INTERNAL USE ONLY

Treatment of enteric infections among Indian infants to improve their response to oral poliovirus vaccine

Protocol Number: CMC EVI 2012

Sponsors: CHRISTIAN MEDICAL COLLEGE

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STATEMENT OF COMPLIANCE

This study will be carried out in accordance with Good Clinical Practice (GCP), as required by applicable local regulations in India, including following the Guidelines of the Indian Council for Medical Research as stated in Schedule Y of the Drugs and Cosmetics Act, local ethical review bodies and the Central Drug Standards Control Organization.

The study informed consent documents will embody the elements of consent as described in the Declaration of Helsinki and the ICH Harmonized Tripartite Guidelines for Good Clinical Practice. All key personnel (all individuals responsible for the design and conduct of this study) will have completed Human Subjects Protection Training prior to interaction with any participants or to having access to their confidential study data.

Indian Council for Medical Research Guidelines for Biomedical Research
Refer to: http://cdsco.nic.in/
http://www.ich.org/products/guidelines.html
http://cme.cancer.gov/c01/
http://www.icmr.nic.in/ethical.pdf

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LIST OF ABBREVIATIONS

CI	Confidence interval
CMC	Christian Medical College
CPE	Cytopathic effect
CRF	Case report form
CRO	Contract Research Organisation
DSMB	Data Safety Monitoring Board
ELISA	Enzyme-Linked ImmunoSorbant Assay
EPI	Expanded Programme on Immunization
GCP	Good Clinical Practice
GPP	Good Pharmacoepidemiology Practice
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent ethics committee
IgA	Immunoglobulin A
IRB	Institutional Review Board
ITT	Intention-to-treat
MoP	Manual of Procedures
mOPV3	Monovalent type 3 oral poliovirus vaccine
N	Number (typically refers to subjects)
NAb	Neutralizing Antibody
OD	Optical density
OPV	Oral polio vaccine
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
RT-PCR	Real-time polymerase chain reaction
SAE	Serious adverse event
SAGE	Strategic Advisory Group of Experts
SOP	Standard Operating Procedure
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	*Treat children in India for enteric infection to improve their response to oral poliovirus vaccine*
Population:	*Generally healthy infants 6-11 months old who lack serum neutralizing antibodies to poliovirus serotype 3*
Study Site:	*Single site trial conducted at Christian Medical College, Vellore, India*
Study Duration:	*18 months from enrollment of the first participant through completion of the final study visit for the last participant*
Subject Participation Duration:	*35 days*
Description of Agents or Interventions:	*Oral monovalent type 3 poliomyelitis vaccine (mOPV3) manufactured by GlaxoSmithKline Biologicals containing at least $10^{5.8}$ $CCID_{50}$ (median cell culture infective doses) of serotype 3 poliovirus, Leon-12a,1b strain produced on human diploid cell culture. This vaccine is an oral suspension with a single dose administered as 2 drops (0.1 ml) measured using a multi-dose dropper supplied with the vaccine.* *Azithromycin, a broad-spectrum macrolide antibiotic, administered at 10mg/kg/day for 3 days using an oral suspension containing 100mg of azithromycin per 5mL.*
Objectives:	***Primary Objectives:*** • *To evaluate the immunogenicity of serotype 3 monovalent oral poliovirus vaccine among Indian infants who have been treated 14 days previously with a 3 day course of oral azithromycin compared with Indian infants who received a placebo.* ***Secondary Objectives:*** • *To evaluate the relationship between the serologic response to mOPV3 and the presence of enteric pathogens in stool samples collected at the time of vaccination*

- *To evaluate the relationship between the serologic response to mOPV3 and serum and fecal markers of gut inflammation and tropical enteropathy.*
- *To evaluate the impact of a 3 day course of oral azithromycin on enteric pathogens identified in the stool of infants at 0 and 14 days after the beginning of treatment.*
- *To evaluate the impact of a 3 day course of oral azithromycin on serum and fecal markers of gut inflammation and tropical enteropathy measured at 0 and 14 days after the beginning of treatment.*
- *To evaluate poliovirus shedding and fecal IgA 7 and 21 days after vaccination with mOPV3 among infants who developed serum neutralizing antibodies after vaccination with mOPV3 compared with those who did not*

Exploratory objectives

- *To compare the in vitro lymphocyte response to poliovirus antigen and non-specific stimuli in blood samples taken from a subset of infants who developed serum NAb after vaccination with mOPV3 compared with those who did not develop serum NAb*
- *To characterize the T cell population in blood samples taken from a subset of infants who developed serum NAb after vaccination with mOPV3 compared with those who did not develop serum NAb*
- *To examine the relationship of the in vitro lymphocyte response to poliovirus antigen and the T cell phenotypes from blood with the serum and fecal markers of intestinal inflammation and tropical enteropathy*
- *To evaluate the relationship between the serologic response to mOPV3 and the levels of retinoic acid in serum*

Hypotheses:

Primary Hypothesis:

- *Treatment of Indian infants aged 6-11 months found to lack serum neutralizing antibodies to serotype 3 poliovirus with a three day course of azithromycin will increase the immunogenicity of a subsequent dose of mOPV3*

Description of Study Design: Single centre, randomized placebo controlled trial with 2 groups enrolling a total of approximately 750 infants

Intervention Groups:

Study Group	***Sample Size***	***Description***
Group 1 (azithromycin)	*375*	*Infants found to lack serum NAb to serotype 3 poliovirus will be enrolled in the study and begin a three day course of azithromycin in oral suspension. At 14 days infants will be vaccinated with a single dose of mOPV3. A stool sample will be taken at the time of enrolment and at the time of vaccination (0 and 14 days). A blood sample will be taken at 35 days after enrolment for all infants to assess seroconversion. In a subset of 150 infants a blood sample will also be taken at the time of vaccination and a stool sample 7 and 21 days after vaccination (days 21 and 35).*
Group 2 (placebo)	*375*	*Infants found to lack serum NAb to serotype 3 poliovirus will be enrolled in the study and begin a three day course of placebo oral suspension. At 14 days infants will be vaccinated with a single dose of mOPV3. A stool sample will be taken at the time of enrolment and at the time of vaccination (0 and 14 days). A blood sample will be taken at 35 days after enrolment for all infants to assess seroconversion. In a subset of 150 infants a blood sample will also be taken at the time of vaccination and a stool sample 7 and 21 days after vaccination (days 21 and 35).*

Total:	*750*	

Estimated Time to Complete Enrollment:

18 months

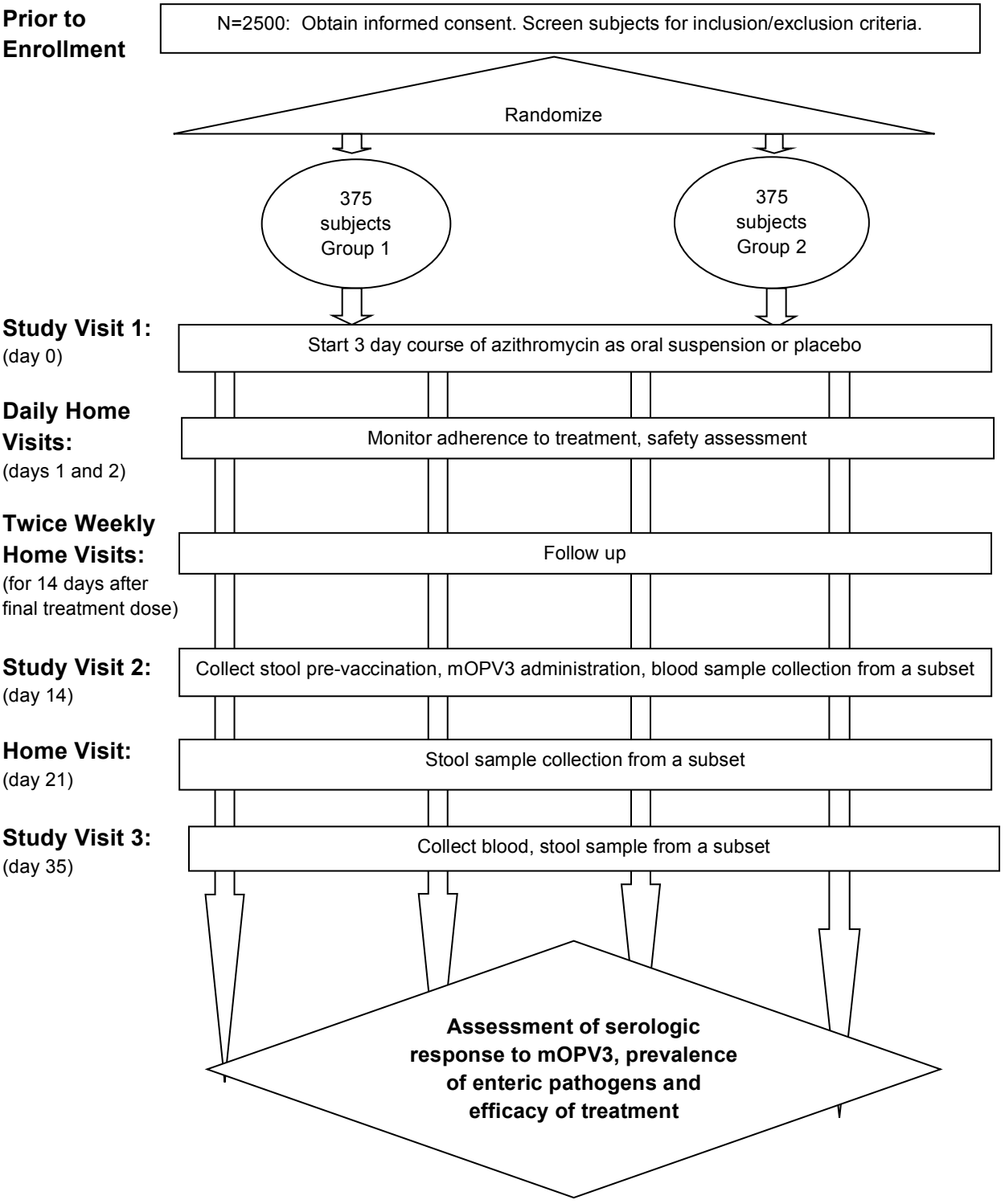
Justification of Sample Size

We estimate that a total of approximately 750 children will need to be recruited in this study to provide 90% power to detect an effect of treatment on the immunogenicity of OPV; this is based on an estimated prevalence of treatable enteric infections of 40%, an average 60% seroconversion after administration of mOPV3, an assumption that the relative immunogenicity of mOPV3 among currently infected infants compared with uninfected infants is 33% and a drop-out rate of 10%. Under the same assumed effect size this sample size will provide 99% power for a secondary comparison of OPV immunogenicity among infants who are shown to be infected with the pathogens of interest versus uninfected infants at the time of administration of the vaccine. The number of infants that will need to be screened for poliovirus antibodies to recruit the required number of infants will depend on seroprevalence. We therefore carried out a seroprevalence survey among 100 infants aged 6-11 months who were outpatients in the main CMC hospital in Vellore. We found 39% of infants had undetectable serum neutralizing antibodies to serotype 3 poliovirus (at a 1 in 4 dilution), compared with 7% and 9% for serotypes 1 and 2 respectively. This means that approximately 2000 infants would need to be screened, which we have inflated to 2500 to ensure we recruit sufficient infants if seroprevalence changes during the course of the study. Screening will stop as soon as the required number of infants is enrolled in the study.

