, Medical Monitors, CRAs, and MCRI and designee contacts can be found in the SRM.

15.2 Quality control and Quality Assurance

15.2.1 Monitoring

Study monitoring will be performed in accordance with applicable regulations, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), and MCRI and designee SOPs.

Before the start of the trial, a representative of MCRI or designee will contact the investigational site to ensure facilities are adequate and discuss responsibilities with the site staff with regards to following the protocol and regulatory and ethical requirements.

During the trial, a CRA from MCRI or its designee will regularly contact and visit the site to monitor study progress, confirm protocol, regulatory and ethical adherence, confirm data accuracy and provide information and support.

A separate unblinded study monitor will conduct monitoring visits at the pharmacy. This monitor will be unblinded to the treatment allocation of participants through their contact with pharmacy personnel and records. The nominated unblinded monitor will ensure that accountability and dispensing records are adequately maintained and ensure that appropriate storage conditions for the vaccine are maintained.

The PI agrees to allow the CRA direct access to all relevant documents, including electronic records, and to allocate their time and the time of their staff to the CRA to discuss findings and any relevant issues.

Site staff will be provided with CRA and back up contact details in the event they have queries or require assistance.

15.2.2 Audits and Inspections

An audit is a systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analysed and accurately reported according to the protocol, MCRI's SOPs and those of MCRI's designees, GCP and the applicable regulatory requirements.

Authorised representatives of MCRI, its designee, a regulatory authority, or the IEC may visit the centre to perform audits or inspections. The investigator should contact MCRI or designee immediately if they are contacted by a regulatory agency about an inspection at their centre. If an audit or inspection occurs, the PI, site coordinating investigators, and institutions agree to allow the auditor/inspector direct access to all relevant documents and allocate their time and the time of their staff to the auditor/inspector to discuss findings and any relevant issues.

15.3 Training of Staff

Each individual involved in this trial should be qualified by education, training, and experience to perform his or her respective tasks.

Site staff may be trained for this study at investigator meetings and initiation visits by MCRI, or their designees.

The PI will maintain a record of all staff involved in the trial at each site. The PI will ensure that appropriate training relevant to the trial is given to all these staff, and that they will receive any new information relevant to the performance of this trial.

15.4 Changes to the Protocol

Trial procedures will not be changed without the agreement of MCRI.

If it is necessary for the trial protocol to be amended, the amended protocol must be approved by the IEC, unless the immediate safety of participants is involved.

If a protocol amendment requires a change to the PICF, approval of the revised PICF by MCRI or their designee and by the IEC is required before the revised form can be used.

MCRI or their designee will distribute amendments and new versions of the protocol to the PI. The PI will be responsible for submitting to the IEC.

MCRI or their designee will also distribute amendments and new versions of the protocol to the regulatory agencies. Regulatory approval must be obtained prior to implementation.

15.5 Trial Agreements

The PI and site coordinating investigator at each centre must comply with all the terms, conditions and obligations of the trial agreement for this trial. In the event of any inconsistency between this protocol and the trial agreement, the trial agreement shall prevail.

15.6 Trial Timetable and Termination

The planned start date for this trial is mid to late-2017. The proposed completion date is in 2019.

MCRI reserves the right to terminate the trial at any stage for any reason including commercial considerations. In the event of early termination, MCRI and designees will work with the PI and site staff to ensure all participants are appropriately discontinued and follow up for safety.

15.7 Ethics and regulatory review

The protocol, PICF, and other patient-facing documents will be submitted for approval to the IEC and regulatory agency, and must be approved or given a favourable opinion in writing as appropriate. The investigator must submit written approval to MCRI or designee before they can commence recruitment or enrol any participants into the trial.

Any amendment to the protocol will be sent to the IEC and regulatory agency. No deviations from or changes to the protocol will be implemented without documented

approval/favourable opinion from the IEC and regulatory agency of an amendment, except where necessary to eliminate an immediate hazard to a trial participant, or when the changes involve only logistical or administrative aspects of the trial.

