SCIENTIFIC DISCUSSION

1. Introduction

Rotavirus infection is the leading cause of severe acute gastroenteritis (GE) in infants and young children throughout the world. The incidence of rotavirus infections is highest in children between 6 and 24 months of age.
Rotavirus is transmitted mainly by the fecal-oral route through close person-to-person contact and through fomites. After the incubation period (2 -4 days), there is an abrupt onset of watery diarrhoea and vomiting, which can result in (severe) dehydration. Other common clinical findings include fever and abdominal distress. Viral shedding peaks at about day 3 of illness and then declines. The symptoms typically last from 3 to 9 days.
A recent review of epidemiological data estimated that, worldwide, rotavirus causes annually approximately 111 million episodes of gastroenteritis requiring home care, 25 million clinic visits, 2 million hospitalizations, and 352,000-592,000 deaths in children less than 5 years of age. New surveillance data suggest that the mortality rate is now estimated to be as high as 608,000 deaths annually worldwide.

The rotavirus belongs to the Reoviridae family of viruses. Rotaviruses carry three important antigenic specificities: group, subgroup and serotype. Group specificity is mainly conferred by viral protein VP6 (inner capsid protein); subgroup specificity is also determined by VP6 and has been used for characterizing the antigenic properties of various strains in epidemiologic surveys. The serotype specificities are independently determined by the outer VP4 and VP7 proteins. A binary system of rotavirus classification was established, the VP7 serotype is designated as G serotype (VP7 is a glycoprotein) whereas the VP4 serotype is designated as P serotype (VP4 is protease sensitive).
The prevalence of HRV in different regions within the same country can differ during the same year and the prevalence of individual serotypes in the same region can show a yearly change. There is no correlation between disease severity and serotype.

To date, the only licensed rotavirus vaccine has been a tetravalent (G1-4) RRV rhesus/human reassortant vaccine marketed as RotaShield by Wyeth-Lederle in 1998. Less than a year after licensure, the vaccine was withdrawn as it became clear that there was an increased risk of intussusception (IS) during the first few weeks after vaccination. As a consequence, occurrence of IS has become an important safety parameter in the evaluation of any new rotavirus vaccine. The majority of the IS cases occurred in infants who were 4 months old or older at first vaccination. This suggests that the risk of IS may have been reduced by restricting first immunization to infants younger than 4 months old.

GlaxoSmithKline Biologicals has submitted a marketing authorisation application for Rotarix (human rotavirus, live, attenuated) in accordance with Article 8.3(i) of Directive 2001/83/EC. The active ingredient of Rotarix is a live attenuated strain of human rotavirus (HRV), which belongs to the G1 serotype and the P[8] genotype. Rotarix is indicated for the active immunisation of infants from the age of 6 weeks for prevention of gastro-enteritis due to rotavirus infection. Two doses of Rotarix should be administered. The first dose may be administered from the age of six weeks and no later than the age of 14 weeks. There should be intervals of at least 4 weeks between doses. It is preferable that both doses should be administered before the age of 16 weeks. Both doses must be given by the age of 24 weeks.

2. Quality aspects

Introduction

The finished product is presented as a powder and solvent for suspension for oral use, containing not less than $10^{6.0}$ $CCID_{50}$ of human rotavirus. The lyophilised vaccine must be stored in the refrigerator at 2°C - 8°C. The lyophilised powder is presented in a vial (Type 1 glass) with a butyl rubber and flip-off cap. The finished product contains the following excipients: sucrose, dextran, sorbitol, amino acids and Dulbecco's Modified Eagle Medium (DMEM). Prior to administration, the vaccine must be reconstituted with 1 ml of diluent containing calcium carbonate, xanthan and sterile water in a prefilled syringe (Type 1 glass) fitted with a butyl rubber plunger stopper and a rubber tip cap. An overage is applied both for the lyophilised powder and the diluent in order to guarantee a potency at release of ≥ 6.2 $\log_{10}$ $CCID_{50}$ per vial and a nominal volume per dose of reconstituted vaccine of 1 ml respectively. For reconstitution, a transfer system is included, consisting of two plastic parts assembled together: a vial adaptor whose spike will pierce the vial stopper, and a soft hose that will fit tightly the syringe tip.

Active Substance

- ***Manufacture***

Manufacturing process

The active substance is manufactured at Wavre (Belgium).

Production of HRV is based on the seed lot principle. The seed lots are produced on a Vero cell substrate and the working seed (WS) is derived from the master seed (MS) lot by one additional passage. Due to limited yields, it is not possible to produce adequate amounts of rotavirus bulk directly from the working virus seed. An intermediate virus culture, derived from the working seed, is produced and then used as inoculum for the bulk vaccine production. The process followed to produce the intermediate virus culture used for inoculation is the same as that followed for the bulk vaccine, up to the virus harvest step. No clarification or DNA removal step is applied to this intermediate HRV culture inoculum. The inoculum is frozen at -70°C in sterile polyethylene containers for up to 24 months.

Vero cells derived from the manufacturer's working cell bank (WCB) at passage 136 (P136) are expanded to P142 in serum-free culture conditions. The cells are grown using T-flasks and multi-tray units (cell factories). For each passage, when the cell sheets reach confluence, the cells are washed with washing buffer and harvested using irradiated trypsin solution. The trypsin is then neutralised, and the cell suspension is diluted with medium before sub-passage. A part of the cell suspension at production level is transferred to the QC laboratory for control of uninoculated cultures and the remaining cultures are used for rotavirus propagation.

On the day of virus inoculation, virus inoculum is thawed and diluted in culture medium that contains trypsin. Confluent cell monolayers are washed after which the washing medium is removed and replaced by the diluted Rotavirus working seed or inoculum. The inoculated cell cultures are incubated at 37°C ± 1°C . In general, cytopathic effect is observed after 5 to 7 days of incubation with resulting cell layer detachment.

The culture medium is harvested and distributed in sterile polyethylene containers and frozen at –45°C for up to 24 months. Samples of the virus harvest are taken for QC testing.

Before the last steps of bulk production, harvests are thawed and pooled in a mixing vessel. Clarification is performed in order to eliminate the Vero cell debris. DNase treatment of clarified bulk

is carried out with Benzonase after which oligonucleotides are further removed by ultrafiltration: HRV bulk is submitted to a 5 to 6 fold concentration followed by diafiltration at constant volume with 5 to 6 volumes of medium. The applicant has validated the re-use of ultrafiltation/diafiltration cartridges for 6 runs with a goal of 20 runs. The Company has committed to provide validation data for the reuse of the cartridges from the next purification campaign. The purified bulks are finally sterilised by filtration through a 0.2 µm membrane.

After sterile filtration, the HRV purified bulks are filled into sterile polyethylene containers and further frozen at –45°C for up to 72 months. The bulks are then transferred for formulation and filling from the Wavre site to the Rixensart site under controlled temperature conditions. Compatibility between bulk vaccine and primary container/closure materials was retrospectively demonstrated through real-time stability studies.

Control of materials

Virus Seed Lots

The 89-12 HRV strain was isolated from the stool sample of a 15-month old infant with mild rotavirus diarrhoea in Cincinnati, USA in 1988. The original isolate was passed 26 times in Primary African Green Monkey Kidney cells (AGMK) in order to prepare seed material, referred to as P26 (J. Gamble Institute of Medical Research, Cincinnati). Initial development of the human rotavirus (HRV) vaccine was done by Virus Research Institute (VRI), now AVANT, an American Biotechnology company. The vaccine developed by AVANT was further passaged on an approved AGMK cell line to P33 (DynCorporation, MD, USA).
GSK Biologicals' HRV vaccine was developed from the P33 virus material. The P33 material was propagated on Vero cells to generate the Master Seed (P40) RVCL25A99, identified as RIX4414; the Working Seed RVCL01B99 was derived from this MS.
The production of this MS and WS was carried out on Vero cell substrate derived from WCB P136/93, originally used at the company for the production of the inactivated poliomyelitis virus (IPV). Those seed materials, produced in 1999, have been used to prepare clinical lots for Phase I and Phase II studies. However, these initial HRV seeds were not fully compliant with the TSE guideline that came into operation in May 2001 that required compliance for virus seed materials of new products (Joint CPMP/CVMP Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products). The main reason for non-compliance was the use of gelatin-derived amino acids in the preparation of the virus seeds.
Consequently, new virus seeds (MS RVCL13E02 and WS RVCL29G02) were produced in 2002 in compliance with the CPMP/CVMP TSE Guideline. These new seeds have been produced in serum-free culture on Vero cells derived from a new working cell bank prepared and stored without any addition of serum. The new seed materials have been used to produce consistency bulks for Phase III and are being used for commercial HRV vaccine production.

Specifications and test methods for HRV master and working seeds are described in GSK Biologicals' monographs. The testing program was established based on the current ICH and Ph. Eur. requirements for live attenuated viral vaccines. The identity and homogeneity of the RIX4414 isolates, virus seeds and further passages have been demonstrated by molecular characterisation. Genetic stability was further confirmed by direct comparison of the entire genomic sequence of two distinct HRV harvests derived from new seed materials with those of both initial and new working seeds. Full genomic sequences, as determined from these materials, were shown to be identical, clearly indicating high stability of HRV strain from seed storage throughout routine manufacturing process. To monitor the stability, the Applicant has committed to submit data in order to set acceptance criteria and action limits for HRV MS, WS and inoculum.
The current inoculum, which is used in actual production of the vaccine, is expected to last to 2008. This would infer that there will be many years of storage for the current WS. Therefore, the Applicant has committed to provide the stability data generated for the inoculum during the production campaign and data for the WS every two years.

Cell substrate

The Vero cell line was derived from an African Green Monkey kidney in 1962 at the Chiba University in Chiba, Japan. The cells were brought at P93 to the laboratory of Tropical Virology National Institute of Health (USA). The cell line has been further submitted to the National Institute of Health (USA) and, later, to the American Type Culture Collection (ATCC) at P113. Cells were received at GSK Bio laboratories at P124 from ATCC stock, no. CCL-81. A MCB was established in 1983 at passage 133 (MCB P133/83F). Two WCBs were established from this MCB and successively used in Rotavirus vaccine production.

Routine production of HRV commercial vaccine lots will be carried out on Vero cells derived from the serum-free WCB VEROSF/RIX4415/WCB. For the production of this WCB, 3 ampoules of MCB P133/83F were thawed and 3 passages were performed successively on cell factories in serum-free growth medium. At P136, cells were trypsinised, centrifuged and suspended in cryopreservation medium.

The cell bank qualification includes control testing at the stage of MCB, WCB and End-of-production cells (P145). The testing program was established based on current EU, WHO and FDA (USA) guidelines. The virus titres obtained during the on-going production campaign will be reported to indirectly support the stability of the WCB for 6 years. The Applicant has committed to submit stability data of the WCB in serum-free storage conditions on an annual basis.

Reagents

The materials used during manufacture of the rotavirus vaccine, together with their pharmacopoeial references were provided. The only compounds not described in a pharmacopoeia are: Benzonase, bovine serum, EGF, L-Glutamine, Ferric (III) Nitrate, Soybean Trypsin Inhibitor and VP-SFM medium. For these, the applicant referred to in-house monographs and has established specifications that provide assurance that the quality of the product is adequate for the intended use.

Trypsin is the only product of animal origin (porcine). It is obtained from porcine pancreas of US origin. Bovine serum was included in the culture medium used for adaptation of the Vero cells to serum-free culture conditions (first passage from the master cell bank). No bovine serum is used in routine production of HRV vaccine.

Controls of critical steps / Process validation

Three critical manufacturing steps have been identified during which in-process controls are performed: cell substrate preparation, virus inoculation/propagation, and harvest processing. Appropriate in-process control tests (HRV harvest potency titre, temperature during HRV harvest thawing, effectiveness of Benzonase treatment, concentration during ultrafiltration) and acceptance criteria have been provided.

The process followed to produce the intermediate inoculum is the same as followed for the bulk vaccine, up to the virus harvest step; no clarification or DNA removal step is applied. The HRV inoculum is QC tested according to the same tests, methods and specifications as those applied to the HRV bulks at the cells and virus harvest level.

For the potency titre of the HRV harvest, the Applicant has committed to set interim consistency limits which after the on-going production campaign will be based on 18 harvests; the interim limits will be revised when data from at least 30 harvests are available.

The robustness and consistency of production of the HRV active substance have been demonstrated through two production campaigns: the first one using the initial seed materials and the second one

using the new seed materials intended for commercial production. Regarding the purification process, the filtration steps applied exclude the possibility for intact Vero cells to be present in the product. In addition, the residual DNA content in commercial bulks was consistently very low and the Benzonase treatment has been shown to efficiently reduce the DNA fragment size. The Applicant has committed to complete the validation package on the effectiveness of the DNA degradation process by measuring residual high molecular weight DNA from at least 30 purified bulks for statistical significance.

Manufacturing process development

Vero cell cultures used to prepare the initial Phase I/II clinical lots were derived from a WCB produced with serum containing medium. A serum-free WCB was established and used from late Phase II clinical studies and afterwards; this WCB is also used for commercial production.
New seed materials have been produced in 2002, on Vero cells derived from the serum-free WCB. , The new seed materials have been used in Phase III vaccine development and are also used for commercial production.

Regarding the production scale, the harvest scale was doubled from initial development bulks to consistency bulks. For the consistency bulks, two harvests were pooled before purification. The current harvest scale for commercial vaccine bulk production is the same, but prepared from one single harvest. Future HRV production might reach double the size of the current harvest scale, but is outside the current scope of this marketing authorisation application.

- ***Characterisation and specifications***

Molecular characterisation studies have confirmed the genetic stability of the HRV vaccine strain throughout the *in vitro* passages that are routinely applied during the HRV production process, from the pre-master seed material (P38) up to P43 production lots and after 3 additional passages (P46). No sequence heterogeneity has been detected in the cloned material in the 6 nucleotide positions which were found to be variable in VP4 and VP7 coding genes of the non-cloned virus strain. Full genomic sequence from two different HRV harvest material and two different working seed viruses were shown to be identical, indicating the high stability of HRV strain upon seed storage and throughout routine manufacturing process.
A monkey neurovirulence test has been performed on Rotavirus Vaccine WS RVCL29G02 according to the Ph. Eur. recommendation for live attenuated vaccines. There was no unexpected clinical or histopathological evidence of involvement of the central nervous system attributable to the inoculated virus.

Potential impurities may originate from two major sources: the cell substrate (proteins, lipids and DNA) and the reagents used in the production and purification of the bulk vaccine (e.g. Benzonase). It was demonstrated that these residuals are effectively removed during the purification process.

Quality control tests (identity, potency, sterility, extraneous agents) are performed at various stages of the preparation of the vaccine bulks (cells, supernatant at the end of the observation period, single harvest and purified bulk). Specifications applied to the HRV bulks are based on ICH and Ph. Eur. requirements relevant for live attenuated vaccines.