Primary Statistical Analyses

The proportion of infants with detectable serum NAb to poliovirus at a 1 in 8 dilution will be compared between Group 1 and 2 using a two-sided Fisher's Exact test with significance level alpha equal to 5%.

Flow diagram



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

In common with other oral vaccines, the immunogenicity and effectiveness of oral poliovirus vaccine (OPV) is impaired in lower-income countries [1]. In India, reduced effectiveness of OPV, especially in northern states, has permitted persistent circulation of wild-type polioviruses despite good coverage achieved during frequent mass vaccination campaigns [2].

Potential contributing factors to the reduced immunogenicity of OPV in lower-income settings include a high prevalence of diarrhea, infection of the gut with other pathogens, malnutrition, micronutrient deficiencies, interference by breast milk antibodies and tropical enteropathy [1, 3]. Although several of these factors may be involved, an important role for other enteric infections is supported by the consistent findings of reduced immunogenicity of OPV when given during the high season for enteric infections or when given to children with diarrhea [4-6]. However, the identity of the enteric infection(s) responsible and the underlying immunological mechanisms are not known. A small number of studies have found reduced immunogenicity of OPV among children excreting enteroviruses [7, 8] or with *Shigella* associated diarrhea [9]. However, these studies have had small sample sizes and there is a risk of publication bias; other studies have not found an association between enteroviruses and the immunogenicity of OPV [10].

Infants aged 6-11 months are the most common age-group reported with poliomyelitis in India, due to a persistent gap in immunity to specific serotypes that in part reflects the relatively poor immunogenicity of OPV [2]. The prevalence of enteric infections rises steeply in this age group in India, with the rate of increase dependent on location, socio-economic and other factors. Cohort studies in urban slums have identified a high prevalence of pathogenic bacteria among 6-11 month old infants both in the presence and absence of diarrheal disease. In a cohort study in Vellore, the most common enteric pathogens identified by ELISA or PCR in single stool samples taken from healthy infants in this age group were *Campylobacter* (38%) and pathogen strains of *E. coli* (EAEC or EPEC 18%). These bacterial pathogens were also commonly found among infants with diarrhea (combined prevalence of 54%).

2.2 Rationale

We hypothesize that infection with pathogenic bacteria inhibits the adaptive immune response to the live-attenuated poliovirus vaccine, potentially as a result of the stimulation of local innate immune effectors that limit virus replication and/or the induction of local T-regulatory cell environment that prevents a systemic response to the vaccine. We propose to test this hypothesis in an RCT of the effect of treatment with azithromycin on the immunogenicity of a

subsequent dose of OPV given 14 days later to infants 6-11 months old who lack serum NAb against poliovirus serotype 3.

Azithromycin is a broad spectrum macrolide antibiotic and has been shown to provide effective treatment for *Campylobacter* and *Vibrio cholerae* following a single dose [11, 12], *Shigella* following a 4 day course [13] and is recommended for travelers diarrhea caused by enterotoxigenic *E. coli* [14, 15]. Bacteriological cure in these studies was observed in 100% of children 7 days after a single dose of azithromycin for *Campylobacter*, in 94% of adults 2 days after a 4 day course of azithromycin for *Shigella* and in 96% of patients infected with Shiga-toxin producing enteroaggregative *E. coli* after 14 days of treatment [11, 13, 15].

We propose to administer a 3-day course with azithromycin to provide effective treatment of the most common enteric pathogens identified among healthy infants aged 6-11 months in India. We expect infection and innate immune effectors, including inflammation to have cleared 14 days after the initation of treatment whilst reinfection will remain low (approx. 10%). At this point we will administer OPV to children in the treatment and control arms to examine the impact of treatment on the immunogenicity of this vaccine. If treatment improves the immunogenicity of OPV this may offer a solution to oral poliovirus vaccine failure in lower income settings, although further work would be needed to refine the treatment regimen, particularly if specific pathogens were more commonly associated with vaccine failure in the control arm of our study. In addition, this approach may offer at least a partial solution to the compromised immunogenicity of other oral vaccines, such as oral rotavirus vaccines that are likely to be included in routine vaccination schedules in a number of lower-income countries in the future.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

2.3.1.1 Serotype 3 monovalent oral poliovirus vaccine (mOPV3)

mOPV3 has been routinely used during mass vaccination campaigns in India since 2005 and the trivalent formulation, which also includes serotypes 1 and 2, is included in the routine EPI schedule in India and recommended for routine use among all infants. Extremely rare cases of paralysis have been observed in vaccinees or in non-immune subjects living in close contact with the person recently vaccinated with oral trivalent poliomyelitis vaccines, which contains serotype 3. The vast majority of post-vaccination paralysis occurred after the first dose of the vaccine. The incidence of paralytic poliomyelitis associated with vaccination in vaccinees has been estimated in several studies in several areas, according to various methods. In the United States, an incidence of less than 1 case per 1 million doses administered was reported in infants and their close entourage between 1980 and 1994 [16]. In India in a study in 1999 the risk of vaccine-associated paralysis was somewhat lower at 1 case per 4.6 to 4.6 million doses administered [17].

Non-specific symptoms, such as fever, vomiting, diarrhoea and allergic/anaphylactic reactions have rarely been observed after vaccination with the GlaxoSmithKline Biologicals oral trivalent poliomyelitis vaccine.

2.3.1.2 Azithromycin

Azithromycin is a broad spectrum macrolide antibiotic with a long half-life in mucosal tissues of 2-4 days (based on assessment of lung, tonsil and genital tract) [18]. It is well tolerated and results in few side effects. Following a 3 day course at a high dose of 30mg/kg given to pediatric patients side-effects occurring in more than 1% of patients were diarrhea observed in 2.6%, short-term abdominal pain in 1.7% and vomiting in 2.3% [19]. Mass treatment of populations (typically all individuals over 6 months old) with a single dose of azithromycin has been routinely performed as part of trachoma control programmes in a number of countries in Asia and Africa with no significant adverse events attributable to the drug reported [20, 21]. Furthermore, the prevalence of childhood diarrhea has been found to decline significantly in the month after mass treatment [22, 23].

Any infant found to develop severe diarrhea, of small volume, frequent, and associated with pain and blood in stools after treatment with azithromycin will be referred to hospital and in addition tested for *C. difficile.*

2.3.1.3 Serum Collection

Participants may experience some discomfort and pain from venipuncture for collection of 3 or 5 ml of blood. Blood drawing is occasionally associated with bleeding at the puncture site and rarely, an infection may occur at the site of phlebotomy. The participant may experience discomfort, pain, redness, swelling, bruising and/or local hardness at the puncture site. Blood will only be taken by a trained health worker according to standard procedures to minimize these risks.

2.3.1.4 Stool Collection

Stool will only be collected following a natural bowel movement. There are no risks to the study participant in collecting stool.

2.3.1.5 Participant Privacy

Personal identifiers, including birth date and sex, will be collected and recorded on study case report forms (CRFs) and each participant will be given a study identification card with the parent's photo and study identification number. Other information collected by study staff and recorded on a linking document containing the study participant identifier will include the name of the participant, name of the parents, study identification number, location/address of residence, and other identifiers important for ensuring follow-up throughout the study period. The study identification number on the card and the linking document provides the link between the participant and his/her study information. Although this linking document will be stored

separately from the study CRFs containing study data, a potential risk for “loss of confidentiality” does exist. Whenever feasible, use of identifiers, such as name and addresses, will be avoided and the unique study identification numbers will be used. Paper-based records (i.e., CRFs) will be kept in a separate, secure location with controlled access and will only be accessible to personnel involved in the study. This linking document will be destroyed once data is verified. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Individual participants will not be identified in any study related reports, and all study results will be reported in aggregate only.

Biological specimens (i.e., serum and stool specimens) will be identified by study participant number, and specimen collection date. Following collection, the specimens will be processed according to specimen handling/processing guidelines and stored at the CMC laboratories for analysis. The links between study data and the study specimens will not be destroyed, but no personal-identifying information associated with the study will be stored on site with the specimens.

2.3.2 Known Potential Benefits

2.3.2.1 Serotype 3 monovalent oral poliovirus vaccine (mOPV3)

We will screen approximately 2,500 infants for serum NAb to poliovirus serotype 3. Despite opportunities for vaccination with trivalent OPV through routine and supplementary immunization activities, we have recently found the prevalence of detectable NAb to this serotype to be low (61%) compared with serotypes 1 and 2 (93% and 91%) among 6-11 month old infants attending CMC hospital outpatient department. We will enroll infants found to lack serum NAb to serotype 3 poliovirus in this study and administer a dose of mOPV3, which we estimate to have a 60% probability of inducing an immune response. Infants who are found to remain seronegative at the end of the study will be offered a dose of inactivated poliovirus vaccine, which is expected to seroconvert the majority of these infants (>90%) based on recent experience with this vaccine in India [24].
Through study participation, the infant and consenting parent will also contribute greatly needed information regarding how we might improve the efficacy of OPV and other oral vaccines, such as that for rotavirus.

