Project Brief: CVIA 082 Study

Full Title of Study/Programme	A Phase II, Observer-blinded, Randomized, Active-controlled Study to Examine the Immunogenicity and Safety of Rotarix® and RV3-BB when Coadministered/Boosted with Trivalent P2-VP8 Subunit Rotavirus Vaccine Candidate in Healthy Infants in South Africa
Technical Focus Area	Research Pediatric
Rationale	Diarrhea is the second-leading cause of infectious disease death worldwide among children under the age of five. Rotavirus is the leading cause of severe diarrhea, resulting in an estimated 215,000 (range: 197,000–233,000) deaths in 2013 which corresponds to 3.4% of all deaths in children
Primary Objectives	Primary Objectives: Immunogenicity 1. To assess and compare the immunogenicity of each standalone LORV to matching regimens that include co-administration with TV P2-VP8, measured by LORV-specific serum anti-rotavirus IgA antibody concentration. 2. To evaluate the boosting effect of TV P2-VP8 in infants receiving a standalone LORV primary series, measured by LORV-specific serum antirotavirus IgA antibody concentration. 3. To assess and compare the immunogenicity of a birth dose of RV3-BB boosted with TV P2-VP8 to TV P2-VP8 administered alone, measured by RV3-BB specific serum anti-rotavirus IgA antibody concentration. Safety To assess the safety and tolerability of LORVs and TV P2-VP8 when administered independently, concomitantly or in a prime-boost regimen
Secondary Objectives	Immunogenicity 1. To further evaluate and compare the immunogenicity of each standalone LORV to matching regimens that include co-administration with TV P2- VP8, measured by serum anti-P2-VP8 IgG antibodies and neutralizing antibodies to each of the 3 strains of rotavirus from which TV P2-VP8 is derived. 2. To further evaluate the boosting effect of TV P2-VP8 in infants receiving a standalone LORV primary series, measured by serum anti-P2-VP8 IgG and neutralizing antibodies to each of the 3 strains of rotavirus from which TV P2-VP8 is derived. 3. To further evaluate and compare the immunogenicity of TV P2-VP8 when administered alone or after priming by a birth dose of RV3-BB, measured by serum anti-P2-VP8 IgG antibodies and neutralizing antibodies to each of the 3 strains of rotavirus from which TV P2-VP8 is derived. 4. To assess and compare the immunogenicity of co-administered LORVs and 2 doses of TV P2-VP8 (partial series) to a matching regimen receiving standalone LORV, measured by LORV-specific serum anti-rotavirus IgA antibodies, anti-P2-VP8 IgG antibodies and neutralizing antibodies to each of the 3 strains of rotavirus from which TV P2-VP8 is derived. 5. To assess and compare the immunogenicity of LORVs either coadministered or boosted with TV P2-VP8 to a regimen receiving TV P2- VP8 alone, measured by LORV-specific serum anti-rotavirus IgA antibodies, anti-P2-VP8 IgG antibodies and neutralizing antibodies to each of the 3 strains of rotavirus from which TV P2-VP8 is derived. 6. To assess and

	compare the immunogenicity of LORVs either coadministered or boosted with TV P2-VP8 to a regimen receiving TV P2- VP8 after priming with birth dose of RV3-BB, measured by serum antirotavirus IgA antibodies, anti-P2-VP8 IgG antibodies and neutralizing antibodies to each of the 3 strains of rotavirus from which TV P2-VP8 is derived. 7. To assess and compare the immunogenicity of each LORV alone to a regimen receiving TV P2-VP8 alone, measured by LORV-specific serum anti-rotavirus IgA antibodies, anti-P2-VP8 IgG antibodies and neutralizing antibodies to each of the 3 strains of rotavirus from which TV P2-VP8 is derived
Primary Endpoint/Outcome	• Immunogenicity Serum anti-rotavirus IgA antibodies will be measured in all samples using Enzyme-Linked Immunosorbent Assay (ELISA) using LORV-specific viral lysates prepared from either the RV3 or 89-12 strains. The primary immunogenicity endpoint is the geometric mean concentration of LORVspecific serum anti-rotavirus IgA antibody. Further, · The between-arm comparisons (all comparisons except for LORV alone vs TV P2-VP8-boosted LORV) will be performed by computing Geometric Mean Concentration (GMC) ratios for LORV-specific serum anti-rotavirus IgA antibody and corresponding confidence intervals. Between-arm comparisons will be conducted using samples obtained 4 