For the potency assay, two methods of titration have been evaluated during Phase II clinical development of HRV vaccine. Both methods have the same objective of determining the infectious titre of rotavirus by inoculation of the virus on MA-104 cells. The first method was used in-house for release of initial clinical lots. This method requires counting of fluorescent foci after ~18 hours of cell infection and allows the determination of the titre expressed in ffu (foci forming unit). The second method was developed in-house and determines the dose infecting 50% of the cell culture and rotavirus titres are expressed in $CCID_{50}$. The latter method will also be used for identity testing of HRV, since a specific monoclonal antibody (VP7 specificity) is used in the test. A summary of the validation study for the test has been provided.

RVC021A44/Q is a lyophilised vaccine used as a reference standard for the potency assay. Its established titre is 6.5 $\log_{10}$ $CCID_{50}$/vial. The Applicant presented the procedures to perform the qualification of a new reference preparation and provided the acceptance criteria for any new reference.

Batch analysis results have been provided for HRV harvests and derived purified bulks that were used to prepare clinical and stability vaccine lots from early phases of vaccine development up to Phase III, as well as for the six commercial bulks produced in 2004; all complied with the specifications in force at the time of their release. For bulks used in Phase I/II development, the potency test virus titres are only expressed in ffu. For consistency bulks used in Phase III development, virus titres are expressed in $CCID_{50}$ in addition to ffu but for routine release of commercial lots the bulk virus titres are expressed in $CCID_{50}$ only.

- ***Stability***

The proposed shelf-lives are 24 months at –45°C for the virus harvest and 72 months at –45°C for the purified bulk. Three HRV harvest lots have entered a stability study for 24 months at –45°C and this storage has been demonstrated not to affect their potency and their suitability for further purification steps. Three derived HRV purified bulks are being followed in real-time stability for 72 months at –45°C, according to an approved stability protocol. The data available to date show that there is no potency loss upon storage at –45°C for at least 24 months. Additional data generated on Phase II purified bulks produced with a different Working Virus Seed and source of cell substrate from that used now, also supported the proposed shelf-life of 72 months at -45°C. The Applicant committed to submit further results on an annual basis.

Finished Product

- ***Pharmaceutical Development***

Formulation development

In initial development, the vaccine was buffered by pre-administration of a commercially available antacid, given 10-15 minutes prior to administration of the rotavirus vaccine. The current pharmaceutical form is a lyophilised vaccine to be reconstituted with an antacid diluent before oral administration, allowing to administer the HRV vaccine without pre-administration of a buffer.

The current HRV lyophilised vaccine formulation includes sucrose, dextran, sorbitol and amino acids as rotavirus stabilisers during lyophilisation (lyoprotectant). DMEM is added for bulk dilution which is applied to achieve the targeted final titre. The current liquid diluent used for vaccine reconstitution includes calcium carbonate as antacid to buffer gastric acids and to prevent HRV inactivation during passage through the stomach. Xanthan is added as thickening agent in order to increase the diluent viscosity and avoid rapid sedimentation of calcium carbonate during the diluent filling. The amount of calcium carbonate per dose has been set based on the antacid capacity as measured by the so-called "Baby Rossett-Rice" method which simulates the conditions in a two to six month-old baby stomach..

Manufacturing process development

For the formulated active substance, a short (1 day) and a long (2 days) lyophilisation cycle was established and validated.

For the diluent, initially, the sterilisation was performed at the bulk level and filling of sterile syringes was carried out in aseptic conditions. However, the sterilisation step had an impact on the xanthan thickening properties, and the diluent sedimentation rate was increased after sterilisation. Therefore, for diluent lots used in Phase III studies and for commercial production, the sterilisation is performed

on the filled syringes by autoclaving at 121.5°C for at least 20 minutes. Filling and stoppering operations are automated and take place in aseptic conditions and filling equipment is sterilised by autoclaving. The containers and closures used for the filling are sterilised before use. Sterility testing has been performed on all diluent lots produced so far. However, the diluent is a turbid liquid suspension and the method requires a sub-passage of the diluent suspension which entails a significant risk of false negative samples. Based on the information collected during the production process (in-process controls and monitoring) and the validation of the terminal sterilisation, it was accepted to replace sterility testing of samples with parametric release for diluent batches when satisfactory data from at least 30 commercial lots are available; this process was GMP inspected.

The compatibility of the vaccine and the diluent with the container-closure components has been demonstrated through stability studies. The compatibility of diluent components with the HRV active ingredient has also been validated by reconstitution assays and by clinical evaluation of the vaccine.

- ***Manufacture of the Product***

Formulation, filling and lyophilisation of the vaccine is performed at GSK Bio, Rixensart (Belgium). Commercial batch sizes may vary between several tens of thousands to maximum 168,000 vials and 180,000 diluent syringes.

The formulation of the HRV final bulk vaccine is carried out in aseptic conditions (Class 100 laminar air flow/ Grade A) in a refrigerated airlock. Stainless steel tanks are sterilised by steam (121.5°C for 60 minutes). Glassware, transfer and filtration devices, connections are sterilised by autoclave at 121.5°C for 30 minutes. Pure and pyrogen-free WFI produced by distillation is added to the formulation tank via transfer lines fitted with 0.2 µm filters.

For the manufacture of the lyophilised powder, the HRV purified bulk is thawed and then further mixed with the appropriate volume of DMEM dilution medium. The stabiliser solution (lyoprotectant) is prepared separately and then transferred into the HRV mixture and stirred. At this stage, samples are taken for QC sterility testing. Prior to filling, the final bulk is stored at 2-8°C in sterile formulation tank, under positive relative pressure.
The dilution medium and stabiliser solution are sterilised by filtration through 0.2 µm membranes.
The final bulk vaccine is aseptically filled into 3-ml depyrogenised and sterilised glass vials (type I), using an automatic filling/stoppering machine. The HRV final bulk is maintained under constant agitation during the whole filling operation. After filling, the vials are automatically partially stoppered and are transferred to a freeze-dryer for subsequent lyophilisation.

The formulation of the diluent is carried out at room temperature and takes place in mobile stainless steel tanks. At the end of the formulation, the mixture is kept in stainless steel tanks under continuous stirring until filling. The diluent bulk is distributed aseptically into 1.75 ml sterile, siliconised syringes (drawn glass, type I), using an automatic filling/stoppering machine. Diluent is maintained under constant agitation during the whole filling operation. After filling, the syringes are automatically closed with grey butyl plunger stoppers. After filling the syringes are sterilised by autoclaving at 121.5°C for at least 20 minutes. Compatibility of the primary containers for the HRV vaccine and the diluent has been demonstrated through stability studies.

Approved, inspected containers (vials and syringes) coming from the filling and/or lyophilisation are placed in boxes, palletised, quarantined and stored. For the HRV lyophilised vaccine, a preliminary storage at –20°C for no longer than 2 years may be applied prior to final labelling and packaging. For the diluent, appropriate stability data are being generated to validate its storage at non-refrigerated temperatures in addition to the storage at 2-8°C.

There is no intermediate produced between thawing of the purified bulks and end of lyophilisation. In-process controls and monitoring carried out at the successive steps of HRV vaccine preparation are described.

There is also no intermediate produced from diluent preparation to filling in glass syringes. In-process controls and monitoring carried out during diluent formulation and filling are described.
Aseptic manipulations during vaccine formulation and filling, the lyophilisation process and the terminal sterilisation of the diluent syringes have been validated.
The robustness of the production process was established retrospectively on the basis of the satisfactory batch analysis results obtained for 18 batches (including 6 commercial lots).

- ***Control of excipients***

The excipients comply with Ph. Eur. except for the amino acids for which applicable tests, specifications and methods of analysis are provided.

- ***Product Specification***

The HRV lyophilised vaccine is tested and released independently from the liquid diluent.
The product in the final container is tested for description (appearance), identity and potency ($CCID_{50}$ method), sterility, moisture content and pH. The proposed specification for potency, not less than 6.2 $\log_{10}$ $CCID_{50}$ per vaccine vial is set in order to guarantee the minimum titre of not less than 6.0 $\log_{10}$ $CCID_{50}$ per nominal dose of vaccine up to the end of shelf life. This latter lower limit in active ingredient was set based upon efficacy results obtained during clinical development of the vaccine.
The diluent in the final container is tested for description (appearance), identity (calcium salts and carbonates), pH, volume and calcium carbonate content.
The control tests performed for release of the HRV lyophilised vaccine are done after reconstitution with sterile water. However, the Applicant must first perform potency testing on the final vaccine lot where the product is reconstituted with diluent, until potency data have been submitted from three final batches of finished product that have been released with different batches of diluent (containing the same lot numbers for all of the excipients). In addition, to verify whether new batches of diluent excipients do not have a negative effect on the potency, the Applicant committed to undertake potency testing on a final vaccine lot when new diluent batches are introduced.
The specifications have been satisfactorily justified and the test methods have been adequately described and validated.
The reference standard is identical to the one described in the active substance section.

The origins of potential impurities present in the HRV vaccine are discussed in the active substance section. No impurity is introduced in the diluent throughout the formulation/filling/packaging steps.

Batch analysis results are presented for all HRV vaccine lots produced from early phases of Rotarix clinical development, as well as for the first six HRV vaccine lots produced for commercial purpose. All the vaccine batches tested complied with the specifications in force at the time of their release.
General information batch analysis results were presented for diluent lots produced from early phases of Rotarix clinical development. All the diluent batches tested from early stages of HRV vaccine development complied with the specifications in force at the time of their release.

- ***Viral safety and TSE***

The use of cell substrates in the production of the HRV vaccine could support bacterial and fungal growth. Therefore, starting materials are tested for microbiological purity according to the relevant requirements, and possible contamination during production is monitored.

No materials of bovine origin are used in the current routine production process of Rotavirus vaccine, including the master seed (MS RVCL13E02) and the current working seed (RVCL29G02), except for lactose (from bovine milk fit for human consumption) which is used as a stabiliser for porcine trypsin.

However, a few materials of bovine origin including donor calf serum, lactalbumin hydrolysate and amino acids were used in the preparation of the Vero master cell bank, MCB P133/83F, produced in 1983. Information on materials of animal origin used in the preparation of the MCB P133/83F have previously been reviewed and approved for Infanrix Penta and Infanrix Hexa.
Also donor calf serum was used in initial stages of adaptation of Vero cells to a serum-free culture process prior to the preparation of the Vero MWCB RIX4415, produced in 1999; a copy of the certificate of suitability has been provided.

Virus testing of starting materials was based on ICH, WHO and Ph. Eur. requirements. As the Company AVANT reported the presence of Bovine Parainfluenza type 3 virus (bPIV-3) in the AGMK cell line initially used for propagation of HRV, the applicant decided to test the P33 parent seed for bPIV-3 by inoculation of appropriate cell substrates (MRC5 and Vero cells), treat the P33 seed with ether before cloning by limit dilution and to conduct appropriate *in vitro* control tests for bPIV-3 at all stages of production (MS, WS, inoculum, harvest); no evidence of the presence of bPIV-3 was found.

In conclusion, the applicant has taken all the necessary precautions to avoid any contamination during production and has put in place appropriate control tests throughout manufacturing of HRV vaccine.

- ***Stability of the Product***

Stability data have been provided on the HRV lyophilised vaccine (on all clinical lots and on three lots validating the 1 day lyophilisation cycle) and on the vaccine after reconstitution with the diluent. The proposed shelf life for HRV lyophilised vaccine is 36 months at +2°C to +8°C. There is no potency loss upon storage at both –20°C and +2°C/+8°C. There is a trend towards an increase in moisture content over time, but no impact on the vaccine potency has been demonstrated. There is also no loss of potency after incubation of the lyophilised vaccine at 37°C for 7 days. Real time stability studies on commercial lots at the recommended storage temperature of +2°C to +8°C up to 36 months are ongoing.
A preliminary storage of the vaccine in final container for up to 24 months at –20°C may be applied prior to final labelling and packaging operations. The shelf life at +2°C to +8°C starts from the date the vaccine is removed from the freezer for final labeling and packaging. There is no potency loss after 2 years of storage at -20°C. Cumulative stability studies are ongoing in order to validate the storage of the lyophilised vaccine for 24 months at –20°C followed by 36 months at +2°C to +8°C.

The reconstituted vaccine has been shown to be stable for 24 hours at both 2°C-8° and 18°C-25°C.

A shelf-life of 36-months is currently proposed for the diluent, which may be stored either in the refrigerator (2-8°C) or at ambient temperature (up to 37°C). The diluent must not be frozen. Satisfactory stability data have been generated for seven diluent lots used in clinical studies of Phase II; real-time, long-term stability studies have been initiated on three Phase III diluent lots, in order to extend the proposed shelf-life from 3 to 5 years of storage at either 2-8°C or "ambient" temperature (25°C and +37°C).

Discussion on chemical, pharmaceutical and biological aspects

During the evaluation of Rotarix, no major objections were identified. A number of other concerns were identified, including the need of further stability data on the serum-free WCB, setting acceptance criteria and defining a maximum storage period for the "inoculum", and the effectiveness and validation of the Benzonase treatment.
The Applicant also claimed that the validation of the rotavirus identity test demonstrated the specificity of the VP7 specific monoclonal antibody, 2C9. Consequently, the use of the VP7 specific MAb in the potency test has made the separate identity test redundant. The Applicant has submitted additional data demonstrating the virus titre reduction in the presence of specific neutralising antibody

observed for the final products released up to now, in order to document that the identity test can be accepted as part of the potency assay.
For the HRV purified bulks, a shelf life of 72 months at –45°C was considered acceptable. The Applicant has committed to submit the results that will be generated during the long-term real-time stability studies according to the protocol given in the MAA.

The proposed tests and specifications for the control of the lyophilised vaccine and the diluent are considered adequate. To rule out any impact of the mode of reconstitution (water or diluent), the Applicant committed to submit potency data from three final vaccine batches that have been released with different batches of diluent (with the same production lot numbers for all of the excipients).

The proposed shelf-life of the HRV vaccine is 36 months at 2-8°C. The potency data, which also verifies the identity, is supportive of a 36 month shelf-life at 2-8°C. However, the Applicant has committed to generate additional stability data up to 36 months, which also includes the tests for description (appearance), sterility, moisture, pH, and abnormal toxicity. Studies are currently being conducted to determine if an extension of the shelf-life of the lyophilised vaccine for up to 5 years, by combining storage at -20°C (24 months) followed by 36 months at 2-8°C, is acceptable.

In conclusion, all quality issues are resolved and several commitments are made by the applicant, to provide further information post-approval.

3. Non-clinical aspects

Introduction

The non-clinical programme includes two non-GLP conform preliminary pharmaco-dynamic studies and one GLP-compliant repeated dose toxicity study.

