SCIENTIFIC DISCUSSION

Name of the medicinal product:	RotaTeq
Applicant:	Sanofi Pasteur MSD, SNC 8, rue Jonas Salk F-69367 Lyon Cedex 07 France
Active substance:	Human-bovine rotavirus reassortants
Common Name:	Rotavirus vaccine, live, oral
Pharmaco-therapeutic group	Viral vaccines
(ATC Code):	Not yet assigned
Therapeutic indication(s):	RotaTeq is indicated for the active immunisation of infants from the age of 6 weeks for prevention of gastroenteritis due to rotavirus infection. In clinical trials, efficacy was demonstrated against gastroenteritis due to rotavirus of serotypes G1P1[8], G2P[4], G3P1[8], G4P1[8], and G9P1[8].
Pharmaceutical form(s):	Oral solution
Strength(s):	One dose (2 ml) contains: rotavirus serotype G1 not less than 2.2×10^6 IU rotavirus serotype G2 not less than 2.8×10^6 IU rotavirus serotype G3 not less than 2.2×10^6 IU rotavirus serotype G4 not less than 2.0×10^6 IU rotavirus serotype P1[8]not less than 2.3×10^6 IU
Route(s) of administration:	Oral use
Packaging:	tube (LDPE)
Package size(s):	1, 10

3.1 Introduction

Sanofi Pasteur MSD submitted a complete and independent application (in accordance with Article 8.3 of Directive 2001/83/EC, as amended) for RotaTeq, a live, oral, pentavalent rotavirus vaccine consisting of five human-bovine reassortant rotavirus strains designated as G1, G2, G3, G4 and P1.

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 aged less than 5 years. New surveillance data suggest that the mortality rate is now estimated to be as high as 608,000 annual deaths 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).
In RotaTeq, all reassortants are composed of the bovine rotavirus strain WC3 (Wistar Calf 3) genome background. Four of the reassortants express one of the VP7 human outer capsid glycoproteins (G1, G2, G3, and G4 serotype), along with the VP4 bovine attachment protein from the P7[5] WC3 strain. The fifth reassortant expresses the VP4 human attachment protein of P1[8] serotype specificity, while retaining the VP7 bovine outer capsid protein of the G6 WC3 strain.
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 two 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.

3.2 Quality aspects

Introduction

The finished product is presented as an oral solution. The vaccine must be stored in a refrigerator at 2°C - 8°C. The vaccine is supplied as a 2 ml single dose presented in a pre-filled squeezable tube (low density polyethylene), with a twist-off cap (high density polyethylene) in a protective bag. The finished product contains the following excipients: sucrose (1080 mg), sodium citrate, sodium dihydrogen phosphate monohydrate, sodium hydroxide, polysorbate 80, culture media and purified water. The formulation contains buffers to protect the viruses from gastric acid and a stabiliser.

One dose (2 ml) contains:	
rotavirus serotype* G1	not less than 2.2 x 10^6 IU[1, 2]
rotavirus serotype* G2	not less than 2.8 x 10^6 IU[1, 2]
rotavirus serotype* G3	not less than 2.2 x 10^6 IU[1, 2]
rotavirus serotype* G4	not less than 2.0 x 10^6 IU[1, 2]
rotavirus serotype* P1[8]	not less than 2.3 x 10^6 IU[1, 2]

* human-bovine rotavirus reassortants (live), produced in Vero cells.

[1] Infectious Units
[2] As lower confidence limit (p = 0.95)

Active Substance

- ***Manufacture***

Manufacturing process

The active substance is manufactured at West Point, Pennsylvania, USA.

Each monovalent vaccine bulk is produced by expanding Vero cells from a frozen vial through a series of expansion steps. Cell expansion is performed by propagating Vero cells, from the manufacturer's working cell bank (WCB), in single-use plastic tissue culture ware. The cells are initially propagated in tissue culture flasks, but are then expanded into multiple-layer tissue cultureware called cell factories (CFs). In this way, Vero cells are initially expanded from a vial of cryopreserved Vero cells up to manufacturing scale.

The cells are then inoculated with aliquots of rotavirus stock seed and harvested to yield harvested virus fluids (HVF), which are frozen and stored at ≤ −60 °C until the next process step is initiated. The HVF is thawed and mixed with a culture media solution, and then clarified by microfiltration. This filtrate is concentrated by ultrafiltration and then sterile filtered through a 0.2 µm membrane. The resulting filtered virus fluids (FVF) are frozen and stored at ≤ −60 °C. FVF is thawed and redispensed into aliquots appropriately sized for use in the RotaTeq formulation and filling process. These aliquots, the redispensed virus fluids (RVF), are frozen and stored at ≤ −60 °C in stainless steel containers until needed.