weeks after the last vaccination within each regimen being compared. · The within-arm evaluation of boosting (participants immunized with LORV alone versus TV P2-VP8-boosted.) will be performed using the geometric mean fold rise (GMFR; post-boost [Week 18] vs pre-boost [Week 14]) of LORV-specific serum anti-rotavirus IgA antibody and its corresponding confidence interval. Safety Proportion of participants reporting: • Immediate adverse events, those that occur within 30 minutes of vaccination. · Local and systemic solicited adverse events (AEs) for 7 days following each vaccination. · Unsolicited AEs through 28 days after each study vaccination. · Serious Adverse Events (SAEs), including intussusception, through 28 days after last study vaccination
Secondary Endpoint/Outcome	Immunogenicity Serum anti-rotavirus IgA binding antibodies using LORV-specific viral lysates, anti-P2-VP8 IgG binding antibodies and TV P2-VP8-specific serum neutralizing antibody titers (using vaccine homologous strains) will be measured in all serum samples. For each comparison, immune responses will be evaluated in the sample obtained 4 weeks after the last vaccination for a given regimen. · Between- and within-arm comparisons for serum IgA will be performed as defined for primary objectives using GMC ratios and GMFRs, respectively. · In addition, both primary and secondary objectives involving serum IgA antibody levels will be further evaluated as described below: o Seroconversion rate for anti-rotavirus IgA antibodies (defined as percentage of participants with

	serum IgA antibody concentration ≥ 20 U/mL in participants with baseline concentration of < 20 U/mL). o Seropositivity rate for anti-rotavirus IgA antibodies (defined as percentage of participants with anti-rotavirus IgA antibody concentration ≥ 20 U/mL) will be determined at each time point. o Seroresponse rate for anti-rotavirus IgA antibody defined as follows: § ≥ 3-fold increase in concentration between pre-vaccination and 4 weeks after the last vaccination for the regimen § ≥ 4-fold increase in concentration between pre-vaccination and 4 weeks after the last vaccination for the regimen. § ≥ 4-fold increase in concentration between pre-vaccination and 4 weeks after the last vaccination for the regimen, in participants who were seropositive at baseline. Secondary objectives involving anti-P2-VP8 IgG antibodies will be evaluated by comparing: · Geometric Mean Concentration (GMC) ratios and Geometric Mean Fold Rise (GMFR) and their corresponding confidence intervals. · Seroresponse rate defined as percentage of participants with a ≥ 4-fold increase in IgG concentration between pre-vaccination and 4 weeks postvaccination. Secondary objectives involving Neutralizing antibody (NAb) titers will be evaluated comparing: · Geometric Mean Titer (GMT) ratios and Geometric Mean Fold Rise (GMFR) and their corresponding confidence intervals. · Seroresponse rate, using two definitions: · Percent of participants with a ≥ 2.7-fold increase in NAb antibody titer between pre-vaccination and 4 weeks post-vaccination paired serum samples · Percent of participants with a ≥ 4-fold increase in NAb antibody titer between pre-vaccination and 4 weeks post-vaccination serum samples. Wherever comparisons of anti-P2-VP8 IgG antibodies and neutralizing antibody levels are made between cohorts (participants with baseline measurement taken at birth, versus baseline measurement taken at W6) or where the timing of 4 week-post-last-dose sample is not common between arms (e.g., W14 vs W18), and where use of the baseline visit is necessary (GMFR, seroresponse rates), comparisons will be performed using both the observed values (e.g., observed fold-rises from the baseline measurement), as well as the adjusted fold-rises. For the adjusted fold-rises, the exponential decay rate of maternally derived antibody will be estimated, for each of these immunological assays, using the means (log scale) of the baseline data from those with measurements taken at birth and those with the baseline measurement taken at the W6 visit. As this involves assumptions that the two cohorts a) began with the same mean level of maternally derived antibody, and b) have common maternal antibody decay rates, additional alternative values may be considered from historical data sources. The GMFR and seroresponse rates will be recomputed using these adjusted fold rises. The statistical analysis plan will contain further details, such as the exclusion of particular participants from the decay rate estimation, and sensitivity analyses