Pharmacology

The selection of a non-clinical animal model for the human rotavirus strain RIX4414 has been based on the fact that mice are not susceptible to human strains and piglets seem only susceptible to a limited number of human rotavirus strains. In contrast, in neonatal Lewis rats, human group A rotavirus was shown to replicate, spread, and induce disease for up to 10 days post-inoculation and rotavirus-induced diarrhoeal disease was shown to be age-dependent.

The immunogenicity and viral shedding of the RIX4414 rotavirus vaccine strain was investigated in 3 studies, two exploratory studies and one “vaccine take report”-study, which was part of the repeated dose toxicity study

- Primary pharmacodynamics (*in vitro/in vivo*)

Development of a preclinical model in Fischer F344 rats to study human rotavirus strain

In the first exploratory study the Fischer F344 rat was used to study the susceptibility of different human rotavirus strains (Wa & RIX4414) at different ages (5-day versus 21-day old rats). The virus suspensions were administered intragastrically to groups of 5 rats, either 5 days old or 21 days old, twice at 2 weeks interval with HRV strain Wa or RIX4414 (10^6 ffu, without the addition of $CaCO_3$, which is present in the Rotarix vaccine candidate as an antacid). A control group of rats received the cell culture medium. Blood samples were taken on day 14 and 28 post dose 2 for anti-rotavirus antibody evaluations. The 5 sera of each group were pooled. An ELISA method developed by the applicant was used to evaluate the specific antibody responses. Seroconversion was defined as a 2-fold increase of optical density (OD). Viral shedding was analysed in the faeces by an ELISA developed by the applicant. Presence/absence of diarrhoea was monitored.

No rotavirus specific response was observed in 5-day old rats regardless of the human rotavirus strain administered.

A weak level of anti-rotavirus antibodies was measured at days 14 and 28 after administration of the Wa strain in 21-day old rats while a high level of antibodies was measured after administration of the RIX4414 strain.

No diarrhoea or viral shedding was observed regardless the age of the animals and the rotavirus strain administered. The applicant concluded that 21-day old Fischer 344 rats were more susceptible to human rotavirus strains than 5-day old animals.

Evaluation of seroconversion and viral shedding associated with RIX4414 with or without $CaCO_3$ in Fischer F344 rats

In the second exploratory study seroconversion and viral shedding induced by different doses of virus administered either alone or with $CaCO_3$ of different granularity was studied in 21-days old Fischer F344 rats. Groups of 5 rats were administered intragastrically twice (on days 0 and 14) with the different viral preparations of RIX4414 (10^6 ffu, 10^5 ffu or 10^4 ffu) formulated on $CaCO_3$. A positive control group was administered 10^6 ffu of RIX4414 alone. A placebo group of animals was administered with $CaCO_3$ alone.

Stool samples were taken from day 3 to day 7 post-dose 1 for viral shedding evaluation, which was assessed by detection of rotavirus specific RNA by an RT-PCR assay developed by the applicant.

Viral shedding peaked between day 4 and day 6-post virus administration and was present in the stools of each group of animals receiving the virus (10^4 ffu, 10^5 ffu or 10^6 ffu of RIX4414).

Blood samples were taken on day 14 after the first dose and day 28 after the second dose for specific anti-rotavirus Ig evaluations, using a direct ELISA.

Seroconversion was observed in 20 to 80 % of animals receiving 10^6 ffu of RIX4414 with or without $CaCO_3$.

Vaccine take report

The vaccine take/exposure of RIX4414 was investigated in the absence and or presence of $CaCO_3$ as part of the repeated dose-toxicity study in the Fischer F344 rat.

Groups of young Fischer 344 rats, (15 males and 15 females in each group), received oral 0.5 ml doses of either saline (control group), the antacid alone (80 mg of $CaCO_3$), the Rotavirus vaccine strain (RIX4414 at $10^{6.1}$ ffu) or the Rotavirus vaccine candidate (RIX4414 at $10^{6.7}$ ffu + 80 mg $CaCO_3$). Each treatment was administered orally on 4 occasions at 14 days interval. Blood samples from a subset of 10 rats of each group were taken on day 27 (13 days post second administration) and day 70 (28 days post fourth administration). Antibodies against Rotavirus were measured at these time-points in individual sera in order to assess seroconversion.

Stools were taken on day 4, 5, 6 after the first administration and on day 4, 5 and 6 after the second administration. Viral shedding in stool samples was assessed by detection of specific nucleic acid sequences of Rix4414 measured by RT-PCR.

Oral administration of Rotavirus in young Fischer rats induced seroconversion in 20 % of RIX4414 recipients and 10 % of Rotavirus vaccine candidate recipients (RIX4414 + $CaCO_3$).

Rotavirus administration induced viral shedding in 20 % of RIX4414 recipients and in 80 % of Rotavirus vaccine candidate recipients (RIX4414 + $CaCO_3$). Vaccine take was evidenced in 40 % of the RIX4414 recipients and 80 % of the vaccine candidate recipients.

- Safety pharmacology

Native rotavirus replicates in the intestine. Human rotavirus is known to cause gastroenteritis, diarrhoea, vomiting, fever and abdominal distress, intracytoplasmic eosinophilic inclusions in the intestinal villi and vacuolization, atrophy of the villi.

In the repeated dose toxicity study there was no evidence of loose stools, effects on bodyweight, food consumption, rectal temperature or histopathological signs of intestinal infection, excluding intestinal disturbances induced by the candidate vaccine.

There were no lesions induced by the candidate vaccine in lungs or heart after one and up to 4 oral administrations to young Fischer F344 rats. Therefore, a single dose study on the cardiovascular or respiratory system, as recommended for human medicines by ICH S7A, was not warranted.

Pharmacokinetics

Animal bio-distribution data in a relevant animal model, which closely mimics the viral replication, tropism and spread as in the human situation, are not available.

Toxicology

- Single dose toxicity

No separate study was performed to assess the single dose toxicity of the candidate vaccine. However, full investigations were performed after the first oral vaccine dose administration in rats, during the GLP-compliant repeated dose toxicity study.

- Repeat dose toxicity (with toxicokinetics)

The objective of the repeated dose toxicity study was to determine the toxicity of the test item (HRV/ $CaCO_3$ all in one formulation) following one or four oral (gavage) administrations to 21-day old Fischer 344 rat at two-week intervals (days 0, 14, 28, 42), in comparison with the reference item (HRV without $CaCO_3$).

Morbidity/mortality, clinical examinations, ophthalmological examination, body weights, food consumption, body temperature and clinical laboratory determinations were used as criteria to disclose any toxic effects. All surviving animals were killed for necropsy on days 5, 47 and 70. Selected organs/tissues from all animals were weighed and examined histopathologically.

No histopathological changes were seen in the intestinal villi. No pathognomonic epithelial syncytia and no intracytoplasmic eosinophilic inclusions were present in the ileum (as commonly seen during infection by Rotaviruses). Standard examination of the lymphoid organs did not reveal any changes related to the administration of the test item.

Up to four administrations of the rotavirus vaccine by the oral route at two-week intervals to 3-week old Fischer 344 rats were not associated with toxicological changes or histopathological (including gut draining mesenteric lymph nodes and Peyer's patches) lesions on days 5, 47 or 70. No differences were seen between the test and control group.

- Genotoxicity *in vitro* and *in vivo* (with toxicokinetics) /Carcinogenicity (with toxicokinetics)

Studies on genotoxic and carcinogenic potential are not applicable, since no novel adjuvants and/or additives need to be tested for genotoxicity (Note for guidance on preclinical pharmacological and toxcicological testing of vaccines, CPMP/SWP/465/95)

- Reproductive and developmental studies

Taking the intended target population for the vaccine, which includes newborns, infants and toddlers and not humans of childbearing age, as well as the nature of the product into account, studies on reproductive and developmental toxicity are not necessary (CPMP/SWP/465/95 and ICH S6).

- Local tolerance

A specific study on local tolerance has not been performed. Microscopic investigations of oesophagus, stomach, small and large intestine were reported in the repeated dose toxicity study in the rat and were not associated with histopathological lesions.

- Other toxicity studies

The potential for hypersensitivity and autoimmune reactions by Rotarix has not been investigated in an animal model. Even large-scale clinical trials cannot prove that a vaccine is completely safe in this respect. Ultimately, post marketing surveillance studies are the only tool to study whether a new vaccine can cause autoimmune disease or allergic reactions.

Ecotoxicity/environmental risk assessment

Neither the attenuated virus nor any excipient will enter the environment in quantities that raise ecological concern. Since the PEC levels are lower than the threshold or very close to the threshold of 0.001 µg/L, the Phase I ERA for the Rotarix vaccine is adequate and a Phase II assessment is not required.

4. Clinical aspects

Introduction

During the clinical development program a total of 15 clinical trials (phase I, II and III) were conducted in 20 countries. In these trials 72,111 subjects were enrolled and vaccinated. Of them, 38,441 subjects received the HRV vaccine. In addition, 1,851 subjects were enrolled and vaccinated in ongoing study 024, for which interim immunogenicity data obtained in 285 subjects are presented at in this report.

Immunogenicity of the HRV vaccine was evaluated in ten clinical trials. A total of 4,802 subjects (3,635 vaccines and 1,167 placebo recipients) were included in the according to protocol (ATP*) cohort for immunogenicity. Final immunogenicity data are available for a subset of 4,517 subjects. From one study (024) interim data (285 subjects) are available.

In Rota-023 the pivotal phase III trial 63,225 subjects were enrolled and vaccinated (31,673 vaccine and 31,552 placebo recipients). The mean duration of the efficacy follow-up was 8 months. First year efficacy results have become available. A part of the efficacy subset (13 000 subjects planned) is being followed for efficacy and safety up to 24 months of age.

The applicant has also submitted the Interim Study Report of study Rota-024 (related to the immune response to oral polio vaccine (OPV) and Rotarix).

During the evaluation procedure the applicant submitted the interim immunogenicity results of study Rota-036, which is a double-blind, randomised, placebo-controlled trial conducted in six European countries (Czech Republic, Finland, France, Germany, Italy and Spain) and which is evaluating the efficacy, safety and immunogenicity of two doses of Rotarix ($10^{6.5}$ CCID50) in healthy infants in co-administration with specific childhood vaccines including Infanrix Hexa. Only the results from the Czech Republic (post dose 3) and Finland (post dose 2) were submitted.

Safety data with the candidate HRV vaccine are available from:

- Eight studies with complete final data (003, 004, 005, 006, 007, 014, 021, 033) (7,369 subjects vaccinated)
- One study with final safety data (023), with respect to the primary safety objective (intussusception (IS) within the 0-30 day period after any dose) as well as to SAEs up to minimum one month post dose 2 is available (63,225 subjects vaccinated)
- SAEs arising from all ongoing studies up to the data lock point of August 31, 2004 are presented
- Supportive safety data are available from 3 studies with the mHRV vaccine (016, 020, 021) (893 subjects vaccinated)
- With respect to the incidence of IS, the applicant submitted the final study report of study Sero-Epi-IS-204 (999910/204), which estimates the incidence of IS in children < 24 months old in hospitals involved in study Rota-023. This study report is submitted as background information to assess the incidence of hospital-related IS in unvaccinated children under 2 years of age in the same countries as study Rota-023 (except, for the inclusion of Costa Rica and exclusion of Venezuela and Finland).

Clinical trials were performed with two formulations of HRV vaccine: the "candidate" HRV vaccine and the “modified” HRV vaccine. The same vaccine viral strain, RIX4414, is used in both the "candidate" and "modified" HRV vaccines.

The candidate HRV vaccine is the formulation for which the license is being sought and is referred to as the "HRV vaccine". The active ingredient of the HRV vaccine is a live attenuated virus derived from HRV strain belonging to G1 serotype and P8 genotype (G1P[8]). The lyophilised vaccine strain

is reconstituted in a calcium carbonate buffer directly prior to administration. The following trials were conducted with the HRV vaccine:

Clinical studies with HRV vaccine

Study no (Phase)	**Country**	**Age at vaccination (doses)**	**Concomitant vaccination**	**HRV viral concentration**	**N HRV vaccine (placebo)**
001 (Phase I)	Belgium	18-44 years (1dose)	None	Minimum $10^{6.0}$ ffu*	22 (11)
002 (Phase I)	Germany	1-3 years (1 dose)	None	$10^{4.7}$ ffu* $10^{6.4}$ ffu*	11 (6) 6 (3)
003 (Phase II)	Finland	2, 4 months (2 doses)	None	$10^{4.7}$ ffu* $10^{4.1}$ ffu $10^{4.7}$ ffu $10^{5.8}$ ffu	32 (16) 32 (16) 32 (16) 32 (16)
004 (Phase II)	Finland	2, 4 months (2 doses)	None	$10^{4.7}$ ffu	270 (135)
005 (Phase II)	USA and Canada	2, 4 months (2 doses)	DTPa, IPV, *S. pneumoniae* and Hib	$10^{5.2}$ ffu $10^{6.4}$ ffu	212 209 (108)
006 (Phase II)	Brazil, Mexico and Venezuela	2, 4 months (2 doses)¶	DTPw-HBV /Hib OPV deferred	$10^{4.7}$ ffu $10^{5.2}$ ffu $10^{5.8}$ ffu	569 570 570 (567)
007 (Phase II)	Singapore	3, 4 months (2 doses)	DTPa-IPV/Hib and HBV	$10^{4.7}$ ffu $10^{5.2}$ ffu $10^{6.1}$ ffu	510 648 653 (653)
013 § (Phase II) (Immuno.)	South Africa	6, 10, 14 weeks (3 doses) 10, 14 weeks (2 doses)	DTPw-HBV /Hib + OPV	$10^{6.5}$ $CCID_{50}$	190 190 (95)
014 (Phase II)	South Africa	6, 10 weeks[1] 10, 14 weeks[2] (2 doses)	DTPa/Hib+ OPV or DTPa-IPV/Hib	$10^{5.2}$ ffu $10^{5.2}$ ffu	148# 149# (150)
021ϕ (Phase II)	Panama	2, 4, 6 months (3 doses)	DTPw-HBV/Hib; OPV delayed-	$10^{5.2}$ ffu	177 (25)
033 (Phase III)	Mexico, Columbia, Peru	2-4 months (2 doses)	DTPw/HBV/Hib OPV deferred	$10^{6.5}$ $CCID_{50}$ (lot A) ($10^{6.1}$ ffu) (lot B) ($10^{5.8}$ ffu) (lot C) ($10^{6.1}$ ffu)	243 241 246 (124)

023‡Phase III (safety arm)	Argentina, Brazil, Chile, Colombia, Dominican Republic, Honduras, Mexico, Nicaragua, Panama, Peru, Venezuela, Finland	2, 3 to 4 months (2 doses)	DTPw-HBV /Hib OPV deferred†	$10^{6.5}$ $CCID_{50}$	31,673 (31,552)
Total					**37,635 (33,493)**
Total target population					**37,596 (33,473)**