Each monovalent vaccine bulk (G1, G2, G3, G4, and P1) is prepared independently. All vaccine bulk intermediates are produced under aseptic manufacturing conditions using pre-sterilized equipment and filter-sterilized culture media. At the end of production, all vaccine bulks are subjected to a final 0.2-µm-sterile-filtration step. Subsequently, all materials contacting the vaccine bulk are pre-sterilized and all open aseptic manipulations are performed under Class 100 conditions.

Control of materials

Virus Seed Lots

At the lab of the Children's Hospital of Philadelphia (Pennsylvania, USA), bovine and human rotavirus were allowed to form reassortants and antisera for VP7 and VP4 for the bovine and human strains was used to inhibit the growth of the parent viruses in order to allow growth of the reassortant. The purpose of co-infection was to allow for exchange of the RNA segment encoding for either the major (VP7) or the minor (VP4) surface protein of a human rotavirus for the corresponding RNA segments of the bovine WC3 rotavirus. Individual progeny viruses were plaque purified and their RNA segments were examined by electropherotyping to confirm that the desired genetic composition was achieved.

The reassortant viruses were transferred from the Children's Hospital of Philadelphia to Merck. Master seeds for the five reassortants were produced in Vero cell cultures that had been expanded from the pilot-scale WCB. The inoculated cultures were then harvested and frozen to produce the harvested master seeds. The harvested master seeds were thawed, clarified by microfiltration, and frozen, resulting in the microfiltered master seed. Finally, a portion of each of the microfiltered master seeds was thawed and refrozen to produce the final master seeds, which are used in the subsequent production of stock seeds.

Pilot-scale stock seeds for the five reassortants were manufactured independently by a single passage of the appropriate master seed in Vero cell cultures that had been expanded from the pilot-scale WCB. The pilot-scale stock seeds were used for production of material for clinical lots and process validations lots (used to demonstrate clinical consistency), and are used for manufacture of early market supply. The manufacturing-scale stock seed process and the manufacturing scale stock seeds will be used from the point that pilot-scale stock seeds are consumed. The manufacturing-scale stock seed process differs from that used at pilot-scale due to the increased batch size of full-scale manufacturing.

Cell substrate

The Vero cell line was derived from the kidney of a normal adult African green monkey in 1962 by T. Yasumura and Y. Kawakita at the Chiba University in Chiba, Japan. In 1964, the cell line was transferred to the Laboratory of Tropical Virology, National Institute of Allergy and Infectious Diseases, NIH, USA by B. Simizu. The cells were maintained in Medium 199 supplemented with 5% foetal bovine serum (FBS). The cell line was then submitted to the Animal Cell Culture Collection. Vero cells for the manufacture of the rotavirus vaccine bulks were obtained from the American Type Culture Collection (ATCC) as Continuous Cell Line 81 (CCL81).

The Vero cells were expanded to create a WCB; the cell bank was tested for egg safety, cell line identity, absence of retroviruses by reverse transcriptase and electron microscopy, animal safety in adult and suckling mice, rabbit and guinea pig, and tumorigenicity in nude mice. Cell fluids were tested for sterility and mycoplasma.

The pilot-scale WCB was used to support pilot-scale stock seed production, Phase III clinical lot manufacture, process validation, and early manufacture of marketed material.

The manufacturing-scale WCB process will be used for all future WCB production. The WCB was tested for cell line identity, sterility, mycoplasma, animal safety, tissue culture safety, tuberculosis in vitro and egg safety.

Reagents

All raw materials and culture media are controlled and tested to standards appropriate for their intended use; selected tests and specifications are provided.

Controls of critical steps / Process validation

The controls that have been implemented for routine manufacturing represent a subset of the critical process parameters (CPPs) or critical quality attributes (CQAs) that were used for process validation. Any deviation from the parameters in the routine-manufacturing control set will require investigation. These parameters are also part of the drug substance specifications.

Process validation lots were manufactured to demonstrate that the vaccine bulk manufacturing process conformed to the validation specifications according to established EMEA guidelines. Process validation was successfully completed for each of the five reassortants – G1, G2, G3, G4, and P1). All

lots met all CPPs and CQAs; therefore, validation of the vaccine bulk manufacturing process has been successfully completed.