2.3.2.2 Azithromycin

Azithromycin is known to be effective against the most common enteric pathogens found in stool samples from infants in Vellore aged 6-11 months (*Campylobacter*, pathogen strains of *E. coli*), which have a combined prevalence of 45% in community stool samples and 54% in diarrhoeal samples. Administration of these two medicines is expected to effectively treat at least 75% of these infections and result in a significant reduction in associated symptoms (see above).

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Objectives:

1. To evaluate the serologic response to serotype 3 monovalent oral poliovirus vaccine among Indian infants who have been treated 14 days previously with a 3 day course of oral azithromycin compared with Indian infants who received a placebo.

3.1.2 Secondary Objectives:

1. To evaluate the relationship between the serologic response to mOPV3 and the presence of enteric pathogens in stool samples collected at the time of vaccination
2. To evaluate the relationship between the serologic response to mOPV3 and serum and fecal markers of gut inflammation and tropical enteropathy.
3. To evaluate the impact of a 3 day course of oral azithromycin on enteric pathogens identified in the stool of infants at 0 and 14 days after the beginning of treatment.
4. To evaluate the impact of a 3 day course of oral azithromycin on serum and fecal markers of gut inflammation and tropical enteropathy measured at 0 and 14 days after the beginning of treatment.
5. To evaluate poliovirus shedding and fecal IgA 7 and 21 days after vaccination with mOPV3 among infants who developed serum neutralizing antibodies after vaccination with mOPV3 compared with those who did not

3.1.3 Exploratory objectives

1. To compare the *in vitro* lymphocyte response to poliovirus antigen and non-specific stimuli in blood samples taken from a subset of infants who developed serum neutralizing antibodies after vaccination with mOPV3 compared with those who did not
2. To characterize the T cell population in blood samples taken from a subset of infants who developed serum neutralizing antibodies after vaccination with mOPV3 compared with those who did not
3. To examine the relationship of the *in vitro* lymphocyte response to poliovirus antigen and the T cell phenotypes from blood with the serum and fecal markers of intestinal inflammation and tropical enteropathy
4. To evaluate the relationship between the serologic response to mOPV3 and the levels of retinoic acid in serum

3.2 Study Outcome Measures

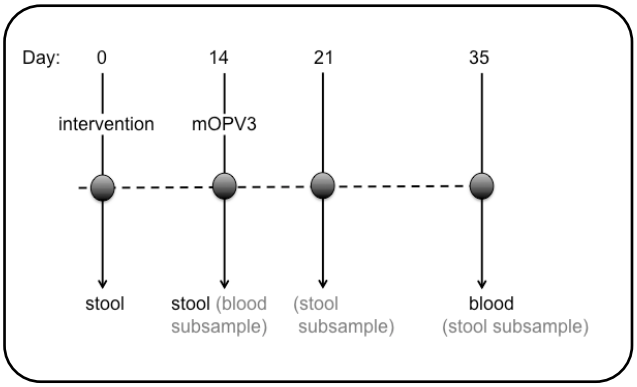
1. A serological response to mOPV3 ('seroconversion') will be defined as the detection of poliovirus neutralization by the serum sample taken at day 35 at a dilution of 1 in 8 or greater.
2. Geometric mean titers (GMTs) of poliovirus-specific serum NAb will be used in addition to seroconversion for the secondary and exploratory objectives.
3. Enteric pathogens detected in stool samples using a Taqman card-based RT-PCR assay will be scored as present or absent based on the detection of amplified products.
4. Fecal and serum biomarkers of tropical enteropathy and intestinal inflammation will be scored as present or absent based on a cut-off measurement of fluorescence intensity in the magnetic or cytometric bead-based analysis determined during the course of the study.
5. Poliovirus shedding will be quantified in stool samples taken at 7 and 21 days after vaccination with mOPV3 using RT-PCR following a standard protocol
6. Fecal IgA will be quantified using a time-resolved fluorescence assay to be established at CMC
7. Lymphocyte proliferation and T cell assays will be performed on blood samples from a subset of infants and used for the exploratory objectives
8. Serious adverse events (SAEs) will be defined as any event observed by study staff and/or reported by parent at any time during the study that meets one of the following conditions:

    - Death.
    - Life threatening.
    - Requires inpatient hospitalization or prolongation of existing hospitalization.
    - Results in a persistent or significant disability or incapacity.
    - Important medical events that may not results in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

4 STUDY DESIGN

Healthy infants aged 6-11 months living in Vellore will be identified during household visits and screened for serum neutralizing antibodies to poliovirus through the collection of a 3ml venous blood sample and standard neutralization assay [25]. Those infants without detectable neutralizing antibodies to serotype 3 poliovirus will be invited to participate in the study. Neutralisation assays will be completed as soon as possible and preferably within 1 week to ensure infants can be recruited as soon as possible after collection of the initial blood sample.

In total we aim to recruit 750 infants aged 6-11 months to the study and expect to screen approximately 2500 based on our recent measurement of the prevalence of poliovirus-specific serum NAb in this age group. Those children recruited to the study will be randomized to receive either placebo or a 3 day course of oral azithromycin treatment at the time of recruitment (see box). Treatment will involve prescription of syrup containing 40 mg of azithromycin per ml of suspension with between 1 and 5 ml to be taken once daily depending on body weight. Serotype 3 monovalent OPV (mOPV3) will be administered at 14 days to infants in both arms of the study and serum neutralizing antibody titers to this poliovirus serotype will be measured in blood samples taken 21 days later. Any infants remaining seronegative to poliovirus serotype 3 at the end of the study will be offered a dose of inactivated poliovirus vaccine to ensure protection against poliomyelitis. Infants will be monitored for any adverse events following treatment throughout the course of the study. Any infants that develop diarrhea following antibiotic treatment will be assessed for symptom severity and referred to follow-up where appropriate, including testing for *C. difficile*.



The primary outcome will be seroconversion to serotype 3 poliovirus 21 days after administration of mOPV3. Secondary outcomes of interest include the prevalence of enteric infections in stool samples taken at the time of recruitment, the impact of treatment on enteric infection in stool samples taken 14 days later and the association between enteric infections at days 0 and 14 and the response to OPV in the control arm. The presence of *Campylobacter*, diarrheagenic *Escherichia coli, Giardia*, *Cryptosporidium*, rotavirus, norovirus and other enteric pathogens in the stool samples will be tested using PCR. Stool samples will be frozen at -80°C and stored to ensure availability for potential additional approaches to assess enteropathogen prevalence and intestinal microbiota.

We will also assess the hypothesized role of intestinal innate immune activation in the failure of OPV in a number of ways. In frozen serum from blood samples taken from a subset of children at the time of vaccination we will measure markers of gut inflammation and integrity including intestinal fatty acid binding protein [26], soluble CD14 [27], citrulline [28, 29] and endotoxin core

antibody [30] using ELISA based methods. The relationship between these markers and the serological response to mOPV3 will be examined. In these same serum samples we will measure inflammatory cytokines, particularly IFN-γ, TNF-α and IL-17A using flow cytometry based methods, and if there is sufficient volume we will also measure retinoic acid. We will also use ELISA methods to measure markers of gut inflammation in frozen stool samples collected from these same children at the time of vaccination, including fecal calprotectin [31, 32], myeloperoxidase [33], and a marker of enteropathy, α-1 anti-trypsin [34]. Current and intervening symptoms of diarrhea will be recorded at each visit. In addition, at baseline we will collect information on breastfeeding, past use of antibiotics, height, weight and demographic variables.

Innate immune activation and the cellular immune response to OPV will be examined by collecting blood samples at the time of administration of OPV and 21 days later in a subset of 50 infants from each study arm (total 200 samples of 3 ml each—taken from the overall 5 ml collection). Aliquots of PBMCs will be stimulated with poliovirus, vero cell culture supernatant, staphylococcus enterotoxin B or toll-like receptor-3 (TLR3) agonist and the CD4 T cell response will be examined using FACS and cytokine profiling [35]. The cell populations of unstimulated PBMC samples will also be examined directly through FACS analysis to determine T cell phenotypes and expression of the gut-homing receptor CCR9 and the integrin $\alpha 4\beta 7$.

We will also collect stool samples from a subset of infants 7 and 21 days after vaccination and measure poliovirus shedding as well as fecal IgA. In addition to examining innate immune function, these assays aim to test the hypothesis that oral vaccination of children who are exposed to frequent enteric infection can result in induction of poliovirus-specific IgA in the intestine through T-cell dependent and/or T-cell independent activation of B cells but without class-switching to generate a systemic (serum IgG) response due to the presence of T-regulatory cells [36, 37].

An independent Data Safety Monitoring Board (DSMB) will provide safety monitoring for the study. They will determine formats for reporting adverse events and constitute rules for stopping the study if required.

Table 1 Sample numbers to be collected and laboratory procedure

Time (day of study)	Sample	n	Laboratory procedure
Screening (Oct 2012-Sep 2013)	2-3 ml blood	2500	1. Poliovirus neutralizing Ab serotype 3 - 1:4 and 1:8 dilutions - 1 week processing time
Enrolment (0)	Stool	750	1. RT-PCR for pathogens 2. subsample of 300 samples (150 from each arm) tested for markers of inflammation/integrity using ELISA (calprotectin, alpha-1 anti-trypsin, myeloperoxidase)
Vaccination (14)	Stool	750	1. RT-PCR for pathogens 2. subsample of 300 samples (same 150 from each arm tested from time of enrolment) tested for markers of inflammation/integrity using ELISA (calprotectin, alpha-1 anti-trypsin, myeloperoxidase)
	3-5ml blood	300	1. 50 of these samples from each arm (100 in total) will be rapidly processed and transit time recorded for FACS analysis and cytokine assay 2. ELISA for markers of inflammation (intestinal fabp, soluble CD14, citrulline and endotoxin core antibody) and for inflammatory cytokines (IFN-γ, TNF-α and IL-17A); we will also measure retinoic acid if there is sufficient blood volume available (50µl required)
Follow-up (21)	Stool	300	In the same 300 infants giving blood at the time of vaccination: 1. RT-PCR for poliovirus shedding
Follow-up (35)	3-5ml blood	750	1. 50 of these samples from each arm (100 in total) will be rapidly processed and transit time recorded for FACS analysis and cytokine assay 2. Poliovirus neutralizing Ab serotype 3 - full dilution
	Stool	300	Among the 300 infants with a stool sample at day 21: 1. serotype 3 poliovirus IgA 2. RT-PCR for poliovirus shedding

Potential participants for this study live in the area surrounding the Christian Medical College in Vellore. The Christian Medical College (CMC) is located in the town of Vellore, Tamil Nadu in southern India. The institution was founded in 1900, with the mission to serve the needs of the local community and to train medical and paramedical professionals in holistic and ethical medicine. This institution grew from an eight-bed hospital to a 2200 bed tertiary/referral care hospital which has >1.4 million outpatient visits and over 120,000 admissions annually. In addition to the large hospital, CMC also provides primary care to urban and rural populations in and around Vellore through the community health program. The institution's involvement in health and development is a continuum from the building of toilets to training in income generation, disease prevention and education, as well as clinical referrals along a chain of

primary, secondary and tertiary care services. CMC's strategy for community services provides health care that is accessible, affordable and acceptable, involves community participation, and addresses the issues of disease prevention and health promotion.