N = number of subjects enrolled who received at least one dose of HRV vaccine or placebo
*: Vaccine reconstituted with water and Mylanta® or equivalent antacid administered 10 to 15 minutes before
vaccination. All other studies, vaccine reconstituted with calcium carbonate buffer;
§ titer reported on the final release protocol;
¶ A subset of 121 subjects in Brazil received three doses of study vaccine/placebo ;
[1] For subjects enrolled in the first study part; [2] For subjects enrolled in the second study part.
Two subjects enrolled in the HRV+OPV group and one subject enrolled in the HRV+IPV group did not receive HRV vaccine.
ϕ Study 021 comparative trial between modified formulation and vaccine formulation: the arm receiving the modified formulation is presented in Table 2. The titers at release are different between the two formulations because the modified formulation contains CaCO3. Both vaccine lots used in this clinical trial were produced using the same bulk, using the same amount of viral particules
§ study 013: final immunogenicity analysis is presented, analysis of reactogenicity and safety is ongoing.
‡ study 023: final safety data obtained up until 30-90 days post dose 2 and efficacy data up to year 1 are reported; further safety follow-up and the efficacy part is ongoing. † OPV administration in Latin American countries only

Ongoing Clinical studies with GSK Biologicals' HRV vaccine at the time of dossier submission

Study no. Phase II	**Country**	**Age at vaccination (doses)**	**Concomitant vaccination**	**HRV viral concentration**	**N HRV vaccine (placebo)**
013§ Phase II (reactgenicity, safety)	South Africa	6, 10, 14 weeks (3 doses) 10, 14 weeks (2 doses)	DTPw-HBV /Hib + OPV	$10^{6.5}$ $CCID_{50}$	190 190 (95)

023†† Phase III (efficacy, safety follow-up)	Argentina, Brazil, Chile, Colombia, Dominican Republic, Honduras, Mexico, Nicaragua, Panama, Peru, Venezuela, Finland	2, 3 to 4 months (2 doses)	DTPw-HBV /Hib OPV deferred	$10^{6.5}$ $CCID_{50}$	31,673 (31,552)†
024‡ Phase III	Brazil, Panama, Colombia, Argentina	2, 3 to 4 months (2 doses)	DTPw-HBV /Hib OPV	$10^{6.5}$ $CCID_{50}$	1234 (617)
028-029-030 Phase III	Singapore (028) HongKong (029) Taiwan (030)	3, 4 months (2 doses) (028) 2, 4 months (2 doses) (029, 030)	DTPa-IPV/Hib	$10^{6.5}$ $CCID_{50}$	4500 (4500)
036 Phase IIIb	Czech Republic Finland Germany Italy Spain France	3, 4 months 3, 5 months 2, 3 months 3, 5 months 2, 4 months 2, 3 months	Hexavalent (Meningitec in Spain and Prevenar® in Germany)	$10^{6.5}$ $CCID_{50}$	2660 (1330)

N: number of subjects enrolled to receive HRV or placebo (actual or planned)
§ study 013: final immunogenicity analysis is presented, analysis of reactogenicity and safety is ongoing.
† study 023: final safety data obtained up until 30-90 days post dose 2 and efficacy data up to year 1 are reported. Further safety follow-up and the efficacy part (in a subset of 20,170) subjects are ongoing.
‡ study 024: interim data on the serum anti-rotavirus IgA response and OPV response at 1 to 2 months after the second HRV vaccine dose on the entire immunogenicity cohort are presented

Clinical studies with modified HRV vaccine

An attempt was made to incorporate the calcium carbonate buffer and the lyophilised RIX4414 vaccine into a single vaccine formulation during phase II development. The mHRV has not been further developed. The data from studies conducted with the modified HRV vaccine are considered as supportive for safety. The following trials were conducted with the mHRV vaccine

Study no (Phase I)	Country	Age at vaccination (doses)	Concomitant vaccination	HRV viral concentration (modified formulation)	N modified HRV vaccine (placebo)
015 (Phase I)	Bangladesh	2-4 years (1 dose)	None	$10^{5.8}$ ffu $10^{6.7}$ ffu	30 30 (30)
020 (Phase II)	Costa Rica	2, 4, 6 months (3 doses)	OPV + DTPw-HBV/Hib	$10^{5.2}$ ffu $10^{5.8}$ ffu $10^{6.7}$ ffu	50 99‡ (26) 50
			DTPa-HBV-IPV/Hib	$10^{5.8}$ ffu	99 (26)
021ϕ (Phase II)	Panama	2, 4, 6 months (3 doses)	DTPw-HBV /Hib OPV delayed	$10^{5.8}$ ffu	177§ (26)
016 (Phase II)	Bangladesh	10-14 weeks 6-10-14 weeks (2 & 3 doses)	OPV and other EPI vaccines	$10^{6.7}$ ffu $10^{6.7}$ ffu	136 136 (68)
Total					**807 (176)**
Total target population					747 (146)

N = number of subjects enrolled who received at least one dose of mHRV vaccine or placebo
‡ one subject in study 020 enrolled in the $10^{5.8}$ ffu mHRV+OPV group did not receive mHRV vaccine
ϕ Study 021 was a comparative trial between modified formulation and vaccine formulation: The arm receiving the HRV formulation is presented in Table 1. The titers at release are different between the two formulations because the modified formulation contains CaCO3. Both vaccine lots used in this clinical trial were produced using the same bulk, using the same amount of viral particules.
§ one subject in study 021 enrolled in the mHRV group did not receive mHRV vaccine

(*Note:* in study Rota-021, the immune response elicited by the mHRV was compared to that elicited by the HRV vaccine).

Claimed indication:

Rotarix is indicated for the prevention of gastro-enteritis caused by Rotavirus, serotype G1 and non G1 serotypes (such as G2, G3, G4, G9).

Approved indication:

Rotarix is indicated for the active immunization of infants from the age of 6 weeks for prevention of gastro-enteritis due to rotavirus infection (see section 4.2).

In clinical trials, efficacy was demonstrated against gastro-enteritis due to rotavirus of types G1P[8], G3P[8] and G9P[8] (see sections 4.4 and 5.1).

The use of Rotarix should be based on official recommendations

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacokinetics

Pharmacokinetic studies are not applicable for this vaccine (Note for guidance on clinical development of new vaccines (CPMP/EWP/463/97))

Pharmacodynamics / Dose finding studies

The quantitative composition per nominal dose (1 ml) of reconstituted HRV vaccine is given in below

Ingredients		Quantity (per nominal dose: 1 ml)
Lyophilized vaccine in glass vial		
Active ingredient: RIX4414 strain		
	Range in clinical trials	$10^{4.1}$-$10^{6.4}$ ffu/dose ($10^{4.5}$-$10^{6.8}$$CCID_{50}$/dose)
	Commercial/final formulation	$10^{6.0}$ $CCID_{50}$/dose
Excipients:	Sucrose	9 mg
	Dextran	18 mg
	Sorbitol	13.5 mg
	Amino acids	9 mg
	DMEM (1)	2.25 mg
Liquid diluent ($CaCO_3$-based) in pre-filled syringe		
Excipients:	Calcium carbonate	60 mg
	Xanthan	3.25 mg
	Water for Injections	q. s. ad 1.0 ml

1 Dulbecco's Modified Eagle Medium

Early studies performed with a precursor vaccine (Bernstein DI 1999 and 2002) demonstrated that 2 doses of $10^{5.0}$ plaque-forming units (pfu) 89-12 had 89% vaccine efficacy.

During the clinical development program the applicant evaluated a range of viral concentrations The quantitative composition per nominal dose (1 ml) of reconstituted HRV vaccine as specified above was used. The potency of all clinical lots administered during HRV vaccine development has been evaluated using two different titration methods, and titres in focus forming units (ffu) and in cell culture infective dose (CCID50) are compared. Based on the results, viral concentration of $10^{5.5}$ ffu could be considered as representative of $10^{6.0}$ CCID50. As indicated in the proposed SPC, the end of shelf life titres should not be less than $10^{6.0}$ CCID50. The placebo was prepared by reconstituting the lyophilized excipients in the glass vial with the liquid calcium carbonate-based diluent contained in the pre-filled syringe. The reconstituted placebo had the same visual appearance as the reconstituted HRV vaccine.

The results on vaccine take, seroconversion rate and geometric mean concentrations (GMCs) were evaluated in dose-escalating studies -003, -005, -006, -007 and –021.

In study 003 higher serum anti-RV IgA seroconversion rates and GMCs were observed with increasing vaccine viral concentrations. This trend was most striking after the first vaccine dose.

In study 005 no statistical difference in the combined vaccine take between the HRV $10^{5.2}$ group and the HRV $10^{6.4}$ group was observed (p=0.153).

In study 006 there was a trend towards greater immunogenicity with an increase in viral concentration. However, seroconversion rates and vaccine take rates in the three vaccine groups post dose 2 were similar. A trend toward higher anti-rotavirus IgA antibody GMCs post dose 2 was observed in the HRV $10^{5.8}$ group.

In study 007 the seroconversion rates were higher in subjects receiving the HRV at a viral concentration of $10^{5.2}$ ffu than in subjects receiving the HRV vaccine at $10^{4.7}$ ffu. No further increase in seroconversion rates was observed in subjects who received the HRV vaccine at a viral concentration of $10^{6.2}$ ffu. However, vaccine take rates were close to 100% for all the three groups.

In study 021 three doses of HRV $10^{5.2}$ ffu and mHRV $10^{5.8}$ ffu, respectively, were compared. Seroconversion rate and , GMCs in the HRV vaccine group had a higher trend. Based on these results the further development of the mHRV vaccine was abandoned.

In study Rota-033 the lot-to-lot consistency of three lots of the HRV vaccine in terms of immunogenicity two months after Dose 2 was studied. For any pair of the three HRV vaccine lots, the limits of 90% CI for the ratio of serum anti-RV IgA antibody GMCs were within the pre-specified limits (0.5-2). Consistency of the manufacturing process was therefore demonstrated.

Clinical efficacy

Immunogenicity of the HRV vaccine was evaluated in ten clinical trials. A total of 4,802 subjects (3,635 vaccines and 1,167 placebo recipients) were included in the according to protocol (ATP*) cohort for immunogenicity. Final immunogenicity data were available for a subset of 4,517 subjects (ATP immunogenicity cohort).

- Eight of these clinical trials were completed ([Rota-003, Rota-004, Rota-005, Rota-006, Rota-007, Rota-014, Rota-021 - HRV arm, and Rota-033).
- Two trials were still ongoing. The final immunogenicity analysis is presented from study Rota-013 and an interim immunogenicity analysis from study Rota-024.

All ongoing trials are being conducted with the HRV.

Vaccine efficacy was evaluated in four trials conducted in different regions of the world; Europe (Rota-004), Latin America (Rota-006), Asia (Rota-007), and Latin America and Finland (023)

- In studies Rota-004 and Rota-006, a total of 2,214 subjects (1,637 vaccinees and 577 placebo recipients) were followed-up over the first efficacy follow-up period and a total of 802 subjects (573 vaccines and 229 placebo recipients) were followed up for up to 22 month.
- In Rota-007 the vaccine efficacy was evaluated in the total vaccinated cohort, comprising 2,421 subjects (1,779 vaccines and 642 placebo recipients), over the combined first and second year efficacy period.
- In Rota-023 the pivotal phase III trial 63,225 subjects were enrolled and vaccinated (31,673 vaccine and 31,552 placebo recipients). The mean duration of the efficacy follow-up was 8 months. A part of the efficacy subset is being followed for efficacy and safety up to 24 months of age.

Methods

Study Participants

The following table gives an overview of number of subjects enrolled in the double blind placebo controlled studies to evaluate the efficacy/immunogenicity of the candidate HRV vaccine (target population)

Study	Age	Number of subjects in the HRV group			Number of subjects in the placebo group		
		Total	ATP cohort for		Total	ATP cohort for	
(country)	At inclusion	Cohort	Immunogenicity	Efficacy (2nd year)	cohort	Immunogenicity	Efficacy (2nd year)
Study 003 (Finland)	6-12 weeks	128	116	-	64	61	-
Study 004 (Finland)	6-12 weeks	270	209	245 (241)	135	112	123 (120)
Study 005* (USA-CAN)	6-12 weeks	421	331	-	108	79	-
Study (006) (Bra, Ven, Mex)	6-12 weeks	1709	1153	1,392 (332)	567	373	454 (109)
Study 007 (Singapore)	11-17 weeks	1811	480	1,779§	653	160	642§
Study 013 (South Africa)	5-10 weeks 10-17 weeks	379	296	-	96	65	-
Study 014 (South Africa)	5-10 weeks 10-17 weeks	181 119	123 90	-	90 60	68 46	-
Study 021 (Panama)	6-12 weeks	177	152	-	25	18	-
Study 023 (Finalnd, Latin America) pivotal study	6-12 weeks	31,673	393	9009 (**)	31,552	341	8858 (**)
Study 024 (Bra, Pan, Col, Arg)	6-12 weeks	1,234	191#	-	617	94#	-
Study 033	6-12 weeks	731	494	-	124	91	
Total HRV		38,797	3,635	3,416	33,091	1,167	1,219

*In study 005 a small subset of 83 infants (67 vaccinees and 16 placebo recipients) were followed for GE in order to provide logistical information for conducting future rotavirus efficacy trials.
** The 2 year efficacy data are not available yet.
§ In study 007 efficacy was an endpoint but no accurate calculation was possible due to the low number of rotavirus GE stool samples (See Section 2.5.4.3.2). Efficacy was calculated on the total vaccinated cohort for the combined efficacy follow-up period.
# number of subjects included in the total enrolled cohort with available interim immunogenicity results.

All subjects had to fulfil the following inclusion criteria:

- Male or female between the ages specified in the protocol at the time of the first vaccination.
- Free of obvious health problems as established by medical history and clinical examination before entering into the study.
- Written informed consent obtained from the parents or guardians.
- Born after a normal gestation period (between 36 and 42 weeks).
- In study Rota-013, only subjects whose mothers were HIV negative were enrolled. The HIV status of mothers was therefore ascertained before infants were enrolled.
- In study Rota-024, only subjects who the investigator believed that their parents/guardians could and would comply with the requirements of the protocol were enrolled in the study.