Study Design	• This is a phase II, observer-blinded, multi-center, randomized and activecontrolled study enrolling healthy infants 0-6 days of age or 6-8 weeks of age. • The study will enroll infants in six arms divided into two cohorts with Cohort A enrolling infants at birth (0-6 days) and Cohort B enrolling infants at approximately 6 weeks of age. Within each cohort, the enrolled infants will be randomized to the three arms in a ratio of 6:6:5 (see below for cohort designation). Cohort A participants will receive: RV3-BB/TV P2-VP8 boost (arm 1); RV3-BB/TV P2-VP8 co-administered (arm 2); RV3-BB primed TV P2-VP8 (arm 3), while participants in Cohort B will receive: Rotarix® /TV P2-VP8 Boost (arm 4); Rotarix® /TV P2-VP8 co-administered (arm 5); or TV P2-VP8 alone (arm 6). • Rotarix® and RV3-BB will be administered orally whereas TV P2-VP8 will be administered intramuscularly in antero-lateral aspect of thigh. The study will be conducted as an observer-blinded study wherein the treatment assignment within a cohort will be blinded, although allocation to a cohort will be unblinded due to the difference in age at enrolment between the two cohorts. To maintain the blind within cohorts, infants allocated to the arms receiving only an oral rotavirus vaccine or only TV P2-VP8 vaccine on Visit 2 and 3 will receive a dose of injectable placebo or an oral placebo (PLC-OA or PLC-OB depending on the cohort), respectively, such that all infants will receive both an oral and injectable administration during these visits. • The initiation of enrollment within a cohort will be staggered, with the target to initiate enrolment of Cohort B after 6 weeks of initiating enrolment in Cohort A, in order to increase the likelihood that participants are similar in terms of baseline characteristics and any environmental exposure to wild-type rotavirus that may be circulating. • A blood sample will be obtained from all the participating infants prevaccination at Visit (V) 1 (for Cohort A)/V2 (for Cohort B) and postvaccination at week 14 and week 18 in both cohorts (appendix I). All serum samples will be tested for serum IgA antibodies using two separate ELISA assays that employ viral lysates derived from either rotavirus strains 89-12 or RV3. Serum samples will also be tested to quantitate anti-P2-VP8 IgG binding antibodies and neutralizing antibodies against the 3 rotavirus vaccine strains expressing P[4], P[6] and P[8] antigens contained in the TV P2-VP8 vaccine. • Active follow-up for vaccine reactogenicity (solicited reactions) over the 7-day period following each vaccination will be conducted on all participants receiving study vaccination. In addition, surveillance for unsolicited AEs will be carried out over the period between first study vaccination and Visit 5 (approximately 28 days after last

	vaccination with IP) and SAEs will be collected until the study is completed. • All participants in all the study arms will receive a challenge dose of Rotarix® at visit 5. Participants in study arms which received non-licensed candidate vaccines (RV3-BB and/or TV P2-VP8; arms 1-3 and 6 in Table 1 below) will receive another (second) supplemental doses of Rotarix® (standard of care in South Africa) with an interval of 4 weeks (Visit 6). Participants in other study arms will receive PLC-OB at this visit to maintain blinding. Safety of supplemental doses of Rotarix® will be ascertained by following up these infants for SAEs at Visit 6 and a phone call as Visit 7. • Stool sample will be collected in all participants 4 and 7 days after the challenge dose with Rotarix® at Visit 5 to evaluate vaccine viral shedding. Viral shedding will be investigated by EIA and PCR in stool specimens collected at these timepoints. • The partially blinded safety data will be reviewed by a PSRT (Protocol Safety Review Team) and by an independent Data and Safety Monitoring Board (DSMB). In addition, the DSMB will also perform unblinded data review at periodic intervals and as necessary on request by the PSRT team.