The Department of Gastrointestinal Sciences has substantial experience working in urban and semi-urban areas of Vellore. CMC has a long standing positive reputation with the community, and because families are familiar with CMC staff, recruitment rates into studies have generally been high.

5 STUDY ENROLLMENT AND WITHDRAWAL

The study population will be drawn from children living in Vellore town. Parents will be contacted through the existing Demographic Surveillance System and informed about the study.

5.1 Subject Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for screening.

- Infants 6-11 months old.
- Live in area under surveillance.
- Available for follow up for duration of study (approximately 35 days after enrolment).
- Parents/guardians of infant are able to understand and follow screening procedures and agree to participate in the screening process by providing signed informed consent.

In addition, subjects must meet the additional following inclusion criteria to be eligible to enrol in the randomized controlled trial.

- No current medical condition as determined by medical doctor that precludes study involvement.
- Parents/guardians of infant are able to understand and follow study procedures and agree to participate in the study by providing signed informed consent.
- Do not have detectable serum NAb to serotype 3 poliovirus

5.2 Subject Exclusion Criteria

Subjects meeting any of the exclusion criteria will be excluded from study participation.

- Infant or infant's mother has syndromic or documented evidence of being immune-compromised.
- Infant has received OPV since initial screen for poliovirus antibodies.
- Infant has history of chronic diarrhea (>14days).
- Infant is receiving immunosuppressant medication.
- Infant has a history of allergic reaction to previous doses of OPV.

5.3 Temporary Exclusion Criteria

Study treatment or placebo will be postponed for any eligible participant until recovery if the child meets any of the following criteria on the day of planned enrolment:

- Infant has a history of antibiotic use in the preceding month.
- Has an acute febrile illness (≥38.0°C axillary).
- Is recommended for hospitalization at the vaccination visit.

Study staff will visit the infant to assess eligibility according to the temporary exclusion criteria for the following two weeks until the child is able to participate, meets a permanent exclusion criteria or the study ends.

5.4 Elimination Criteria During Study

A child will be eliminated from the study if any of the following occur:

- The infant has a severe adverse reaction after treatment with azithromycin.
- The infants receives unscheduled polio vaccination during the study.
- A physician determines the child is ineligible to continue with the study.

5.5 Treatment Assignment Procedures

This study is a double-blinded, placebo controlled, randomized clinical trial design that will be conducted in a single centre in India.

5.5.1 Randomization Procedures

Eligible infants will be randomly allocated to one of the two study groups. The randomization sequence will be computer generated using a blocked randomization procedure with variable block sizes determined by an independent statistician. The allocation code for each subject will be concealed in sequentially numbered opaque covers. Prior to opening the covers, the study personnel responsible for allocation, will record the study ID, cover sequence number, date and time in the randomization register. The randomization covers will be opened and utilized only sequentially. Any change in sequence of allocation will be recorded and intimated to the PI and recorded appropriately.

5.5.2 Adherence to Randomization

Assigned arm will be recorded on the study CRF at the time of enrollment. As the participant returns for each study visit, the study team will verify the study identification number and the assigned arm before proceeding with procedures for each visit.

In the cases of breaking of the allotment schedule (i.e., the participant is provided with incorrect intervention), the investigator will record this appropriately.

5.5.3 Reasons for Withdrawal

Parents are free to withdraw study participants at any time. The investigator may also withdraw participants if the participant develops a reaction to the vaccination or any exclusion criteria. Participants will not be withdrawn from the study because they do not complete vaccination according to schedule or do not complete the course of treatment (or placebo) according to schedule.

5.5.4 Handling of Withdrawals

In the case of premature withdrawal for any reason, the investigator should exert his/her best effort to:

- Conduct an interview, if possible, to determine if the reason for withdrawal was due to an allergic reaction to the vaccine or treatment or another illness or adverse event.

- Document the reason for premature withdrawal on the CRF.

5.5.5 Termination of Study

There are no reasons expected that would result in suspension and/or termination of the study. However, it is possible that ethical review committees overseeing this study may suspend and/or terminate the study for any reason.

6 STUDY INTERVENTION

6.1 Study Products Descriptions

6.1.1 Azithromycin

Azithromycin is a broad spectrum macrolide antibiotic with a long half-life in mucosal tissues of 2-4 days (based on assessment of lung, tonsil and genital tract) [18]. An oral suspension 'Zithrox' manufactured by MacLeods Pharmaceuticals Ltd. (Mumbai) will be used in the study. After reconstitution in water the oral suspension contains 100mg of azithromycin per 5mL and the following inactive ingredients: sucrose, colloidal silicon dioxide, sodium phosphate, tribasic hydroxypropyl cellulose and artificial flavours (Table 2).

Azithromycin is licensed for use in infants in India from 6 months of age by the Drug Controller General of India and is available on the public and private sector. We will use a dosage of 10 mg/kg given once a day for 3 days, based on the recommended dosage and duration of treatment for bacterial infections (Table 3).

6.1.2 Monovalent serotype 3 oral poliovirus vaccine (mOPV3)

The oral monovalent type 3 poliomyelitis vaccine (mOPV3) to be used is manufactured by GlaxoSmithKline Biologicals and contains at least $10^{5.8}$ $CCID_{50}$ (median cell culture infective doses) of serotype 3 poliovirus, Leon-12a,b strain produced in Vero cells (Table 2). It is licensed for use in India by the Drug Controller General of India and will be donated by the manufacturer for the study. The vaccine is an oral suspension with a single dose administered as 2 drops (0.1 ml) measured using a multi-dose dropper supplied with the vaccine (Table 3).

Table 2: Product Formulation and Presentation

Product	Formulation	Presentation	Storage
Zithrox	100 mg azithromycin/5mL oral suspension Inactive ingredients: sucrose, colloidal silicon dioxide, sodium phosphate, tribasic hydroxypropyl cellulose and artificial flavours	Bottle containing azithromycin dihydrate powder for reconstitution in water.	Store dry powder below 30°C. Store constituted suspension between 5°C to 30°C and discard when full dosing is completed.
mOPV3	$10^{5.8}$ $CCID_{50}$ type 3 Sabin polio virus per dose (2 drops equal to approximately 0.1mL) Excipients: hexahydrated magnesium chloride, polysorbate 80, L-arginine,	Liquid in multidose vial of 20 doses administered using plastic squeeze dropper	The vaccine is potent if stored: • at -20°C or less until the expiry date indicated on

	purified water The vaccine fulfils WHO requirements for poliomyelitis vaccine.		the cap of plastic dispenser • at between +2°C to +8°C for up to 6 months. The expiry date on the box refers to product kept at -20°C in integral packaging.

Table 3: Product Dosage and Administration

Administration Schedule	Product	Dose	Route
Study Visit 1 (3 day course)	azithromycin or placebo	1/day	Oral
Study Visit 2 (single dose)	mOPV3	1	Oral

6.1.3 Acquisition

Study vaccines and drugs will be procured by the CMC Pharmacy for use in this study. The investigator or study coordinator, as applicable, will be personally responsible for vaccine receipt and management or will designate a person who will be responsible for these activities. The acknowledgement of receipt of vaccine will be dated and signed by the person in charge of vaccine and drug procurement.

The person in charge of vaccine receipt will verify that the cold chain was maintained during shipment of vaccine (mOPV3) through review of temperature monitoring devices and/or cold chain monitoring cards. In case of any problem or deviation, he/she will alert the study PI immediately.

6.1.4 Formulation, Packaging, and Labeling

Azithromycin will be provided as a bottle containing azithromycin dihydrate powder for reconstitution in water.

Azithromycin and placebo will be provided in an identical container/ packaging, labelled with the appropriate alphabet letter, according to the randomization code provided by the statistician. Only designated pharmacy staff involved with the study will have access to the key linking Study Groups to alphabet codes.

mOPV3 will be provided in its commercial presentation. It is provided in a liquid formulation in a 20 dose vial along with a plastic dropper for administration.

6.1.5 Product Storage, and Stability

mOPV3 stock will be stored at -20°C (+/- 5°C) at a central storage facility at the Department of Gastrointestinal Science, which has appropriate back-up power generators. Every month supplies sufficient for 1 month will be transferred to vaccine refrigerators. The vaccine will be transferred on a daily basis to the study clinic using cold storage boxes with ice. Vaccine vial monitors (VVM) will be checked for exposure to high temperatures prior to use of the vaccine. If any VVM indicate exposure to high temperatures then all the mOPV3 in the cool box will be destroyed and a fresh lot of vaccines will be transferred from the Department of Gastrointestinal Science.

Azithromycin will be stored in a cool dark room at approximately 25°C and transferred daily to the study clinic according to requirements.

6.2 Dosage, Preparation, and Administration of Investigational Product

Each day a sufficient number of bottles of azithromycin and placebo, labeled with appropriate alphabet codes will be transported to the study clinic from CMC pharmacy by a test article team. The first dose of the oral suspension will be administered under supervision of a study nurse. Subsequent doses over the 3 day course will be administered at home by the parent of the infant. Visits on the second and third day by the study staff will monitor compliance and replace any broken bottles with stock labelled with the same alphabet code.

mOPV3 will be transported daily to the study clinic in cool boxes with ice and administered as 2 drops to the infant at Study Visit 2 by a study nurse. Should a subject regurgitate or vomit after vaccination with mOPV3, a replacement dose will *not* be administered.