The following were exclusion criteria:

- Use of any investigational or non-registered drug or vaccine other than the study vaccine within 30 days preceding the study vaccine or placebo, or planned use during the study period.
- Planned administration of a vaccine (including routine paediatric vaccines) not foreseen by the study protocol within 14 days before and after any dose of study vaccine.
- Chronic administration (defined as more than 14 days) of immunosuppressive or other immune-modifying drugs since birth (topical steroids were allowed).
- Use of antibiotics within 14 days preceding the study vaccine administration and during the first 7 days after vaccine administration.
- Any clinically significant history of chronic gastrointestinal disease or other serious medical condition as determined by the investigator.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection.
- History of allergic disease or reaction likely to be exacerbated by any component of the vaccine.
- Acute disease at time of enrolment: acute disease was defined as the presence of moderate or severe illness with fever i.e. temperature ≥100.4°F (≥ 38.0°C) as measured by a rectal thermometer.
- Vaccination should be postponed if the subject had gastro-enteritis within the past 7 days. In Rota-005 and 013 this criterion led to exclusion.
- Household contact with an immunosuppressed individual or pregnant woman.
- Administration of immunoglobulins and/or blood products since birth or planned administration during the study period.
- Previous confirmed occurrence of rotavirus gastro-enteritis.

In study 014 and 013 there were two additional exclusion criteria:

- Previous routine vaccination except BCG, HBV and OPV vaccination at birth (should be documented in the CRF).
- History of/or intercurrent polio disease

In Rota-024 there was one additional exclusion criterion: children unlikely to remain in the study area for the duration of the study

Hypersensitivity reaction due the vaccine and previous occurrence of intussusception (Rota-005, 006, 007, 013, 014, 021 and 033) were considered as absolute contraindications to further administration of HRV vaccine or placebo. Rectal temperature ≥ 100.4°C (≥ 38°C) and gastro-enteritis within 7 days preceding the study vaccine administration constituted a contraindication to administration of vaccine or placebo at that point in time; the subject could be vaccinated at a later date, within the time window specified in the protocol, or withdrawn at the discretion of the investigator.

The contraindications to the routinely co-administered vaccines are described in each of the protocols.

Design and Treatment

All studies were double blind with respect to rotavirus vaccination and were placebo controlled.

All studies were randomised. A randomisation blocking scheme was used to ensure that the balance between treatments was maintained. The randomisation number uniquely identified the vaccine dose to be administered to any subject.

In studies Rota-003 and Rota-004 feeding 1 hour prior to vaccination was prohibited. In all other studies feeding was unrestricted with respect to vaccination.

The vaccination schedule in the target population consisted of two doses of 1 ml from the age of 5 weeks onwards. An interval of at least one month was respected between the 2 doses: vaccination schedules such as 6-10 weeks, 10-14 weeks, 2-4 months, 3-4 months were assessed. The immunisation schedules used were based on the respective official country recommendations.

No routine paediatric vaccines were co-administered with the study vaccine in trials Rota-003 and Rota-004. In studies 005, 006, 007, 013, 014, 020, 021, 024 and 033, the concomitant administration of the HRV vaccine with other routinely administered vaccines was assessed for the following

antigens: DTPa, DTPa-IPV and Hib, DTPw-HBV and Hib, HBV, Hib, S. pneumoniae, OPV (oral polio vaccine) and IPV

In studies Rota-006, Rota-021 and Rota-033, the administration of OPV was separated from the administration of HRV or placebo and the co-administered routine vaccines by at least two weeks. In studies Rota-013, Rota-014 and Rota-024 OPV was co-administered with the HRV vaccine.

Objectives

The objectives for all studies were to assess efficacy and/or immunogenicity and safety of the HRV vaccine (see also study 023).

Outcomes/endpoints

Clinical efficacy was defined as prevention of rota virus gastro enteritis (RVGE) and defined as an episode of gastroenteritis occurring at least two weeks after dose 2, in which rotavirus other than vaccine strain was identified in a stool sample collected not later than 7 days after onset of symptoms. In studies 004 (Finland) and 007 (Singapore), GE was defined as presence of diarrhoea and/or vomiting. In study 006, GE was defined as presence of diarrhoea. Diarrhoea was defined as three or more looser than normal stools within a day. Vomiting was defined as one or more episodes of forceful emptying of partially digested stomach contents ≥ 1 hour after feeding within a day. GE episodes were analysed according the Vesikari scale, which takes into account the intensity and duration of diarrhoea and vomiting, the intensity of fever and dehydration as well as the need for treatment (hospitalisation). Two RVGE episodes were considered as two separate episodes if there were 5 or more symptom-free days between the episodes. Time periods during which subjects were to be followed for occurrence of gastroenteritis (GE) were specified in each study protocol.

In study Rota-023, the pivotal Phase III trial, the primary efficacy objective was to determine if two doses of HRV vaccine could prevent severe RVGE caused by the circulating wild-type RV strains.

The diagnosis of RVGE was based on the demonstration of the virus in the stools. Rotavirus antigen in stool samples collected during the efficacy follow-up were analysed by an Enzyme Linked Immunosorbent Assay (ELISA). Comparison of the results of ELISA and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) showed concordance between both methods for all moderate to severe cases. RT-PCR detected more cases; most of these cases were associated with vaccine virus shedding.

Immunogenicity

Immunogenicity of HRV was determined by testing for anti-rotavirus IgA antibodies in paired samples. A seropositive subject was defined as a subject whose antibody concentration was ≥ 20 U/ml, the assay cut-off value. Seroconversion was defined as the appearance of anti-rotavirus IgA antibody concentration ≥20 U/ml post-HRV vaccinations in subjects who were negative for anti-rotavirus IgA prior to the first vaccine administration. A serum sample was collected approximately 6-12 months after the administration of the second vaccine dose in studies 004, 005 and 006.

Immune response to concomitantly administered antigens was determined when appropriate.

Vaccine take

Vaccine take was calculated as a combined endpoint of serum IgA antibody seroconversion and/or antigen positivity in post vaccination stool samples.

Statistical methods

Efficacy analysis was performed on the per protocol cohort (primary analysis) and on the total cohort. A global overview of the number of GE episodes and RVGE episodes reported by the subjects from 2 weeks after dose 2 until approximatively one year of age was provided for studies 004, 006 and 023. The percentage of subjects reporting RVGE (any and severe intensity) was calculated by group with its 95% CI.

Results

- *Main studies*

Study 023 (pivotal study)

Study Rota-023 is the pivotal Phase III trial, which evaluated the safety and the efficacy of the vaccine (co-primary endpoints). In total 63,225 infants were enrolled at 2 and 4 months of age. The analysis of the safety data coming from these 63, 225 subjects up until study visit 3 (30 to 90 days after the 2nd dose of HRV/placebo (median time of 100 days)) is complete. In a subset of subjects the HRV vaccine to prevent severe RVGE (co-primary endpoint) was evaluated. In this subset 20,169 infants (10,159 in the HRV group and 10,010 in the placebo group) from Latin American countries were included. The infants were followed for occurrence of severe GE until 12 months of age (first follow-up period). The mean duration of the efficacy follow-up period starting at 2 weeks post-dose 2 was 8 months. First year efficacy results have become available. A part of the efficacy subset (13 000 subjects planned) is being followed for efficacy and safety up to 24 months of age.

The primary efficacy objective was, to determine if two doses of HRV vaccine could prevent severe RV GE caused by the circulating wild-type RV strains during the period starting from 2 weeks after Dose 2 until one year of age. Assuming a 1.5% incidence of severe RV GE in the placebo group during the observation period, and a 70% vaccine efficacy, the sample size of 20 000 subjects had at least 80% power to detect a lower limit of the 95% Confidence Interval (CI) for the vaccine efficacy above 50%.

The primary efficacy analysis was performed on the according-to-protocol (ATP) cohort (9,009 subjects in HRV group and 8,858 subjects in the placebo group) with severe RVGE caused by the circulating wild-type RV strains as endpoint. Demographic characteristics (age at each dose, gender distribution and race) of the two study groups were comparable.

Severe GE was defined as an episode of diarrhoea with three or more loos stools that required hospitalization and/or WHO treatment plan B or C in a medical facility. Stool samples were tested by ELISA to detect the presence of rotavirus (RV) antigen and were serotyped using a RT-PCR technique.

- **Efficacy of HRV vaccine against severe GE until one year of age**

RV infection accounted for 24% of all severe GE episodes in the placebo group.

Vaccine efficacy against severe RV GE and hospitalized RVGE from 2 weeks after Dose 2 up to Visit 4 – Study Rota-023 (ATP efficacy cohort)

			n/N			Vaccine Efficacy			
				95%CI			95%CI		
Group	N	n	%	LL	UL	%	LL	UL	P-value
Severe RV GE									
HRV	9009	12	0.1	0.1	0.2	84.7	71.7	92.4	<0.001
Placebo	8858	77	0.9	0.7	1.1				
Hospitalised RVGE									
HRV	9009	9	0.1	0.0	0.2	85.0	69.6	93.5	<0.001
Placebo	8858	59	0.7	0.5	0.9				

N = number of subjects included in each group
n/% = number/percentage of subjects reporting at least one specified RV GE episode in each group
95% CI,LL,UL = Lower and upper limits of the 95% confidence interval
P-value = two-sided Fisher's exact test (significant level of a=0.05)

A secondary analysis using the Vesikari scale to assess the intensity of GE episodes showed an efficacy against severe RVGE (i.e. score ≥ 11) was 84. 8% [95% CI: 71.1%, 92.7%].

- **Type-specific protection induced by HRV vaccine**

Multiple RV serotypes were circulating and the number of RV GE cases for each serotype was sufficiently large to make an evaluation of vaccine efficacy against severe RV GE by serotype possible (G1P[8], G2 P[4], G3 P[8] and G9 P[8]).

Vaccine efficacy against severe RV GE by RV G-type from 2 weeks after Dose 2 up to Visit 4 – Study Rota-023 (ATP efficacy cohort):

- G1P[8]: 91.8% [95% CI: 74.1% ,98.4%]

- G9P[8]: 90.6% (95% CI: 61.7%; 98.9%).
- G3P[8]: 87.7% (95% CI: 8.3%; 99.7%).
- G2P[4]: 41.0% (95% CI: -79.2%, 82.4)
- Overall P[8] serotypes: 90.9% (95% CI: 79.2-96.8)

Vaccine efficacy against severe RV GE was evaluated for each non-G1 serotype. Fewer subjects in the vaccine group than in the placebo group reported severe RVGE episodes caused by the G2 P[4] type but the difference did not reach statistical significance due to the limited number of cases observed (6 versus 10, two-sided Fisher's exact P-value = 0.328).

Study 004 (Finland)

The primary objective of the double-blind, randomized, placebo-controlled study was to determine if two doses of HRV vaccine could prevent any RV GE over one season following vaccination (primary objective). The secondary objectives addressed efficacy against severe GE during the first and second season, efficacy against any RV GE during the second season, combined efficacy over the two seasons, reactogenicity and safety. Subjects were randomized in two groups (2:1) to receive HRV vaccine ($10^{4.7}$ ffu) and placebo. Two doses of the vaccine/placebo were administered to healthy infants aged 6 to 12 weeks in a 0, 2-month schedule. All subjects received their scheduled routine vaccinations 14 days apart from the study vaccine doses. Feeding 1 hour prior to vaccination was prohibited.

In total, 405 infants (mean age 8.3 weeks) were enrolled in the study with 270 infants receiving the HRV vaccine. A statistically significant decrease in the percentage of subjects reporting any or severe rotavirus GE was detected in the HRV vaccine group as compared to the placebo group. Two doses of HRV vaccine at viral concentration $10^{4.7}$ ffu were effective in preventing severe rotavirus GE illness.

Vaccine efficacy against any and severe RV GE for 1st, 2nd and combined efficacy follow-up periods in study Rota-004.

	% observed vaccine efficacy (95% CI)		
	1st season N = 245/123	**2nd season N = 241/120**	**Combined N = 245/123**
Any RV GE	**73.0** (27.1; 90.9)	**72.8** (19.9; 91.8)	**71.6** (41.6; 86.8)
Severe RV GE	**90.0** (10.3; 99.8)	**83.4** (7.2; 98.4)	**84.9** (41.5; 97.3)

N = number of subjects included in the vaccine/placebo groups.
Severe was defined as a score with ≥11 points on the 20-point Vesikari scale.

One month after the second dose of HRV vaccine 80.4% of subjects in the HRV vaccine group were serum anti-RV IgA seropositive (GMCs: 164.0 U/ml)

Study 006 (Brazil, Mexico, Venezuela)

The primary objective of the double-blind, randomised, placebo-controlled study was to determine if two doses HRV vaccine could prevent RV GE during the first efficacy follow-up period. The secondary objectives assessed efficacy against severe RV GE during the first efficacy follow-up period, the efficacy against rotavirus serotypes during the first efficacy follow-up period; the efficacy against any and severe RV GE observed during the second efficacy follow-up period; the immune response to concomitantly administered routine vaccinations; and the effect of unrestricted feeding on the immune response to HRV vaccine. Subjects were randomised into 4 groups (1:1:1:1): 3 vaccine groups (viral concentrations: $10^{4.7}$, $10^{5.2}$ and $10^{5.8}$ ffu) and a placebo group. Two doses of the vaccine/placebo were administered to healthy infants aged 6 to 12 weeks in a 0, 2 month schedule. A subset of subject received a third dose (0, 2, 4 month schedule). Routine vaccines (DTPw-HBV, Hib) were concomitantly administered. Oral polio vaccine (OPV) was separated from the study vaccines by a 2-week period. Feeding prior to vaccination was permitted.

A total of 2,276 subjects (mean age 8.3 weeks) were enrolled in the study and a total of 1,709 received the HRV vaccine. A statistically significant decrease in the percentage of subjects reporting any or severe rotavirus GE, as well as those that were hospitalised for rotavirus GE, was detected in the HRV

group as compared to the placebo group. Efficacy to prevent severe rotavirus GE illness ranged from **65.8%** (95% CI, 32.2-83.9) to **85.6%** (95% CI, 63.0-95.6) depending on the viral concentration ($10^{4.7}$, $10^{5.2}$, $10^{5.8}$). Efficacy against a rotavirus GE episode of any severity ranged from **55.7%** (95% CI, 25.3-74.5) to **70.0 %** (95% CI, 45.7-84.4)**.** Protective efficacy against hospitalisation for rotavirus GE, ranged from **65.4%** (95% CI, -1.8 - 90.2) to **93.0%** (95% CI, 53.7-99.8).

Pooling the results from the groups in study 006 receiving the HRV vaccine with viral concentrations of $10^{5.2}$ and $10^{5.8}$ ffu results in a protective efficacy against any, severe and hospitalised rotavirus GE of 62.9%, 78.3% and 86.0%, respectively. This pooling gives a fair estimate of a protective viral concentration of $10^{5.5}$ ffu. These data support the proposed specification of the titres ($10^{6.0}$ $CCID_{50}$) at the end of the shelf-life.