Study Arms	Cohort A(0-6days) Cohort B (6 weeks)
Study Population	Infants aged 0-6 days or 6-8 weeks will be enrolled in this study from two clinical sites in South Africa
Study Sample Size	850 infants
Follow-up/Duration	Total duration is 5-7 months per participant. Participants will be followed from first study vaccination (within 6 days of birth for Cohort A and 6-8 weeks of age for Cohort B) up to 28 days after the last supplemental dose of Rotarix)
Study/Programme Sites	• Wits RHI Shandukani Research Centre (SRC) • RMPRU
Study/Programme Duration	Start Date: Mar 2021 Estimated End Date:
Intervention	Trivalent P2-VP8: Trivalent rotavirus P2-VP8 subunit vaccine candidate (TV P2-VP8) is composed of recombinant, truncated VP8 rotavirus structural proteins representing the P4, P6 and P8 genotypes fused to the P2 CD4+ T-cell epitope of tetanus toxin. The purified proteins are then blended in equal amounts and co-formulated with aluminum hydroxide. Each 0.5 mL dose, to be administered intramuscularly, contains 30 µg of each of the three antigens (derived from a P[4], a P[6] and a P[8] strain of rotavirus), for a total of 90 µg of subunit protein. The three antigens are derived from DS1, 1076 and Wa rotavirus strains and formulated with 0.56 mg of aluminum hydroxide per dose. The vaccine candidate will be stored at 2-8 °C and administered intramuscularly. The vaccine candidate is manufactured by S. K. biosciences (Seoul, South Korea). Live Oral Rotavirus Vaccines (LORV) RV3-BB:

	This is a candidate vaccine based on live attenuated human rotavirus RV3 strain of G3P[6] type containing virus at titer of 9.95 x 106 FFU/mL (range 5x106 –1x107 FFU/mL dose) in serum free media supplemented with 10% sucrose. 1.0 mL of the thawed vaccine is to be administered orally. Vaccine vials will be stored at -70°C (+/-10°C) until thawed, and can be kept at 2-10°C up to 6 hours prior to administration. The vaccine candidate was developed by researchers from the Murdoch Children's Research Institute (MCRI) and manufactured by Meridian Life Sciences (Memphis, USA). Rotarix® : This is a WHO pre-qualified and licensed rotavirus vaccine. Rotarix® is a live attenuated RIX4414 strain of human rotavirus of the G1P[8] type containing not less than 106.0 CCID50 (cell culture infectious dose 50%) of the RIX4414 strain of human rotavirus. Each dose is comprised of 1.5 mL of the liquid vaccine, which will be administered orally. The vaccine is to be stored at 2-8°C. The vaccine is manufactured by GSK Biologicals' (Rixensart, Belgium) and is currently recommended in South African EPI program at 6 and 14 weeks of age. Placebo: Parenteral For both Cohorts: Normal saline by intramuscular administration (NS; PLC-I) will be used as parenteral placebo. 0.5 mL of the solution will be administered intramuscularly. Oral 1) For Cohort A: Clear, pink, cell culture medium/10% sucrose (PLC-OA). Placebo vials are to be stored at -70°C (+/-10°C) until thawed and can be kept at 2-10°C up to 6 hours prior to administration. 1.0 mL of the thawed placebo is to be administered orally. 2) For Cohort B: Clear, colorless, locally licensed Oral Rehydration Solution (PLC-OB). The ORS solution will be stored as per manufacturer's recommendation. 1.5 mL of the solution will be administered orally
Operations	Study Specific
Investigators	• Prof Lee Fairlie, Principal Investigator • Dr Gabriella Benade • Dr Masebole Masenya • Dr Faeezah Patel • Dr Elizea Horne • Dr Mrinmayee Dhar • Dr Alden Geldenhuys
Other Partners & Collaborators	• PATH • RMPRU, Soweto
Sponsors/Donors	PATH
Publications/Key Presentations to Date	None as yet
Progress Update as at Jul 2020	Screened: 9 Enrolled: 7
Frequency of Donor Narrative Report	Monthly
Overall Study/Project Contact	Dr Hermien Gous (hgous@wrhi.ac.za)
Briefing Owner and Date	