6.3 Accountability Procedures for the Study Intervention/Investigational Product(s)

All vaccines and the interventions/placebo will be kept in a secure place at the Department of Gastrointestinal Sciences. The investigator or the person in charge of vaccine management will maintain records of delivery of the vaccine to the trial site, the inventory at the site, the dose(s) given to each participant. The investigator or the person in charge of interventions/placebo management will maintain records of delivery of the interventions/placebo to each enrolled child.

Vaccines will be distributed to the study clinic from the storage centre at the Department of Gastrointestinal Sciences on the day of study vaccination using temperature monitored cold boxes. The quantity of vaccine provided to each clinic on each vaccination day will be dependent upon the estimated number of study participants expected to participate in each study vaccination day. Unused study vaccine will be returned in the temperature monitored cold boxes at the end of each day for secure storage at the Department of Gastrointestinal Sciences. All unused vaccine, interventions and placebos remaining at the end of the study will be destroyed.

The supply of intervention/placebo will be provided to the parent/guardian at the first study visit. The field worker will visit the child daily for three days to supervise one dose and call in the evening to remind the mother about the second daily dose. After three days, the field workers will visit the participant's home and tally used and unused interventions/placebos.

6.4 Concomitant Medications/Treatments

Among concomitant medications, only additional use of antibiotics during the study follow up period will be recorded.

7 STUDY SCHEDULE

7.1 Screening Visit:

Households in the study area with infants in the study age group will be contacted by home visit and invited to participate in the screening for serotype 3 poliovirus-specific antibodies. Each infant who meets the screening inclusion criteria will receive a screening ID and a participant information sheet will be provided. The clinical coordinator will administer informed consent for screening process and 3 ml of blood will be drawn from eligible consenting participants. Basic demographic data of those not consenting for screening will be documented in the first part of the screening form as described in the *CRF completion guidelines*.

7.2 Enrollment/Baseline and administration of study drugs (Study Visit 1)

Infants who do not have detectable serum NAb to serotype 3 poliovirus at a dilution of 1 in 4 or greater will be invited to participate in the main study by visiting the study clinic with a stool sample. At the study clinic:

1. The infant will undergo a routine physical exam by a study physician to assess eligibility for inclusion in the study.
2. Participants who are not eligible based on the inclusion/exclusion criteria will have this noted on a screening form and will be excused from study participation and the participants would be referred to CMC for a dose of IPV that would be paid for by the study.
3. Participants who are eligible based on the inclusion/exclusion criteria will be consented, assigned a unique study identification number, and randomized per defined study procedures into one of the study groups.
4. Parents will be interviewed to collect baseline demographic and health information and the appropriate parts of the CRF will be completed for all study participants.
5. The first dose of azithromycin or placebo will be administered by study staff after the stool sample has been collected, acting as a demonstration for parents on how to administer the medicine at home. Instructions on proper storage and the administration schedule will also be given. Parents will be reminded not to share or substitute products between different study participants or others at home. They will also be instructed to retain the bottle for collection by field workers. The participants will be sent home after thirty minutes of observation for immediate adverse events.
6. Parents will be provided the entire course of intervention/placebo and a stool collection kit.
7. Parents are requested to bring a stool sample collected from their child on the day before or the day of their clinic visit. Field workers will collect empty or partially used bottles at the end of three days.

7.3 Daily visits to monitor treatment adherence and safety

Participants will be visited at home by a field worker on each of the days when intervention / placebo are being administered to monitor compliance. At these visits:

1. Parents will be told of the importance of providing the intervention/placebo according to study procedures and of reporting actual doses administered. If a dose is due when the field worker visits the child the dose will be administered in the presence of the field worker.
2. Field workers will inquire regarding any illness or adverse event that occurred in the time since the last visit and record these on the study CRF. Parents will be reminded to inform the study team immediately if the child has a serious illness or they consider that the child requires hospitalization or examination by a physician. Parents will be reminded to return to the study clinic for their next study visit on day 14 for a dose of mOPV3.

7.4 Safety visits

Twice weekly safety visits will be made to the study participants homes by the field workers until 14 days after the completion of the intervention. At these visits

1. Field workers will inquire regarding any illness or adverse event that occurred in the time since the last visit and record these on the study CRF. Parents will be reminded to inform the study team immediately if the child has a serious illness or they consider that the child requires hospitalization or examination by a physician.
2. Any infant found to have developed severe diarrhea, of small volume, frequent, and associated with pain and blood in stools after treatment will be referred to hospital and in addition tested for *C. difficile.*

7.5 Vaccination visit (Study Visit 2)

The child will visit the study clinic 14 days following enrollment with a stool sample. At this visit:

1. A study clinician will inquire regarding any illness that occurred in the period following enrolment and assess eligibility to continue in the study.
2. The stool sample will be collected.
3. A subset of participants will have approximately 3-5ml sample of venous blood drawn.
4. Participants will be administered a dose of mOPV3.

7.6 Stool sample collection (Home Visit)

The subset of infants who provided a blood sample on the day of vaccination will be requested for a stool sample seven days following the mOPV3 dose (day 21) which will be collected by the study team at home and transported to the laboratory. The parents of these infants will be reminded to bring a stool sample from their child on the day of the final study visit (day 35).

7.7 Final Study Visit (Study Visit 3)

The child will visit the study clinic at 35 days following enrollment where

1. The study clinician will inquire regarding any illness that occurred since the last visit.
2. Participants will have an approximately 3-5ml sample of venous blood drawn.
3. The appropriate parts of the CRF will be completed for all study participants.
4. A stool sample will be collected from the subset of infants who provided a blood sample on the day of vaccination.
5. All participants will be exited from the study. Infants who do not have detectable serum NAb to serotype 3 poliovirus at the end of the study will be referred to CMC to receive a dose of IPV that would be paid for by the study.

7.8 Premature Withdrawal Visit

The investigator may decide to withdraw a participant from the study, should an event which is considered a definite contra-indication occur during the trial. In case of premature withdrawal, the investigator should exert his/her best effort to:

1. Collect all study documentation available on the participant.
2. Conduct a medical interview, if possible, to determine if the participant has had a medical visit since the last study visit and the reason and outcome of the visit.
3. Identify and document any SAEs since the last visit.
4. Document the reason for premature withdrawal on the CRF.

7.9 Unscheduled Visit

Parents/guardians will be instructed that they should take the child to the study clinic immediately if the child becomes unwell for evaluation by a study clinician. Participants who require urgent specialized care will be transported to CMC Hospital for further follow-up.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

A medical history will be taken from the parent/guardian accompanying the child to the clinic at the baseline visit to help study staff determine whether each potential participant is eligible for enrollment. The medical history will include information on socio-demographics; a physical examination (including vital signs and anthropometry measurements); information regarding past illnesses, especially as related to details included in the inclusion/exclusion criteria; the child's vaccination history; and history of antibiotic use in the previous month.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

There will be no clinical laboratory evaluations in this study. Laboratory assays specified in Section 8.2.2 are for research purposes only and are not to be used for clinical diagnosis or treatment.

8.2.2 Special Assays or Procedures

8.2.2.1 Serum: NAb to serotype 3 poliovirus

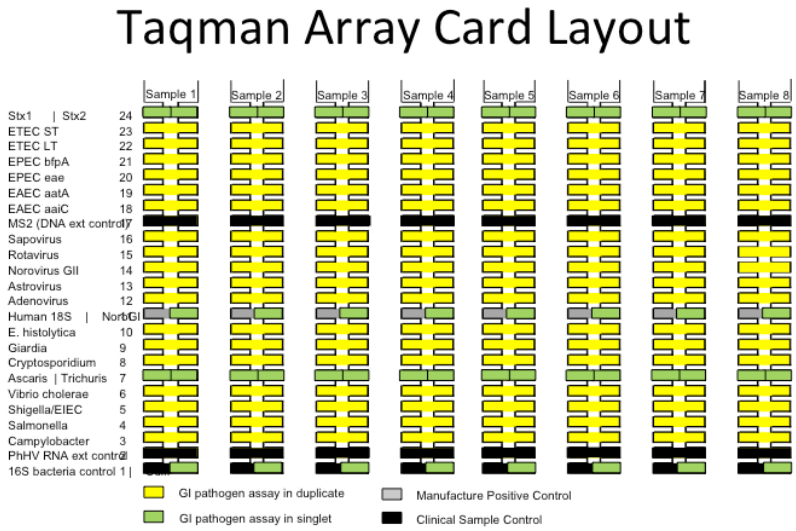
Assessment of neutralizing antibodies to polioviruses will be performed in a micro-neutralization assay. In brief, dilutions of sera are tested for their ability to neutralize Sabin polioviruses and tested for cytopathic effect on Vero cells. The assay has been standardized and qualified in the Clinical Virology laboratory at CMC. Additional information regarding the specifics of this assay is included in a detailed protocol maintained at the study laboratory.

8.2.2.2 Serum: biomarkers of intestinal inflammation

Four biomarkers of intestinal inflammation will be measured in serum in a subset of 300 children at two time points. Human intestinal fatty acid binding protein, soluble CD 14 and endotoxin core antibody are capture based sandwich ELISAs that will be performed per manufacturers' instructions. Levels of IFN-γ, TNF-α and IL-17A will be measured by using a commercially available multiplex fluorescent bead based analyte kit and analysing the samples using a flow cytometer. The serum citrulline is a high performance liquid chromatography assay that has been standardized in the Wellcome Trust Research Laboratory at CMC. If sufficient volumes of serum are available we will also measure retinoic acid in a high performance liquid chromatography assay that has been standardized in the Wellcome Trust Research Laboratory at CMC. Additional information regarding the specifics of these assays is included in detailed protocols maintained at the study laboratory.

8.2.2.3 Stool: RT-PCR for enteric pathogens using Taqman card

Stool testing for enteric pathogens will be carried out on samples collected at the time of initiation of treatment and the time of vaccination. A stool RT-PCR array has been developed in the laboratory of Dr. Eric Houpt, who has agreed to provide both the cards and the training for the assays. The array is based on individual PCR reactions for a range of common enteric pathogens, allowing for the detection of multiple pathogens from a single sample. The layout of the card and the pathogens identified and controls included in the assay are shown in the figure below. After transfer and qualification of the assays, a standard operating procedure will be developed and approved prior to initiation of testing of study samples.



card layout for illustrative purposes only.