Vaccine take for combined doses and anti-HRV IgA seroconversion rates and GMCs post dose 2

	HRV vaccine groups - Viral concentration (ffu)		
	$10^{4.7}$ ffu N = 142	$10^{5.2}$ ffu N = 125	$10^{5.8}$ ffu N = 124
seroconversion rate/% (95%CI)	**60.6** (52.0-68.7)	**62.4** (53.3-70.9)	**65.3** (56.3-73.6)
GMCs (U/ml) (95%CI)	**54.0** (40.9-71.2)	**52.1** (39.7-68.3)	**70.7** (51.9-96.3)
Vaccine take/% (95%CI)	**64.5** (54.6-73.5)	**72.5** (63.1-80.6)	**75.5** (66.2-83.3)

N = number of subjects in the ATP cohort for immunogenicity with available serum anti-RV IgA antibody results post dose 2. 95%CI - 95% confidence intervals
(Combined Vaccine take was assessed in 107, 109 and 106 subjects in the groups receiving the HRV vaccine at viral concentration of 104.7ffu, 105.2ffu and 105.8ffu, respectively).

Seroconversion rates and vaccine take rates in the three vaccine groups post dose 2 were similar. A trend toward higher anti-RV IgA antibody GMCs post dose 2 was observed in the $10^{5.8}$ ffu HRV vaccine group as compared to the other vaccine groups. A subset of 121 infants from Brazil received a third dose of the HRV vaccine or placebo to assess the impact on the HRV immune response. The administration of the third dose seems to further increase the immune response, however some seroconversion was observed in the placebo group, which is indicative of wild type RV infection. The majority of shedding occurred 7 days post first dose and the rate was similar in all groups. The response to HRV was similar in subjects who had been breast-fed and formula fed. The administration of the HRV vaccine did not alter the immune response to the concomitantly administered routine vaccines or the response to the three polio viral strains.

Study 007 (Singapore)

The primary objective of this double-blind, randomised, controlled study was to determine if two doses of HRV vaccine could prevent RV GE during the first efficacy follow-up. The safety, reactogenicity and immunogenicity of the HRV vaccine at 3 viral concentrations were assessed. The immune response to concomitantly administered routine vaccinations and the effect of unrestricted feeding on the immune response to HRV vaccine were evaluated. Subjects were randomised into 4 groups (1:1:1:1): 3 vaccine groups ($10^{4.7}$, $10^{5.2}$ and $10^{6.1}$ ffu) and a placebo group. Two doses of the vaccine/placebo were administered to healthy infants aged 11 to 17 weeks in a 0, 1 month schedule. Routine vaccinations (DTPa-IPV/Hib) were administered concomitantly. Feeding prior to vaccination was permitted.

A total of 2,464 subjects (mean age 13.3 weeks) were enrolled in the study and a total of 1,811 received the HRV vaccine. The study was not conclusive for efficacy due to the smaller number of documented rotavirus GE cases than expected. However, based on the 6 episodes (2 in Group HRV $10^{4.7}$ ffu and 4 in the placebo group) of rotavirus GE detected during the combined 13-month follow-up period, the vaccine efficacy for pooled vaccine groups was 82% (95% CI: -25.9 – 98.4); P-value = 0.046, which is consistent with the vaccine efficacy found in studies 004 and 006. The attack rate of rotavirus GE was much lower (0.6%) than expected (10-12%). The attack rate of rotavirus GE in Singapore may have been overestimated: there being no recent epidemiological reports on acute

gastroenteritis in the general population in Singapore, the sample size of the present study was determined based on the attack rate observed in other published studies. In addition, the parents did not collect any stool sample in 41.5 % of the GE episodes, contrary to instructions that had been given. Thus the statistical power to conclude on the efficacy endpoints was not adequete.

Vaccine take for combined doses and anti-HRV IgA seroconversion rates and GMCs post dose 2

	HRV vaccine groups - Viral concentration (ffu)		
	$10^{4.7}$ ffu N = 146	$10^{5.2}$ ffu N = 145	$10^{6.1}$ ffu N = 154
seroconversion rate/% (95%CI)	**76.0** (68.3-82.7)	**91.0** (85.2-95.1)	**88.3** (82.2-92.9)
GMCs (U/ml) (95%CI)	**123.4** (93.1-163.5)	**219.8** (175.6-275.3)	**171.2** (135.0-217.1)
Vaccine take/% (95%CI)	**100.0** (90.0-100.0)	**97.9** (88.7-100.0)	**97.8** (88.5-100.0)

N = number of subjects in the ATP cohort for immunogenicity with available serum anti-RV IgA antibody results post dose 2. 95%CI - 95% confidence intervals
(Combined Vaccine take was assessed in 35, 47 and 46 subjects in the groups receiving the HRV vaccine at viral concentration of 104.7ffu, 105.2ffu and 106.1ffu, respectively).

The seroconversion rates were higher in subjects receiving the HRV at a viral concentration of $10^{5.2}$ ffu than in subjects receiving the HRV vaccine at $10^{4.7}$ ffu. No further increase in seroconversion rates was observed in subjects who received the HRV vaccine at a viral concentration of $10^{6.1}$ ffu. Vaccine take rates were close to 100%. The majority of shedding was seen 7 days post dose 1. There was no difference in the response to concomitantly administered vaccines in the groups, which received HRV vaccine, and the group, which received placebo.

- Analysis performed across trials (Post-hoc analyses, pooled analyses AND meta-analysis)

Efficacy against RVGE by severity in Rota-004 and Rota-006

In study 023 the primary endpoint was severe RVGE. The applicant presented an additional exploratory analysis of study 004 and 006 to support prevention against any RVGE.

Percentage of subjects who reported RV GE episode(s) and efficacy of the vaccine from day of Dose 1 till the end of the first efficacy follow-up period, by RV GE severity (total vaccinated cohort)

Vaccine efficacy was higher against the most severe forms of RVGE. This finding is expected since, it has also been found that efficacy estimates were higher against the more severe forms of diseases for other vaccines (for instance pertussis and varicella).

It should be emphasized that the studies were not designed to evaluate efficacy against mild or moderate RVGE and that in both studies 004 and 006, formulations with a lower viral titer than in the commercial formulation were tested.

Study	RV GE severity	Group	N	n	n/N	95%CI		Vaccine Efficacy	95%CI	
						LL	UL	%	LL	UL
004	**any**	HRV	270	8	2.96	1.29	5.75	**69.23**	19.91	88.94
		placebo	135	13	9.63	5.23	15.90			
	mild	HRV	270	1	0.37	0.01	2.05	**83.33**	-107.6	99.68
		placebo	135	3	2.22	0.46	6.36			
	moderate	HRV	270	5	1.85	0.60	4.27	**50.00**	-117.3	88.49
		placebo	135	5	3.70	1.21	8.43			
	severe	HRV	270	1	0.37	0.01	2.05	**90.00**	10.63	99.79
		placebo	135	5	3.70	1.21	8.43			
006	**any**	HRV	1618	81	5.01	4.00	6.18	**64.16**	50.26	74.14
		placebo	537	75	13.97	11.15	17.19			
	mild	HRV	1618	21	1.30	0.81	1.98	**0.43**	-177.3	59.22
		placebo	537	7	1.30	0.53	2.67			
	moderate	HRV	1618	25	1.55	1.00	2.27	**58.51**	21.23	77.88
		placebo	537	20	3.72	2.29	5.69			
	severe	HRV	1618	36	2.22	1.56	3.07	**76.10**	62.58	84.88
		placebo	537	50	9.31	6.99	12.09			

N = number of subjects included in each group

n = number of subjects reporting at least one RV GE episode of specified intensity in each group
% = percentage of subjects reporting at least one RV GE episode of specified intensity in each group

95% CI = 95% Confidence Interval; L.L. = Lower Limit, U.L. = Upper Limit

Efficacy against G2P[4]

To allow a more precise estimation of efficacy against severe RV GE due to G2P[4], a meta-analysis of efficacy against severe RV GE (score ≥11 on the Vesikari scale) caused by G2P[4] in studies 004, 006 and 023 was performed. In the analysis all Rotarix efficacy studies with reports of RV GE due to G2P[4] were included. Since in studies Rota-004 and -006 all GE were collected, whereas in study 023 only severe GE were collected, the same definition of severe RV GE across all studies was used, ie score ≥11 on Vesikari scale.

Efficacy analysis	Group	N	n	%	95% CI LL	UL
Meta-analysis 004, 006, 023 - ATP cohort **(VE estimated as rate ratio using Mantel-Haenzel approach)**	**HRV** **Placebo**	10646 9435	5 13	**67.2**	14.8	87.1
Meta-analysis 004, 006, 023 - ATP cohort **(considering exact 95%CI, VE defined as 1-stratified Poisson rate ratio)**	**HRV** **Placebo**	10646 9435	5 13	**70.7**	10.0	92.0
Meta-analysis 004, 006, 023 - total cohort **(considering exact 95%CI, VE defined as 1-stratified Poisson rate ratio)**	**HRV** **Placebo**	12047 10682	6 15	**71.8**	20.6	91.3

Number of subjects included in each group in studies 004+006+023: HRV N =10646, placebo N =9435

n/% = number/percentage of subjects reporting at least one specified severe RV GE episode in each group

95% CI, LL, UL = Lower and upper limits of the exact 95% confidence interval

The vaccine's efficacy against severe RVGE caused by G2P[4] in study Rota-023 was 41.0% (95% CI, - 79.2%; + 82.4%). These results suggest efficacy against this particular serotype. The CHMP consider the prospectively obtained information form study 023 more relevant than the outcome of a meta-analysis.

Co-administration

The studies described below support the CHMP recommendation on co-administration of routine paediatric vaccines. The effect of the administration of the HRV vaccine on the response to routine paediatric vaccines was investigated as part of the clinical evaluation of the HRV vaccine in five trials: **Rota-005**, Rota-006 (see above, main studies), Rota-007 (see above, main studies), **Rota-013 and Rota-014**.

Study 005 (USA)

The primary objective of the study was to assess the reactogenicity and immunogenicity of two doses of HRV vaccine at different viral concentrations in infants of approximately 2 months of age while permitting unrestricted feeding and administration of other routine childhood vaccinations concomitantly with HRV vaccine. The study was double-blind, randomized, placebo-controlled, with three parallel groups. Subjects were randomized (2:2:1) in three groups: 2 groups received the HRV vaccine (viral concentrations $10^{5.2}$ ffu or $10^{6.4}$ ffu) and the third group received placebo. Two doses of the vaccine/placebo were administered to healthy infants aged 6 to 12 weeks in a 0, 2 month schedule. Routine vaccines (DTPa, Hib, IPV, 7Pn) were concomitantly administered and feeding prior to vaccination was permitted.

A total of 529 subjects (mean age 8.5 weeks) were enrolled in the study. A total of 421 subjects received the HRV vaccine.

Vaccine take for combined doses (combined vaccine take) and anti-HRV IgA seroconversion rates and GMCs post dose 2

	HRV vaccine groups: Viral concentration (ffu)	
	$10^{5.2}$ ffu N = 138	**$10^{6.4}$ ffu N = 133**
Combined Vaccine take (%)* *(95% CI)*	***81.5*** *(74.6-87.3)*	***88.0*** *(81.7-92.7)*
seroconversion rate % (95%CI)	**67.4** (58.9-75.1)	**78.2** (70.2-84.9)
GMCs (U/ml) (95%CI)	**80.1** (59.9-107.0)	**117.0** (88.3-154.9)

* primary endpoint. Combined Vaccine take

N = number of subjects in the ATP cohort for immunogenicity with available serum anti-RV IgA antibody results post dose 2. 95%CI - 95% confidence intervals

There was no statistical difference in the combined vaccine take (co-primary endpoint) between the two vaccine groups (p = 0.153). The majority of shedding occurred 7 days post first dose and was similar between the two groups. The vaccine take rates were slightly lower in subjects who had been breast-fed compared to those who had been formula fed. The administration of the HRV vaccine did not alter the response to the routinely administered vaccines.

Study 013 (South Africa)

The main objective of this double-blind, randomized, controlled trial was to compare the immune response to the HRV vaccine after 2 and 3 doses. The secondary objectives were to assess the safety and reactogenicity of the HRV vaccine and evaluate the response to OPV in all subjects. Subjects were randomised in parallel groups with an unbalanced allocation (2:2:1). One vaccine group received two doses of vaccine at 10 and 14 weeks of age, while the second vaccine group received three doses of vaccine at 6, 10 and 14 weeks of age. The third group received three doses of placebo. The HRV vaccine had a viral concentration of $10^{6.5}CCID_{50}$. All routine vaccines, including OPV, were administered according to EPI schedule. All subjects were followed for the occurrence of GE through the RV season

A total of 475 subjects were enrolled (mean age 6.4 weeks), 190 subjects received 2 doses of HRV, 189 subjects received 3 doses of HRV and 96 subjects received 3 doses of placebo.

After two doses of HRV vaccine administered at the age of 10 and 14 weeks concomitantly with OPV, the seroconversion rate was 44.3% (95% CI, 35.6-53.2) which is similar to the seroconversion rate observed after three doses of HRV vaccine co-administered with OPV at the age of 6, 10 and 14 weeks, (44.4%; 95% CI, 35.8-53.2). Seroconversion among placebo recipients was low (1.7%). Therefore no marked increase in response to HRV vaccine was achieved by the administration of a dose at 6 weeks of age.

Study 014 (South Africa)

The primary objective of this double-blind controlled study was to demonstrate that co-administration of HRV vaccine did not decrease the poliovirus immune response one month after the third dose of polio vaccine. The safety, reactogenicity and immunogenicity of the HRV vaccine when concomitantly administered with OPV(oral polio vaccine) or IPV (inactivated polio vaccine) were also assessed. Subjects were randomised in three groups (1:1:1): group 1 (HRV vaccine + OPV + DTPa/Hib); group 2 (HRV vaccine + DTPa-IPV/Hib) and group 3 (Placebo + OPV + DTPa/Hib). The study was conducted in two parts, part 1 prior to the start of the 2002 RV season and part 2 after the end of the 2002 RV season. In part 1, two doses of the vaccine/placebo were administered at 6 and 10 weeks of age. In part 2, two doses of the vaccine/placebo were administered at 10 and 14 weeks of age. The HRV viral concentration was $10^{5.2}$ ffu. Routine vaccinations were concomitantly administered and unrestricted feeding was permitted.

A total of 450 (271 in part 1 and 179 in part 2) subjects (mean age 6.2 weeks in part 1 and 10.9 weeks in part 2) were enrolled in the study.

The co-administration of the HRV vaccine with OPV did not significantly decrease the response to the polioviruses post dose 3. The seroconversion rates, GMCs and HRV vaccine take rates one month post dose 1 and 2 are below.