The deviations from or changes to the protocol which were implemented to eliminate an immediate hazard to a trial participant and the proposed amendment, if appropriate, should be submitted to the IEC and regulatory agency for review and approval as soon as possible.

The PI must submit progress reports to the IEC according to local regulations and guidelines. The PI must also provide the IEC with any reports of SAEs from the trial site in accordance with the IEC requirements and timelines.

MCRI or designee will report to the regulatory agency as required.

15.8 Ethical Conduct of the Trial

The trial will be performed in accordance with the ethical principles in the Guidelines of the World Medical Association's Declaration of Helsinki in its revised edition (Fortaleza, Brazil, October 2013), ICH GCP, the approved study protocol, and applicable regulatory requirements.

15.9 Insurance and Liability

Liability and insurance for this study are addressed in the Clinical Trial Agreement. MCRI will not, under any circumstances, have any responsibility or liability regarding any injury to, or death of, an infant or infant's mother arising from or in connection with delivery or birth of a potential research participant.

15.10 Participant Information and Consent

The investigator at each centre will ensure that the participant's parents/guardians are given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the trial prior to obtaining both preliminary and final study consent. Parents/guardians must also be notified that they are free to discontinue their child from the trial at any time. The parents/guardians should be given the opportunity to ask questions and should be allowed time to consider the information provided.

The participant's parents/guardians' signed and dated informed consent must be obtained before conducting any procedure specifically for the trial. The PI must store the original, signed PICFs. A copy of the signed and dated PICFs must be given to the parents/guardians.

15.11 Data Protection

The PICF will explain that trial data will be stored in a computer database, maintaining confidentiality of participants. Participants in this database will be identified by Screening and Randomisation Number only. The PICF will also explain that for data verification purposes, authorised representatives of MCRI or their designees, regulatory authorities, IECs or sites may require direct access to parts of the hospital or site records relevant to the trial, including the participant's and mother's personal information.

15.12 Archiving

The PI is responsible for the archiving of the trial records for their site. Trial records include the participant files as well as the source data, the Investigator Site File, pharmacy records, and other study documents. Trial records must be archived for at least 15 years (or at least 2 years after the formal discontinuation of clinical development of RV3-BB vaccine).

However, these documents should be retained for a longer period if specified by regulatory requirements or by an agreement with MCRI. It is the responsibility of MCRI to inform the PI/institution as to when these documents no longer need to be retained. Records may not be destroyed without prior written consent from MCRI.

If the PI leaves the investigational site for any reason, the responsibility for all study related records must be transferred to another person at site.

15.13 Emergency Procedures

The PI is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the trial.

If emergency unblinding is required, the site should contact the study Medical Monitor. Contact details are provided in the SRM.

15.14 Publication Policy

The publication policy for the study will be specified in a separate contract(s) between MCRI, and other relevant parties involved with the clinical study.

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17 APPENDICES

17.1 Appendix 1: Modified Vesikari Grading Scale for Gastroenteritis in Children

Non severe diarrhoea < 11 points

Severe diarrhoea ≥11 points

Points / Value / Symptom	1	2	3
Duration of diarrhoea (days)	1-4	5	≥6
Max. number of diarrhoea stools/24hrs	1-3	4-5	≥6
Duration of vomiting (days)	1	2-4	≥5
Max. number of vomiting episodes/24hrs	1	2-5	≥6
Temperature (degrees C)*	37.1 – 38.4	38.5 – 38.9	≥39.0
Dehydration	-	Some (1-5%)	Severe (≥6%)
Treatment	Rehydration	Hospitalisation	-

Reference: (Ruuska ,1990)

*Temperature measurements will be entered into the CRF as axillary measurements and converted at the database level to rectal equivalent temperatures, which are standard for Vesikari scoring.

Modifications

Children who receive oral rehydration solution prior to the dehydration assessment AND who have at least one of the signs of "some" dehydration according to the Integrated Management of Childhood Illness dehydration criteria at the initial clinic assessment receive a score of 2 for 'Dehydration'

Use of NG, IV or six hours of supervised oral rehydration is scored as hospitalisation (score of 2) for 'Treatment'

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