Additional testing for *C. difficile* may be required for some children and will be carried out by a ELISA screening for toxins A and B (Techlab Cat. No. T5015) and confirmed by PCR using published primers.

8.2.2.4 Stool: biomarkers of inflammation and enteropathy

Biomarkers will be measured in the stool of a subset of 300 children at two time points. These will include neopterin, myeloperoxidase and alpha 1 anti-trypsin. These are capture based ELISA and will be performed according to manufacturers' instructions. Additional information regarding the specifics of these assays is included in detailed protocols maintained at the study laboratory.

8.2.2.5 Stool: RT-PCR for serotype 3 poliovirus

For analysis of shedding at 7 and 21 days in a subset of 200 children, a quantitative real-time PCR will be carried out, using RNA extracted using Vx reagents on a Qiaxtractor.

Complementary DNA will be generated from the eluted RNA by reverse transcription using specific and panentero primers. DNA amplification will be carried out in a ABI thermal cycler with detection using Taqman probe hybridization. A plasmid constructed by ligation of a poliovirus 3 region of the VP1 PCR fragment in TOPO-TA 2.1 vector propagated in *Escherichia coli* DH5α cells is used as a plasmid DNA standard for calibration of assay interpretation. Additional information regarding the specifics of these assays is included in detailed protocols maintained at the study laboratory.

8.2.2.6 Stool: poliovirus specific IgA

In an exploratory study on the mucosal antibody response in a subset of 300 children, poliovirus specific IgA in stool will be evaluated at 7 and 21 days. A time resolved fluorescence assay will be developed at CMC based on IgA heavy chain capture, which will detect total stool IgA and total poliovirus specific IgA. After establishment of the assay, a standard operating procedure will be developed and approved prior to initiation of testing of study samples.

8.2.2.7 PBMCs: FACS and cytokine assay

Aliquots of PBMCs will be stimulated with poliovirus, vero cell culture supernatant, staphylococcus enterotoxin B or toll-like receptor-3 (TLR3) agonist and FACS analysis of the CD4 T cell response will be examined. Specifically, we will examine Th1, Th2 and Th17 markers (CD4, IL-17A, IFN-γ and IL-4) and memory responses (CD4, CD69, CD45RO and IFN-γ). Similarly stimulated aliquots of PBMC will be centrifuged and the supernatant examined for cytokines using a multiplex bead-based assay. In particular, we will investigate IFN-γ and TNF-α for Th1, IL-4 and IL-13 for Th2, and IL-17A and IL-22 for Th17 or Th22 bias, IL-21 for follicular helper cell responses, IFN-α as an antiviral response, IL10 and TGF-β as a Th2/anti-inflammatory cytokine and IL-1, IL-6, and TNF-α together with the chemokines MIP-1α and MIP-1β as markers for macrophage activation. The cell populations of unstimulated PBMC samples will also be examined directly through FACS analysis to determine T cell phenotypes (CD3, CD4, foxp3 and CCR6) and expression of the gut-homing receptor CCR9 and the integrin $\alpha4\beta7$ (with CD4 and CD8). In total these assays will require 3.1ml of blood. Additional information regarding the specifics of these assays will be included in a detailed protocol maintained at the study laboratory.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Following universal precautions, 2-5 ml of blood will be collected from participants by venipuncture. Detailed procedures will be detailed in a blood collection standard operating procedure (SOP).

A stool specimen (minimum ½ teaspoon) will be collected by the parent/guardian into a provided universal stool container. Detailed procedures will be detailed in a stool collection SOP.

The blood and stool specimens will be stored (if permission is given by parents) for future evaluation related to the performance of vaccines and enteric infections.

8.2.3.2 Processing of blood samples

Immediately after collection (at the clinic), the sample will be properly labeled with subject identification number, date and time of sampling and transported in an ice box with adequate ice packs to maintain the temperature of 4-8^{0}C within 3 hours of collection to the laboratory. At the laboratory, specimens will be centrifuged at 3000 rpm for 5 minutes before aliquoting serum into at least 3 vials of approximately 300 to 450µl each.

In a subset of children, in whom FACS analysis is planned, blood samples will be collected in a tube with anti coagulant (3.25 ml) as well as a tube without anti coagulant (0.75ml). Serum from the tube without anti coagulant will be separated as described above.

The tube with the anti coagulant will be centrifuged at 2300 rpm for 10 minutes, to separate the plasma which will be stored. PBMC separation will be performed on the remaining cell pellet by following the density gradient method using Ficoll. The PBMCs will frozen down in liquid nitrogen and batch tested as described above in section 8.2.2.7.

8.2.3.3 Processing of Stool

Stool specimens will be sent to the laboratory for storage at 2 to 8°C at the end of each day along with a specimen transfer sheet. The stool samples will be processed as specified in a detailed stool processing protocol maintained at the study laboratory. A 20 percent stool suspension will be made for PCR testing and the remainder kept at -70˚C for storage. All handling will be done to prevent unnecessary freeze-thaw cycles.

8.2.3.4 Specimen Shipment

All testing will be done at CMC, so no shipment is planned.

9 ASSESSMENT OF SAFETY

9.1 Definitions of Safety Parameters

9.1.1 Immediate Adverse Event

An immediate adverse event is defined as a systemic reaction occurring within 30 minutes directly after receipt of the initial treatment.

9.1.2 Adverse Events

ICH E6 Good Clinical Practice Guidelines defines an Adverse Event (AE) as any untoward medical occurrence in a patient or clinical investigation subject, administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product (Test Article), whether or not related to the medicinal product (Test Article).

In this trial, these will include all reported illnesses, physical findings and abnormal laboratory findings up to 14 days following the completion of treatment with azithromycin or placebo including the days of treatment (total of 18 days). These adverse events may be expected or unexpected. The former is any adverse reaction whose nature and severity have been previously observed and documented for the study product. An unexpected adverse event is any adverse reaction not previously observed.

9.1.3 Serious Adverse Events

A serious adverse event (SAE) is defined as an event that meets one of the following conditions:

- Death.
- Life threatening (subject at immediate risk of death).
- Requires inpatient hospitalization or prolongation of hospital stay.
- Results in a persistent or significant disability or incapacity including congenital abnormality.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All SAEs will be recorded and reported as per regulatory guidelines.

Relationship to Study treatment: The clinician's assessment of a SAE's relationship to test treatment is part of the documentation process, but it is not a factor in determining what is or is not recorded in the study. If there is any doubt as to whether a clinical observation is an SAE, the event will be recorded. All SAEs will have their relationship to study treatment assessed using the terms: associated or not associated. To help assess, the following guidelines will used:

- Related: The event is temporally related to the administration of the study treatment ***and*** no other aetiology explains the event.
- Not Related: The event is temporally independent of study treatment and/or the event appears to be explained by another aetiology.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Assessing Immediate Adverse Events

Information on immediate reactions through 30 minutes post initial treatment will be documented on CRFs by a study clinician at the time of vaccination.

9.2.2 Assessing Adverse Events

Information on reactions will be documented on CRFs by a fieldworker during home visits on day 2 and 3 of treatment. Adverse events occurring up to 14 days after the final day of treatment will be documented on CRFs by a field worker during twice weekly home visits.

9.2.3 Assessing Serious Adverse Events

Information on SAEs will be documented through the duration of the study by study clinicians at the study clinic or hospital. SAEs will be collected for the duration of participation or for a minimum of one month in the event of premature withdrawal from the study.

All SAEs occurring at anytime during the study will be recorded on a SAE Form and will be reviewed and evaluated by a study clinician and the DSMB. The relationship of the SAE to study treatment will be evaluated (as outlined in Section 9.1.3) and recorded and reported (as specified in Section 9.3). All SAEs will be followed until satisfactory resolution or until the investigator and/or DSMB deem the event to be chronic or the patient to be stable.

9.3 Reporting Procedures

9.3.1 Reporting Immediate Adverse Events

Information on immediate reactions will be documented on CRFs by a study clinician at the time of treatment and entered into the safety database.

9.3.2 Reporting Adverse Events

Information on reactions up through 14 days post treatment including treatment days (total of 18 days) will be documented on CRFs by a field worker during home visits.

9.3.3 Reporting Serious Adverse Events

All SAEs occurring at any time during the study must be reported to the DSMB, CMC IRB and the DCGI, even if the investigator considers that the SAE is not related to treatment. The study clinician will complete a **Serious Adverse Event Form** within the following timelines of such events:

- All SAEs, deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the Serious Adverse Event Form and sent to CMC IRB and DCGI within 24 hours of site awareness.

9.3.4 Regulatory Reporting

SAEs will be summarized at the end of the study and a summary report will be sent to responsible ethical review committees and the Drug Controller General of India.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

SAEs occurring at any time during the study will be followed through resolution and recorded on a SAE Form. SAEs likely to be related to the product, which persist at the end of the trial will be followed up by the investigator until resolution or until the investigator and/or DSMB deem the event to be chronic or the patient to be stable. The investigator will document the date of final resolution or chronic nature of the SAE on a follow-up SAE report. All SAEs will be reported per regulatory requirements, and compensation will be paid as determined by the Compensation Committee following the CDSCO requirements.

9.5 Safety Oversight by Data Safety Monitor Board (DSMB)

The primary focus of the safety monitoring board is to review independently all serious adverse events and thoroughly investigate SAEs meeting reporting criteria (per section 9.3). Clinical and laboratory data, clinical records, and other study-related records will be made available for review. If necessary, special reports will be prepared by the investigator.

It is the responsibility of the investigators to ensure that the monitoring board is apprised of all new safety information relevant to the study vaccine and treatments in the trial. The board will receive all protocol revisions and may receive other documents related to the study.

A DSMB charter will outline the composition, responsibilities, meeting procedures, and communication strategy for the DSMB. The DSMB consists of the following members, Dr. P.S.S. Sundar Rao (Chairperson, Biostatistician, former Director, The Leprosy Mission), Dr. Thomas Kuruvilla (Paediatrician, Head of Paediatrics, Sundaram Medical Foundation, Chennai), Dr. Ashish Bavdekar (Paediatric Gastroenterologist, KEM Hospital Research Centre, Pune) and Dr. Rajesh Kumar (Public Health Physician, Head of Community Medicine, Post Graduate Institute for Medical Education and Research, Chandigarh).