Vaccine take rates, anti-HRV IgA seroconversion rates and GMCs post dose 1 and 2 in infants receiving 2 doses of HRV vaccine with and without OPV concomitantly administered

	HRV vaccine groups: Viral concentration (ffu)			
	Group 1: $10^{5.2}$ ffu + OPV		**Group 2:** $10^{5.2}$ ffu + IPV	
	Post dose 1	**Post dose 2**	Post dose 1	**Post dose 2**
Part 1:	N = 60	**N = 53**	N = 58	**N = 49**
seroconversion rate % (95%CI)	13.3 (5.9-24.6)	**35.8** (23.1-50.2)	32.8 (21.0-46.3)	**42.9** (28.8-57.8)
GMCs (U/ml) (95%CI)	<20	**28.1** (18.2-43.2)	23.9 (16.3-35.0)	**32.6** (20.7-51.3)
	N = 34	N = 32	N = 40	N = 35
Vaccine take (%) (95% CI)	14.7 (5.0-31.1)	**37.5** (21.1-56.3)	27.5 (14.6-43.9)	**40.0** (23.9-57.9)
Part 2:	N = 41	**N = 38**	N = 42	**N = 42**
Seroconversion rate % (95%CI)	46.3 (30.7-62.6)	**60.5** (43.4-76.0)	61.9 (45.6-76.4)	**54.8** (38.7-70.2)
GMCs (U/ml) (95%CI)	39.0 (23.5-64.7)	**48.6** (29.9-78.9)	64.5 (36.6-113.7)	**56.7** (32.5-98.9)

N = number of subjects in the ATP cohort for immunogenicity with available serum anti-RV IgA antibody results post dose 1 or post dose 2. 95%CI - 95% confidence intervals

The seroconversion after the first dose was lower in subjects receiving OPV co-administered with the HRV vaccine as compared to subjects who received IPV. After the second dose of HRV vaccine, the seroconversion rates and GMCs were similar in both groups.

Study 036

Study Rota-036 is a double-blind, randomized, placebo-controlled trial conducted in the Czech Republic, Finland, France, Germany, Italy and Spain. The study is evaluating the efficacy, safety and immunogenicity of two doses of HRV ($10^{6.5}$ CCID50) in healthy infants in co-administration with specific childhood vaccines including GSK Biologicals' Infanrix Hexa® vaccine. The study vaccine and co-administered childhood vaccines were given according to the local national Plan of Immunisation schedule in each country (3, 4, 5 month schedule in the Czech Republic and 3, 5, 11-12 month regimen in Finland). Subjects aged 9 to 18 weeks at the time of the first dose received a hexavalent vaccine with the first and second doses of HRV vaccine or placebo, and a third dose of hexavalent in accordance with national immunization practices.

In Finland, 300 subjects enrolled at specific centres were part of the immunogenicity and reactogenicity subset. For the Czech Republic, all enrolled subjects were part of the immunogenicity and reactogenicity subset.

Interim data of the anti-HRV immune response are now available on subjects enrolled in the Czech Republic and Finland as well as interim immunogenicity results for concomitant vaccinations (post dose 3 for the Czech Republic, post dose 2 for Finland). The interim results of study Rota-036 (only the part conducted in the Czech Republic and Finland is shown), demonstrated that seropositivity/seroprotection rates as well as geometric mean concentrations of all antibodies to the DTPa-HBV-IPV/Hib antigens were similar between HRV and placebo recipients The immune response observed with HRV ($10^{6.5}$ $CCID_{50}$) co-administered with hexavalent vaccine was as good as the response seen in study Rota-004 where HRV ($10^{4.7}$ ffu) was administered without concomitant vaccination and where 83.6% (95% CI: 78.1-88.2) of subjects had anti-rotavirus IgA antibody concentration ≥ 20 U/ml. Furthermore, immune responses and safety profiles of the co-administered vaccines were comparable. It should however be stressed that the immune response to polio 2 antigen was very low among both HRV vaccine and placebo recipients in Finland when compared with the

Czech republic. The applicant committed to submit the final study report as soon as it becomes available.

Study 024 (Brazil, Panama, Colombia, Argentina), interim analysis of immunogenicity/efficacy

A total of 1,851 subjects were enrolled (mean age 8.3 weeks), 1,234 subjects received 2 doses of HRV; 617 subjects received 2 doses of placebo. Routine childhood vaccines and OPV was administered concomitantly. The efficacy follow-up of this study is ongoing. However an interim analysis has been conducted for subset of 285 subjects (191 received HRV and 94 received placebo) and is presented at this time. The serum anti-RV IgA levels were measured 1 to 2 months after the last dose of HRV.

Serum anti-rotavirus IgA seropositivity rates and antibody GMCs in subjects receiving 2 doses of HRV vaccine concomitantly administered with routine vaccines including OPV in study Rota-024 (ITT):

	N	**HRV vaccine ($10^{6.5}$ $CCID_{50}$)** (95%CI)
Seropositivity rates (95% CI)	191	**60.2** (52.9-67.2)
GMCs (95%CI)	191	**62.5** (48.0-81.4)

N = number of subjects with available serum IgA antibody results post dose 2. 95%CI - 95% confidence intervals

Difference between groups in Polio seroprotection rates after 3rd Polio dose (total enrolled cohort)

Antigen	**Placebo**		**HRV**		**Difference Placebo minus HRV**		
					Value	**95% CI**	
	N	**%**	**N**	**%**	**%**	**LL**	**UL**
Anti-Polio 1	91	100	186	99.5	0.5	-3.5	3.0
Anti-Polio 2	95	100	187	99.5	0.5	-3.4	3.0
Anti-Polio 3	95	97.9	190	94.2	3.7	-2.0	8.4

N = number of subjects with available results

% = percentage of subjects with titres ≥ 8 ED50 (seroprotection)

CI = standardized asymptotic confidence interval; LL = Lower Limit; UL = Upper Limit

The immune response to the polio antigens is not impacted by co-administration of OPV with Rotarix, as shown by the largely overlapping 95% CI. An additional analysis of these results showed that the OPV response in the HRV group was non-inferior in terms of seroprotection rates for polio types 1, 2 and 3 compared to the OPV response in the placebo control group, with a non-inferiority limit of 10%

With respect to the immune response to Rotarix after co-administration with OPV, Rotarix given concomitantly with OPV in study Rota-024 was immunogenic in infants with 60.2% (95% CI: 52.9%; 67.2%) seropositivity rate for anti- rotavirus IgA antibodies. The seropositivity rate tended to be lower as compared to that seen in the other Latin American studies Rota-006 [69.5% (95% CI: 65.2%; 73.6%)] and Rota-023 [77.9% (95% CI: 73.8%; 81.6%)] where no OPV was co-administered, although an overlap in 95% CI's was observed between studies Rota-024 and –006.

- Efficacy in special populations

Data on the efficacy and safety of the candidate vaccine among HIV infected infants are currently missing. Data on the efficacy and safety among prematurely born infants are limited. The applicant committed to perform further studies in immune compromised and preterm infants as part of the Risk Management Plan.

The applicant commits to further study the effectiveness of Rotarix in Africa.

Breastfed infants

To document the impact of maternal antibodies on the immune response, serum levels of maternal antibodies (anti-rotavirus IgG and neutralising antibodies) were evaluated in pre-vaccination blood samples from a subsets of subjects. The results showed a trend towards a limited impact on HRV immunogenicity. However, it should be noted that Rotarix has been demonstrated to induce high protection against RV GE in studies Rota-006 and Rota-023 (overall efficacy of 85% in Rota-023 with the commercial formulation). As shown by data on breast feeding practices more than 90% of infants were breast-fed in these studies.

- *Supportive studies*

Study 003 (Finland)

One hundred ninety two (192) infants (mean age 8.3 weeks) were enrolled in the study with a total of 128 infants receiving the HRV vaccine. The serum anti-RV IgA seroconversion rates and post vaccination GMCs observed in part 1 and part 3 were in similar ranges. In study parts, 2, 3 and 4 higher serum anti-RV IgA seroconversion rates and GMCs were observed with increasing viral concentrations. The seroconversion rates ranged from 72.7% to 95.7% and GMCs in all subjects ranged from 110.3 U/ml to 375.3 U/ml. The trend was more striking after the first dose. The percentage of vaccinees with rotavirus shedding was highest after the first dose and ranged from 37.5% to 60% in the HRV vaccine groups.

Study 021 (Panama)

A total of 406 subjects (mean age 8.0 weeks) were enrolled in the study and a total of 177 and 178 subjects received the HRV and mHRV vaccines, respectively. Seroconversion rate and GMCs in the HRV vaccine group had an higher trend. Based on these results, the further development of the mHRV vaccine was abandoned.

Study 033 (Mexico, Columbia, Peru)

A total of 855 subjects were enrolled (mean age 8.4 weeks), 243 subjects in Group HRV vaccine lot A, 242 subjects in Group HRV vaccine lot B, 246 subjects in Group HRV vaccine lot C and 124 subjects in the placebo group. For any pair of the three HRV vaccine lots, the limits of 90% CI for the ratio of serum anti-RV IgA antibody GMCs were within the pre-specified limits [0.5; 2].

Ratio of serum anti-rotavirus IgA antibody GMCs two months after the second dose between the HRV vaccine groups in study Rota-033 (ATP immunogenicity cohort)

						Ratio of GMCs		
Group	**N**	**GMC**	**Group**	**N**	**GMC**	**groups**	**Value**	**90 % CI (LL-UL)**
HRV lot A	154	83.0	**HRV lot B**	167	59.4	**HRV Lot A over HRV Lot B**	1.40	1.05 - 1.87*
HRV lot A	154	83.0	**HRV lot C**	173	81.2	**HRV Lot A over HRV Lot C**	1.02	0.77 - 1.36*
HRV lot B	167	59.4	**HRV lot C**	173	81.2	**HRV Lot B over HRV Lot C**	0.73	0.55 - 0.97*

N = number of subjects with available data
90% CI = 90% confidence interval; L.L. = lower limit, U.L. = upper limit (Anova model - pooled variance with more than 2 groups)

*Lower and upper limit of the 90%CI within the pre-specified [0.5; 2] limit interval for consistency

The three consecutive production lots of the HRV vaccine were shown to be consistent in terms of seroconversion rates and reactogenicity.

Safety

Safety data with the candidate HRV vaccine are available from:

- Eight studies with complete final data (003, 004, 005, 006, 007, 014, 021, 033) (7,369 subjects vaccinated)
- One study with final safety data (023), with respect to the primary safety objective (intussusception within the 0-30 day period after any dose) as well as to SAEs up to minimum one month post dose 2 is available (63,225 subjects vaccinated)
- SAEs arising from all ongoing studies up to the data lock point of August 31, 2004 are presented
- Supportive safety data are available from 3 studies with the mHRV vaccine (016, 020, 021) (893 subjects vaccinated)
- With respect to the incidence of IS, the applicant submitted the final study report of study Sero-Epi-IS-204 (999910/204), which estimates the incidence of IS in children < 24 months old in hospitals involved in study Rota-023. This study report is submitted as background information to assess the incidence of hospital-related IS in unvaccinated children under 2 years of age in the same countries as study Rota-023 (except, for the inclusion of Costa Rica and exclusion of Venezuela and Finland)

Study 023

In study 023, the pivotal phase III trial 63,225 subjects were enrolled to evaluate the safety of the HRV vaccine with respect to definite IS diagnosed within 31 days after each HRV vaccine dose. Final safety data with respect to the primary safety objective (intussusception within the 0-30 day period after any dose) as well as to SAEs up to minimum one month post dose 2 are included in this report.

- Intersusseption (IS)

A total of 63,225 subjects were enrolled and vaccinated; 31,673 subjects received the HRV vaccine and 31,552 subjects received the placebo. The safety follow-up period was from dose 1 up until study visit 3 (30-90 days post vaccination) for these subjects

Parents/guardians and investigators were informed prior to study start about IS risk and symptoms. Clinical investigations were performed on every case of IS which occurred, and the subjects were followed until the event subsided.

A number of biological samples (stool samples, throat swabs, blood samples (acute and convalescent sera), intestinal and lymph node biopsies if surgery was performed) were collected at the IS treatment site. Several characterization assays (detection rotavirus, enteroviruses and adenoviruses in tissue, stools, throat & rectal swabs; detection of anti-RV IgA and RV RNA in acute and convalescent sera, respectively; and detection of bacterial enteropathogen in stool samples) were performed. In the event that RV was detected, the viral RNA was sequenced to distinguish wild type strains from the vaccine viral strain.

Totally 25 definite IS cases adjudicated by the CEC and diagnosed from Dose 1 until study visit 3 are included in the analysis on definite IS:

- 13 cases (6 in the HRV vaccine group and 7 in the placebo group) were diagnosed within the 31-day risk window after any dose
- 12 (3 in the HRV vaccine group and 9 in the Placebo group) cases were diagnosed beyond the 31-day risk window.

Of the 25 definite IS cases, 10 cases (3 in the HRV vaccine group and 7 in the Placebo group) occurred after Dose 1 and 15 cases (6 in the HRV vaccine group and 9 in the Placebo group) occurred after Dose 2. The primary safety objective was met. The observed Risk Difference of -0.32/10 000

(95%CI: -2.91/10 000; 2.18/10 000) and the Relative Risk of 0.85 (95% CI: 0.30; 2.42) provide evidence of no increased risk of IS in the HRV vaccine group as compared to placebo.

- Serious adverse event/deaths/other significant events

In study 023, there were a total of 99 fatal cases reported up until the data lock point of September 10, 2004. Fifty-six cases were reported in the HRV group (17.7 per 10,000) and 43 (13.6 per 10,000) in the placebo group (p value = 0.198). Various diagnoses have contributed to the fatalities and none of the cases were assessed as related to vaccination by the investigators.

A potential imbalance was noted for one cause of death coded to MedDRA preferred term 'Pneumonia'. Pneumonia was reported by 14 subjects in the HRV vaccine group versus 5 subjects in the placebo group as the primary cause of death.

Given the overlap between pneumonia and bronchopneumonia and the fact that the etiologic pathogen was not recovered in all cases, all deaths coded to these three preferred terms were grouped together for an ad hoc exploratory analysis to evaluate any potential imbalance. There was no potential imbalance noted between groups for fatal SAEs related to various PT categories for pneumonia (based on predefined exploratory < 0.05 significance level).

The majority of subjects who reported a SAE coded to pneumonia were hospitalised, 277/280 subjects in HRV vaccine group versus 273/277 subjects in the placebo group. A temporal association between a primary CoD of pneumonia and HRV vaccination was not established.

SAEs classified under the 'Infections and Infestations' were most frequently reported (234.9 per 10,000 in the HRV vaccine group and 272.2 per 10,000 in the placebo group). The next most frequently reported system organ class category (SOC) was the 'Respiratory, Thoracic, and Mediastinal Disorders' (30.3 per 10,000 in the HRV vaccine group and 28.2 per 10,000 in the placebo group).

SAEs related to the MedDRA SOC 'Nervous system disorders' were evenly distributed between HRV and Placebo groups (29 versus 29 cases). However, when analysing PTs within the Nervous system category, a potential imbalance in favour of the placebo was noted for SAEs classified under the MedDRA PT 'Convulsion'. From Dose 1 until Visit 3, at least one SAE relating to 'convulsion' was reported by 16 subjects in the HRV vaccine group versus 6 subjects in the Placebo group. These apparent signals were then reviewed to evaluate the clinical relevance of the potential imbalance. Additional exploratory group comparisons were performed, indicating that the observed potential imbalance is likely to be a chance finding and not clinically relevant. All cases were assessed as not related to vaccination by the investigators.