9.6 Halting Rules

Given the safety profile of the treatments used in the study a premature halting of the study is unanticipated. Should safety concerns arise, the investigators in consultation with the CMC IRB and the DSMB will decide on the halting of the study. If the study is halted, parents/guardians of subjects will be contacted immediately explaining why the study has been halted and the implications for the safety and/or protection of their child. All children will be eligible for a dose of IPV at CMC.

10 STUDY MONITORING

10.1 Site Monitoring Plan

A study monitor will be designated from the Clinical Data Management Centre. The study monitor will periodically contact the site and perform on-site visits. The extent, nature and frequency of site visits will be based on such considerations as study objectives, study design and complexity, and enrollment rate; periodicity and nature of monitoring activities will be described in the *Monitoring Plan.*

10.1.1 Set-up Visit

The monitor will contact the site prior to the start of the study to discuss the protocol and data collection procedures with site personnel. Prior to enrollment of subjects at the study site, specific regulatory documents must be available, such as independent ethics committee (IEC) approvals, other IRB required approvals, and curriculum vitae for investigator and study staff.

10.1.2 Follow-up Visits

The individual responsible for monitoring the study will be given access to all records necessary to ensure the integrity/validity of the recorded data and will periodically review the progress of the study.

During sites visits and contacts, the monitor will:

- Check and assess the progress of the study.
- Review study data collected.
- Perform source data verification.
- Review regulatory files.
- Identify any issues and address their resolution.

This will be done to verify that:

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

As part of study conduct, the principal investigator in India agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and resolve any relevant issues.

10.1.3 Close-out Visit

Upon completion of the study, the study monitor and the investigator will conduct the following activities:

- Data clarification and/or resolution.
- Accounting, reconciliation, and donation or destruction at sites of unused vaccines and treatments.
- Review of site study records for completeness.
- Return of all study data to CMC.

10.2 Audits and Inspections

For the purpose of compliance with applicable regulatory guidelines it may be necessary for DCGI to conduct a site audit or inspection. This may occur at any time from start to after conclusion of the study.

The local principal investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor to discuss findings and any relevant issues.

10.3 Archiving

In accordance with applicable regulatory requirements, following closure of the study, the investigator/institution will maintain a copy of study documents in a secure and designated location at The Department of Gastrointestinal Sciences, CMC. Essential documents shall be retained for at least five (5) years after the completion or discontinuation of the study.

11 STATISTICAL CONSIDERATIONS

11.1 Overview and study objectives

The data from this study will be analyzed by the study investigators at CMC and Imperial College London. The primary objective of the study is to evaluate the serologic response to serotype 3 monovalent oral poliovirus vaccine among Indian infants who have been treated 14 days previously with a 3 day course of oral azithromycin compared with Indian infants who received a placebo. Secondary and exploratory objectives are defined in sections 3.1.2 and 3.1.3.

11.2 Study Population

The primary analysis will be performed on a per-protocol basis. A per-protocol analysis set will be based on the following:

- all participants satisfying the inclusion/exclusion criteria;
- all participants who received oral poliovirus vaccine, and azithromycin or placebo according to schedule as planned with no significant deviations;
- all participants who remain in the study through blood collection at 35 days after enrolment and have a valid serology laboratory result from that final study visit.

The per-protocol analysis set will be used for analyses of serology and will be considered as the primary approach to the analyses.

Supportive intention-to-treat (ITT) analysis will also be conducted on all enrolled participants who are randomized, received oral poliovirus vaccine and who remain in the study through blood collection at 35 days after enrolment and have a valid serology laboratory result from that final study visit. The ITT analysis set will be used for analyses of serology and will be treated as supportive to the per-protocol results.

Secondary analysis of serum and fecal biomarkers of tropical enteropathy and intestinal inflammation, lymphocyte proliferation assays and T cell assays, poliovirus shedding in stool and fecal IgA will be based on all participants for whom these laboratory results are available.

A subgroup analysis comparing poliovirus type 3 seroconversion between the study arms will be performed on those infants found to have enteric pathogens at baseline as determined by the Taqman card array.

11.3 Description of the Analyses

Primary Criteria Analysis:

- Serologic immune response to mOPV3, defined as the detection of poliovirus-specific serum NAb at day 35 of the study at a dilution of 1 in 8 or greater, will be compared between Groups 1 and 2 using Fisher's Exact test at a two-sided significance level of alpha = 0.05.

Secondary Criteria Analyses:

- The presence or absence of individual pathogens or defined pathogen coinfections will be compared to the serologic response (yes/no) to mOPV3 using Fisher's exact test. The Bonferroni correction will be used to account for multiple comparisons.
- The relationship between the serologic response (yes/no) to mOPV3 and serum and fecal biomarkers of inflammation or enteropathy at the time of vaccination on a continuous or categorical scale will be assessed using the Mann-Whitney or Fisher's exact test, as appropriate.
- The effect of treatment with a 3 day course of azithromycin on the presence of enteric pathogens in stool samples collected at 0 and 14 days will be assessed in the treatment arm using McNemar's test based on a 2 x 2 paired contingency table and a comparison between the treatment and placebo arms made using Fisher's exact test. The Bonferroni correction will be used to account for multiple comparisons.
- The effect of treatment with a 3 day course of azithromycin on the serum and fecal biomarkers of inflammation or enteropathy on a continuous or categorical scale will be assessed using a paired sign or McNemar's test, as appropriate, and comparison between the treatment and placebo arms based on the Mann-Whitney (or t-test if appropriate) or Fisher's exact test. The Bonferroni correction will be used to account for multiple comparisons.
- The relationship between the serologic response (yes/no) to mOPV3 and poliovirus shedding in at least one of the 2 stool samples collected at 7 or 21 days will be assessed using Fisher's exact test.

Exploratory Criteria Analyses (Per-Protocol):

- Additional statistical analysis to meet our exploratory objectives will be performed using appropriate statistical methods to be defined. Any analyses will undergo scientific and statistical peer-review prior to reporting.

11.4 Study Hypotheses

Primary Hypothesis

The null hypothesis is that treatment of Indian infants aged 6-11 months found to lack serum neutralizing antibodies to serotype 3 poliovirus with a three day course of azithromycin will not affect the serologic response to a subsequent dose of mOPV3 when compared with untreated infants. Our alternative hypothesis is that treatment significantly increases the serologic response, where this is defined as detection of NAb to serotype 3 poliovirus in serum taken 21 days after vaccination of study infants who were seronegative at the time of screening for study enrolment.

Secondary hypotheses

- The presence of enteric pathogens in stool samples collected at the time of vaccination with mOPV3 is associated with a reduced serologic response to mOPV3 as defined by detection of poliovirus-specific serum NAb.
- Intestinal inflammation resulting from infection with enteric pathogens and measured by serum and fecal biomarkers is associated with a reduced serologic response to mOPV3 as defined by detection of poliovirus-specific serum NAb.
- Tropical enteropathy resulting from infection with enteric pathogens and measured by serum and fecal biomarkers is associated with a reduced serologic response to mOPV3 as defined by detection of poliovirus-specific serum NAb.
- Treatment of Indian infants with a 3 day course of azithromycin will reduce the prevalence of enteric pathogens in a stool sample collected 14 days after the start of treatment and result in resolution of inflammation as measured by fecal and serum biomarkers
- Poliovirus replication can occur in some infants after vaccination with mOPV3 without stimulating a serologic response, potentially as a result of local intestinal IgA secretion and T-regulatory cell population bias

11.5 Sample Size Considerations

We estimate that a total of approximately 750 children will need to be recruited in this study to provide 90% power to detect an effect of treatment on the immunogenicity of OPV; this is based on an estimated prevalence of treatable enteric infections of 40%, an average 60% seroconversion after administration of mOPV3, an assumption that the relative immunogenicity of mOPV3 among currently infected infants compared with uninfected infants is 33% and a drop-out rate of 10%. Under the same assumed effect size this sample size will provide 99% power for a secondary comparison of OPV immunogenicity among infants who are shown to be infected with the pathogens of interest versus uninfected infants at the time of administration of the vaccine. The number of infants that will need to be screened for poliovirus antibodies to

recruit the required number of infants will depend on seroprevalence. We therefore carried out a seroprevalence survey among 100 infants aged 6-11 months who were outpatients in the main CMC hospital in Vellore. We found 39% of infants had undetectable serum neutralizing antibodies to serotype 3 poliovirus (at a 1 in 4 dilution), compared with 7% and 9% for serotypes 1 and 2 respectively. This means that approximately 2000 infants would need to be screened, which we have inflated to 2500 to ensure we recruit sufficient infants if seroprevalence changes during the course of the study. Screening will stop as soon as the required number of infants is enrolled in the study.

Sample size assumptions

a. The relative immunogenicity among infants currently infected with pathogenic enteric bacteria or protozoa is 33%. In Vellore, where we expect the average immunogenicity of mOPV3 to be 60% and where the prevalence of these enteric infections among infants in the community is approximately 40%, this implies immunogenicity among uninfected and infected infants of 82% and 27% respectively. This would imply significantly lower immunogenicity in communities with a higher prevalence of enteric infections such as Uttar Pradesh.

b. Treatment effectiveness following a 3 day course of azithromycin is 75% for the bacterial and protozoal infections under consideration. This is consistent with available data (see above) and assumes limited resistance to these antibiotics in agreement with isolates of *Campylobacter* and *E. coli* from cases of travelers diarrhea acquired in Goa and Kolkata [38].

c. Reinfection 14 days after treatment is 10%. The combined incidence of *Campylobacter* and pathogenic strains of *E. coli* among infants 6-11 months old in a cohort study in Vellore was approximately 14 per 1000 child days, which implies approximately 10% of infants will be reinfected in the 8 day period between the end of treatment effects and administration of OPV (based on the 3 day terminal half-life reported for azithromycin).

d. The main mode of action of azithromycin is through treatment of enteric pathogens. It has been suggested that azithromycin may have an anti-inflammatory mode of action based on inhibition of the NF-κB pathway and suppression of TREM-1 [39]. Clinical trials of azithromycin given to patients with cystic fibrosis as an anti-inflammatory have not, however, seen clinically significant declines in IL-8 [40] and improvements following administration of azithromycin appear restricted to individuals initially infected with *Pseudomonas* [41]. If inflammation is associated with failure of OPV to induce an immune response, then any anti-inflammatory action of azithromycin will enhance the power of the study to detect an effect of treatment. In addition, the proposed analysis of stools for specific pathogens in conjunction with measurement of inflammatory markers in serum and stools will permit any anti-inflammatory properties of azithromycin to be measured.

11.6 Planned Interim Analyses

Recruitment to the study will occur over an 18 month period. At 9 months an interim analysis of the efficacy of azithromycin against *Campylobacter* and pathogenic strains of *E. coli* will be performed. The prevalence of these pathogens in stool samples collected just prior to treatment (day 0) will be compared with their prevalence 14 days later for all individuals with completed laboratory analysis of these stool samples. If the effect of treatment against the combined prevalence of these pathogens is found to be significantly lower than 53% at day 14 (based on an expected 75% efficacy and allowing for an expected overall 10% reinfection rate; see sample size calculations) based on the likelihood ratio test for a binomial distribution, the study sample size will be increased according to the observed efficacy and sample size calculations described above (up to an upper limit to be defined by the study funders)). An increased sample size will be contingent on the agreement of a supplemental grant from the funders who have requested this interim analysis.

11.7 Safety Review

Serious adverse events (SAEs) will be summarized by group in terms of counts and percentages. Ninety-five percent confidence intervals will be provided by group at the system organ or preferred term level. The SAEs will further be described by severity and in terms of individual listing.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Primary source documents for this study are study data case report forms (CRFs), laboratory report forms, SAE hospital records, and clinic or hospital records of participant visits to clinics or hospitals at any time during the study. Only authorized study staff, ethics committee members and authorized regulatory agencies may have direct access to source documents containing study data.

13 QUALITY CONTROL AND QUALITY ASSURANCE

The study will be conducted in accordance with the procedures specified in the protocol and staff will be guided by the study manual of procedures (MoP). Study data collection forms are designed to guide staff study conduct; forms also include areas for documenting that activities did occur (even if these activities did not require recording of data) and in the appropriate sequence. All study staff must attend mandatory training prior to participant enrollment. A description of staff positions, roles, responsibilities and supervisory duties will be developed and discussed with all staff.

Individual SOPs will be developed and documented for key study procedures and refined/revised as necessary. These SOPs will be included in the study MoP at the site or in the laboratories.

Site and field monitoring will be conducted to ensure that human subject protection procedures and study procedures, including azithromycin and mOPV3 administration and clinical data and biological specimen collection are of high quality and that the study is conducted in accordance with the protocol.

Any paper CRFs will be checked for accuracy before being entered into the database. After data have been entered in the study database, they will be checked systematically by data management staff according to a pre-specified data validation plan. All listings of the database will be reviewed and discussed for assessment of consistency and medical plausibility. The database will be locked after resolution of any queries. An audit trail will be kept of all subsequent changes to the data.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigators will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki or with local regulatory requirements, whichever affords the greater protection to the subject.

14.2 Institutional Review Board

CMC maintains an Institutional Review Board (IRB). This study will be approved by CMC IRB before study start. All amendments will be approved by CMC IRB before implementation, as appropriate.

Imperial College London maintains an IRB (the Imperial College Research Ethics Committee, ICREC). This study will be reviewed by the Joint Research Office of Imperial College London and by the Head of Department. Approval will be made conditional on subsequent CMC IRB approval. Any concerns will result in full ICREC review. In this case the study will need to be approved by ICREC before implementation.

It is the responsibility of the Principal Investigator in India to ensure that this protocol and all amendments are reviewed and approved by the local Institutional Review Board responsible for the study site. In India, this is the CMC IRB. The IRB must also review and approve all informed consent forms and any other written information to be provided to the subject. Written approval from the IRBs shall be obtained prior to study start. All amendments will be approved by these ethics boards before implementation, as appropriate. The Principal Investigator in India or his/her designee shall forward copies of the IRB approvals to Imperial College London prior to the start of the study. The IRB approval letters must identify all documents approved and list the study site, the study investigator, protocol version number, date, version and title, informed consent form version number and date, and the date of approval. A list of IRB members shall be attached to the approval letter.

No deviations from, or changes to, the protocol shall be initiated without prior written IRB approval of an appropriate amendment from CMC IRB, except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study (e.g., change of telephone number, etc.).

The lead investigators will sign the final approved protocol.

14.3 Informed Consent Process

Consent forms will be translated into the local language. The investigator, or a person designated by the investigator, will fully inform the subject's parent(s)/legal guardian(s) of all

pertinent aspects of the study; and individual consent will be documented by a signature and/or signature of an impartial literate witness of the consent form.

Prior to screening and to enrolment to the study, written informed consent will be obtained from a parent/guardian for all participants. Informed consent documents will embody the elements of consent as described in the Declaration of Helsinki and the International Conference on Harmonisation (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice (GCP). Original informed consent forms will be kept in participant folders at the study site by the investigator for possible inspection by regulatory authorities. The subject or the subject's legally acceptable representative must be offered a copy of the signed and dated informed consent forms(s), and any subsequent updates or amendments.

The study monitor shall check the documentation of the individual informed consent forms during each monitoring visit.

Monetary incentives/compensation will not be provided to subjects or their parent(s)/guardian(s) for participation. Participants may be reimbursed for transportation to/from the study site.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

Children of any race/ethnicity and residing in the study area will be recruited for participation in the study. No special recruitment methods will be used to ensure certain levels of participation by any specific minorities residing in the source population. Investigators will emphasize during information sessions that girls and boys are at similar risk of acquiring poliovirus and its complications.

14.5 Subject Confidentiality

Medical information about individual subjects obtained during the course of this study is confidential and may not be disclosed to third parties, except authorized monitors, sponsors, auditors or inspectors. Confidentiality will be ensured by the use of subject number and code for the identification of each subject; these subject number and code will also be used for subject data in the subject files at the site and for the CRFs.

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological specimens in addition to the clinical information relating to participating subjects.

Study participants will not be reported by name in any report or publication resulting from data collected in this study.

Documents and data pertaining to the study will be kept in a locked room under the responsibility of the Principal Investigator. Only study investigators and designated staff will be granted access to the study data and records. Study data will be kept for 5 years after completion of the study.

The investigators will keep individual results confidential to the extent permitted by law. Information will not be released to anyone other than the participant's parent or guardian, or their medical provider, unless required to do so by law. Specimens will be identified by code only and the code key will be maintained by study personnel. The database linking the study identification number and patient identifiers will be maintained by study personnel and will be password protected. Access to this linkage and other confidential data will be strictly controlled.

14.6 Study Discontinuation

Study discontinuation is not expected to occur. However, if the study is discontinued for safety reasons, parents of participants will be informed of the reasons for discontinuation and of the implications/potential consequences for the child. If the study could gain valuable information towards the study endpoint, a serum and/or stool specimen may be collected at the time of study discontinuation.

14.7 Future Use of Stored Specimens

Biological specimens will be maintained until the end of the study to allow time for all study-related testing. The specimens will be maintained at the laboratory conducting the testing. No personal-identifying information will be used to label the specimens.

Participants will be asked whether remaining serum and stool specimens may be stored for up to five years after the study end and tested as part of future research to promote child health apart from the current study. For participants who consent to additional storage and testing, serum and stool specimens will be kept following the end of the study at CMC for future testing.

15 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

The investigator is responsible to ensure the accuracy, completeness and timeliness of the data reported. Data collection is the responsibility of the clinical study staff at the site under the supervision of the primary investigator.

Adverse events must be graded, assessed for severity and causality, and reviewed by the primary investigator or designee.

CMC Central Data Management Centre will conduct data entry, management and quality review.

15.2 Data Capture Methods

All the information required by the study protocol must be recorded on the study CRF. Data entry into CRF will be a combination of electronic and paper forms as detailed in the *CRF completion guidelines.* An explanation must be provided for any missing data.

All source documents and paper CRFs should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, the original entry will be crossed out with a single line, and changes will be initialled and dated. All source documents, electronic entries and laboratory reports will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Data will be entered from the paper CRFs to the study database by a double data entry process verifying the data with the second entry. Free text data (e.g. comments) will be entered only once and compared to the CRFs visually. Whenever possible, electronic CRFs will be completed using laptops or personal digital assistants. Further details will be provided in the study manual of operations.

The investigator or designee must sign and date paper CRFs or approve electronic CRFs online, attesting to his/her responsibility for the quality of all data recorded and that the data represent a complete and accurate record of each subject's participation in the study.

Clinical data (including SAEs) will be entered into the computer database directly using electronic forms or paper CRFs. Laboratory data will be merged with the clinical database by the study investigator or designee. Visit dates and laboratory procedure dates will be recorded on forms. All participants will be assigned a unique study participant number at study enrollment – this study participant number will be included on all forms and in the computer database and will serve to link study data to specific individuals. Data forms will be entered,

verified for accuracy, linked by study participant number, and managed using database management software.

15.3 Types of Data

Data for this study will include clinical and laboratory data (serum, PBMC and stool).

15.4 Timing/Reports

Data will be reviewed on an on-going basis throughout the study using a copy of the study database. The study monitor will also receive a copy of the study database for monitoring purposes.

15.5 Study Records Retention

It is planned that the study data will be archived at the Department of Gastrointestinal Sciences, CMC and the Central Data Management Centre for 5 years after completion of the study.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, GPP, or the MoP. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions will be implemented promptly.
It is the responsibility of the study PI or designee to use continuous vigilance to identify and report deviations to the IRB as per the IRB guidelines. All deviations from the protocol will be addressed in study subject source documents.

16 PUBLICATION POLICY

Following completion of the study, the investigators are expected to publish the results of this research in peer-reviewed scientific journal(s). Statements of authorship will follow the Uniform Requirements for Manuscripts outlined by the International Committee of Medical Journal Editors (http://www.icmje.org/ethical_1author.html).

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry. It will be the principal investigator's responsibility to register this trial with an appropriate Clinical Trials registry (www.ctri.nic.in).

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