Other studies

- Adverse events

Solicited adverse events included irritability, loss of appetite, diarrhoea, fever, vomiting and cough/runny nose. There was no increase in the reactogenicity following HRV vaccination as compared to the placebo administration. There was no significant difference between the respective vaccine groups, receiving vaccine of different potency (viral concentrations in the range of $10^{5.2}$ffu - $10^{6.2}$ffu per dose), nor between the placebo group and the vaccine groups with regard to fever, vomiting or diarrhoea grade/intensity 3 during the 15-day follow-up period.

When routine paediatric vaccines were given concomitantly with HRV vaccine or placebo, the applicant concluded that the solicited adverse events were due to the paediatric vaccines. For this reason the Company proposes to present in the SPC only the pooled incidences of solicited symptoms from subjects receiving the HRV vaccine in the absence of concomitant vaccines (Studies Rota-003 and Rota-004). This proposal was accepted by CHMP.

Unsolicited symptoms classified in MedDRA as infections and infestations (preferred terms: bronchitis, influenza, nasopharyngitis, rhinitis, pharyngitis, rhinitis, upper respiratory tract infection and viral infection) were the most frequently reported symptoms. Of unsolicited adverse events bronchitis was reported in 1.95% and 1.03% of subjects receiving HRV vaccine and placebo, respectively. Only one case of bronchitis was related to vaccination, i.e. 0.02% of 5,543 subjects, and no cases in the placebo group. Nine cases (0.16%) of upper respiratory tract infection were probably

related to administration of the HRV vaccine. However, this incidence was not significantly different from that of the placebo group (p=0.128).

The incidence of pyrexia was similar between HRV and placebo recipients (3.75% versus 3.94%, respectively). Only 0.09% and 0.05% of the cases of pyrexia observed in the HRV and placebo groups, respectively, were related to vaccination. These observations confirm the results of fever seen in the analyses of solicited symptoms.

Similar rates with grade 3 intensity of all unsolicited symptoms reported as causally related to vaccination were seen between HRV and placebo groups.

The use of medication was similar between subjects receiving HRV vaccine and placebo in all studies. There was no increase in the use of medication after the second (and third) HRV vaccine dose as compared to the use after the first dose. There was no increase in the use of medication with higher viral concentrations of HRV vaccine. As could be expected, the use of antipyretics was higher in studies where the subjects had received DTPa or DTPw based vaccines concomitantly with the HRV vaccine than in studies where the HRV vaccine was administered alone.

DEATHS

A total of 18 deaths were reported in studies Rota-003, Rota-004, Rota-005, Rota-006, Rota-007, Rota-014, Rota-016, Rota-020, Rota-021 and Rota-033. Twelve deaths were reported in subjects who had received the HRV or mHRV vaccine (0.19%; 95%CI: 0.10-0.33) vaccine and 6 deaths were reported in subjects who had received the placebo or mPlacebo (0.30%; 95%CI: 0.11-0.65). All fatalities were assessed as not related to the study vaccines by the investigators. Most of the fatalities can be explained by the underlying medical condition. Two fatalities were caused by accidental events. Eight of the 18 deaths occurred in study Rota-014. Three deaths were reported in subjects who had received the HRV vaccine and 5 deaths were reported in subjects who had received placebo. Six out of eight subjects were found to be HIV positive and the cause of death was found to be HIV related. This trial was conducted in South Africa where there is a high prevalence of HIV and a subsequently high infant mortality rate (42 deaths per 1,000 live births). The death rate observed in study Rota-014, part 1, is 26 per 1,000 for a follow-up period of ca.10.5 months after birth was lower than the national and provincial figures for South Africa.

SAEs leading to hospitalisation

The number of subjects with SAEs leading to hospitalisation from Dose 1 up to study visit 3 was reviewed post-hoc as part of the safety analysis given their clinical significance. Of the 1975 subjects with SAEs, 1889 (95.6%) were actually hospitalised. There was significantly less hospitalisation in the HRV vaccine group compared to the placebo group (886 versus 1003, two-sided asymptotic P-value = 0.005).

When hospitalised SAEs under the MedDRA SOC 'Infections and infestations' were analysed excluding the potential impact of GE related events, there was no potential imbalance between the treatment groups (627 in the HRV vaccine groups versus 654 in the Placebo group)

- Safety in special populations

HIV infected infants were excluded from vaccination.

The applicant committed to address the safety of the vaccine in preterm and immune compromised infants in the “Risk Management Plan”.

- Safety related to drug-drug interactions and other interactions

Routine paediatric vaccines were concomitantly administered with the candidate vaccine. There was no difference in reactogenicity and safety between subjects who received the candidate vaccine simultaneously with routine paediatric vaccines and subjects who received routine paediatric vaccines together with placebo.

The widespread prophylactic use of antipyretic treatment in studies Rota-005 and, to a lesser extent, in study Rota-006, precludes a valid conclusion on the impact of the vaccine and concomitantly administered vaccines on the incidence of fever. Children who received prophylactic treatment should be excluded for assessing the incidence of fever in these studies

- Discontinuation due to adverse events

There is no significant difference in the number of drop-outs due to AES in the respective vaccine groups and placebo groups.

5. Pharmacovigilance

▪ Description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements. The company must ensure that this system is in place and functioning before the product is placed on the market

▪ Risk Management Plan

The MAA submitted a risk management plan. The postmarketing safety surveillance will rely on standard pharmacovigilance activities based on passive surveillance and additionally, specific surveillance activities are planned in the early postmarketing period for addressing potential safety risks and missing information:

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Bronchitis	Addressed in study 036	
IS	Active surveillance in EU (PSUR)	
	Observed versus expected analysis	
	PASS study in Mexico	
Pneumonia deaths	PASS study in Mexico	
Vaccine effectiveness	European Rotavirus Surveillance Network	
Genetic stability of vaccine virus	Study Genetic Stability of Vaccine Virus	
Vaccine virus transmission	Addressed in study 052	Rota Study 052
Use in preterm infants	Addressed in study 054	Rota Study 054
Use in immune compromised infants	Addressed in study 022	Rota study 022
Additional efficacy after co-administration of oral polio vaccine	Addressed in study ongoing study 024	

The vaccine contains 9 mg of sucrose as an excipient. This amount is far too low to cause adverse events in patients with rare hereditary problems such as fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency. Therefore these words of caution should not be mentioned in the section 4.3 (contraindications) and can be downgraded to section 4.4.

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

6. Overall conclusions, benefit/risk assessment and recommendation

Quality

The dossier covers the rotavirus active substance manufactured in serum free conditions and the lyophilised rotavirus vaccine as finished product.

The Applicant detailed the manufacturing processes, including the "in process" controls and acceptance criteria. The control of virus seed lots and Vero cell bank system is adequately presented, as well as the genetic stability studies for the Human Rotavirus vaccine RIX4414 strain.
The analytical control procedures are summarised and the validation parameters, acceptance criteria and validation results are reported, as the references to international requirements in force.
The Applicant committed to submit the stability data for the intermediates as for the final vaccine on an ongoing basis. The accepted shelf life for the HRV lyophilised vaccine is 36 months at +2-8°C.
In conclusion, the benefit/risk ratio was found positive with regard to the quality of the product.

Non-clinical pharmacology and toxicology

In a first study, the antibody response was investigated by analysing seroconversion. No induction of diarrhoea and no seroconversion has been observed in the proposed Fisher rat model regardless the age of the animals and the rotavirus strain administered. In a second study, the vaccine take was defined as the combination of seroconversion and/or viral shedding (by RT-PCR analysis of rotavirus mRNA in the stools), which is a more accurate measure of animal exposure than seroconversion alone. The seroconversion and viral shedding induced by different doses of the RIX4414 vaccine administered either alone or with $CaCO_3$ in two different granularities was studied. Although from the data obtained in this preliminary experiment, it cannot be concluded that the addition of $CaCO_3$ has a beneficial effect, there is a clinical need for addition of $CaCO_3$ (neutralisation of the acidic pH of the stomach).

In both preliminary pharmacodynamic studies in Fisher rats, no induction of diarrhea has been observed. The capability of group A rotaviruses to replicate and induce diarrhoea in rats has been suggested to be rat-strain specific. Therefore, the choice of the Fisher rat for the development of the pre-clinical animal model is questioned. An animal model that closely resembles the human disease should have been used. However, CHMP agreed that at the current stage of vaccine development, with protection data documented in humans, further primary pharmacodynamic studies in animals are not approbriate.

Toxicology

No separate study was performed to assess the single dose toxicity of the candidate vaccine. However, full investigations were performed after the first oral vaccine dose administration in rats, during the GLP-compliant repeated dose toxicity study.

The vaccine formulation used in the repeated-dose toxicity study is different from the one used in the final clinical trials. With respect to inherent toxicities of the product, the additional excipient (xanthan) is not assumed to change the safety profile of the RIX4414 final vaccine. Xanthan is widely used as a pharmaceutical excipient and generally regarded as non-toxic. In addition, the differences in the formulation raise no concern with respect to hepatotoxicity (liver enzyme elevations have been reported with human rotavirus disease), but were absent with the formulation used in the repeated-dose toxicity study.

Studies on genotoxic and carcinogenic potential were not performed; this is in line with the note for guidedance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/495/95).

Taking the intended target population for the vaccine, which includes newborns, infants and toddlers and not humans of childbearing age, as well as the nature of the product into account, studies on reproductive and developmental toxicity are not necessary (CPMP/SWP/465/95 and ICH S6).

A specific study on local tolerance has not been performed, since microscopic investigations of esophagus, stomach, small and large intestine were reported in the repeated dose toxicity study in the rat.

The potential for hypersensitivity and autoimmune reactions by Rotarix has not been investigated in an animal model. Even large-scale clinical trials cannot prove that a vaccine is completely safe in this respect. Ultimately, post marketing surveillance studies are the only tool to study whether a new vaccine can cause autoimmune disease or allergic reactions or not.

In conclusion, the applicant has followed a minimalistic approach in the non-clinical support of this new vaccine, which is line with the note for guidedance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/495/95).

Efficacy

The clinical protection elicited by two doses of the candidate vaccine against rotavirus gastroenteritis due to serotype G1P[8], G3P[8] and G9P[8] was demonstrated in the clinical studies. However cross-protection against gastroenteritis caused other serotypes is not sufficiently demonstrated in the clinical trials. The applicant could not demonstrate cross protection against rotavirus gastroenteritis due to serotype G2[P4] type. This is not an unexpected finding, since immune response to HRV is dependent on both, G and P type. Cross protection against strains with neither G1 or P[8] moiety can not be expected. Similarly, immunogenicity (tested in 10 studies) varied across different geographic areas. With a similar viral load, the candidate vaccine provided a difference in seroconversion rates and vaccine take in Finland (Rota-004), Singapore (Rota-007) and Latin American countries (Rota-006)..

Although the efficacy data were broken down with regard to protection against mild, moderate and severe disease and protection against "severe" HRV infection was demonstrated it was agreed that mentioning "protection against severe disease" in the SPC might lead to misunderstanding and have a negative impact on the vaccine uptake.

Data on the efficacy and safety of the candidate vaccine among HIV infected infants are currently missing. Data on the efficacy and safety among prematurely born infants are limited. The applicant committed to perform further studies in immune compromised and preterm infants as part of the Risk Management Plan. The applicant further committed to investigate the efficacy and safety in Africa.

Upon request the applicant provided an analysis of vaccine take and combined seroconversion rates for each HRV viral concentration tested according to feeding habits. It was concluded that breastfeeding had no clinical effect. This is reflected in section 4.6 of the SPC

The vaccine can be given concomitantly with any of the following monovalent or combination vaccines: diphtheria-tetanus-whole cell pertussis vaccine (DTPw), diphtheria-tetanus-acellular pertussis vaccine (DTPa), Haemophilus influenzae type b vaccine (Hib), inactivated polio vaccine (IPV), hepatitis B vaccine and pneumococcal vaccine. Clinical studies demonstrated that the immune responses and the safety profiles of the administered vaccines were unaffected. Concomitant administration of Rotarix and oral polio vaccine (OPV) does not affect the immune response to the respective polio antigens. Although concomitant administration of OPV may slightly reduce the immune response to rotavirus vaccine there is currently no evidence that clinical protection against severe rotavirus gastro-enteritis would be affected. The immune response to Rotarix is unaffected when OPV is administered two weeks apart from Rotarix. This is reflected in section 4.5 of the SPC.

The ongoing study 024 will provide additional efficacy data after co-administration of Rotarix and oral polio vaccine. The applicant committed to provide 1-year efficacy data by mid 2007.

Safety

For all studies, the incidences of solicited general symptoms (irritability, loss of appetite, diarrhoea, fever and vomiting) were comparable between the vaccine and placebo recipients. There was no increased incidence of symptoms neither with increasing number of doses nor with increasing viral concentration. The vaccine did not induce gastroenteritis-like symptoms. A total of 25 cases of intussusception (IS) were diagnosed in study Rota-023, from which 13 were within the 31-day window period after vaccination. The incidence of IS was not significantly different between the vaccine and placebo groups and the upper limit of 95% CI of the difference between these group (2.18/10,000) was lower than the pre-specified limit of 6/10,000. Sporadic, naturally occurring intussusception tends to peak in incidence between 5 to 9 months of age, and occurs only very rarely before that age. The studies have been performed in children younger than 5 months and the data provided by the applicant did not show evidence that intussusception is age related. Taking this into consideration and in order to accommodate the diversity in the European vaccination schedules, it is decided to allow more flexibility for the time point of the second dose. Thefollowing wording for section 4.2 is decided by the CHMP: *The vaccination course consists of two doses. The first dose should be given between 6 and 14 weeks of age. "The interval between doses should be at least 4*

weeks. The second dose should be given preferably before 16 weeks and not later than the age of 24 weeks”>

In study Rota-023, within the unsolicited AEs, there is a very weak signal represented by bronchitis. Presence of a safety signal with respect to bronchitis will be investigated in the ongoing Rota-036 study

Benefit/risk assessment

The major efficacy and safety concerns have been satisfactorily addressed. Clinical studies in Europe and Latin America have clearly demonstrated that two doses of Rotarix confer a clinical protection against rotavirus gastro-enteritis. In the absence of major safety issues, especially the fact that the vaccine did not increase the risk of intussusception, the benefit risk balance is positive.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- Pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns
- No additional risk minimisation activities were required beyond those included in the product information

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of Rotarix indicated for the active immunisation of infants from the age of 6 weeks for prevention of gastro-enteritis due to rotavirus infection was favourable and therefore recommended the granting of the marketing authorisation.