A second sterile filtration step on FVF may be performed following specific unplanned events. This process was prospectively validated using data from six refiltered FVF lots that had previously undergone one complete sterile filtration.

Manufacturing process development

The development process was used for the manufacture of monovalent vaccine bulks for Phase I and II clinical studies (Protocols 001-005). This process used the pre-master seed for inoculation of Vero cell cultures to produce monovalent vaccine bulks. Pre-master seed was also expanded to produce new master seed, in support of long-term, large-scale vaccine manufacture.

In the final manufacturing process, the culture medium was supplemented with 1% (v/v) foetal bovine serum (FBS) to improve cell-expansion-process performance. This process was implemented at pilot scale and manufacturing scale, which differs in scale but not in operating parameters. This process was used to manufacture rotavirus monovalent bulks in support of Phase III clinical study monovalent vaccine bulks used to manufacture pentavalent vaccine in Protocols 006, 007 and 009.

- ***Characterisation and specifications***

RotaTeq is a combination of five human-bovine reassortant rotavirus strains, designated as G1, G2, G3, G4, and P1, respectively. All reassortants are composed of the bovine rotavirus strain WC3 (P7[5], G6) genome background expressing human outer capsid proteins.

RNA electrophoretic migration patterns confirmed the parental origin of the RNA genome segments as being from either the original bovine strain (WC3) or the WI79 original human strain.

Each of the 5 vaccine reassortants was sequenced and a primary amino acid structure deduced from the obtained sequence. Differences between the sequence of either the bovine or human parent and the reassortants were detailed. For the G1-G4 reassortants there was 97% sequence homology.

The genetic stability of the reassortant was monitored for the master seed through production level passage by complete sequence analysis of the 5 reassortants at the two extremes of processing time. Very few differences in the overall sequence were identified which are attributable to either the detection of dimorphic bases or the presence of two RNA variants in the sample.

The presence, identity and content of the reassortant rotavirus strains in each of the individual vaccine bulks were confirmed by assaying samples using the Rotavirus Multivalent Quantitative-Polymerase Chain Reaction-Based Potency Assay (M-QPA). In addition, the reassortants were characterized physically, biochemically and biologically.

The physical characterisation of the vaccine particle measured the amount of infectious triple (protein) layered virus and the amount of non-infectious double-layered virus particles. The majority of the five monovalent virus vaccines have the majority of the particles in the double-layered state (non-infectious). The norm for cultured rotavirus is that the majority of the virus is in the double-layered condition.

The biochemical characterisation measured the total protein for each of the 5 strains and each determination used 5 production lots. There were some minor differences between the reassortant types, but these were not significant. SDS-PAGE followed by immunoblotting showed that the protein profile for each reassortant over a number of batches was consistent.

As vaccine bulks contain both rotavirus proteins and Vero cell components, the amount of rotavirus-specific antigen in the rotavirus bulks was determined. In general, there was no link between viral antigen content and potency but there was a good link between viral antigen content and total protein content, suggesting that viral protein makes up a consistent percentage of the total protein content.

Quantitative polymerase chain reaction (Q-PCR) was used to determine the number of viral genomes present in process validation and production samples. Overall, the amount of genomes/ml of all vaccine lots analyzed, indicated consistency in the manufacturing across the different lots.

The biological characterisation was carried out using polyclonal antibodies generated against each strain. Cross neutralisation was attempted with the antibodies and the results confirmed that the G1-G4 strains were immunogenically similar, due to the fact that they only differed in their VP7 protein, and that the P1 strain was distinct from these.

The initial interaction of rotavirus strains with *N*-acetylneuraminic (sialic) acid residues on the cell surface correlates with the VP4 genotype specificity. The five reassortant rotavirus strains from process validation lots were shown to be sialic acid-independent.

Process-related residuals arise from the vaccine bulk manufacturing processes. Residuals derived from the host cell substrate include proteins and Vero cell DNA. Residuals from medium components include bovine serum, trypsin, and chymotrypsin.

For the Vero cell DNA, a specification of 35 µg/dose was established, based on data supporting that orally administered DNA was on the order of 10,000 times less likely to enter cells as compared to parenteral administered DNA. It is also found unlikely that rotaviruses would incorporate cellular DNA. Specifications were set based upon manufacturing capability.

For the bovine serum, procedures have been implemented to minimize the concentration of bovine serum proteins carried through to the drug product. BSA was measured for a series of process validation and production lots and shown to be at very low levels relative to many parenternally administered vaccines.

During the vaccine bulk manufacturing process, porcine trypsin solution is added to ensure efficient cleavage of the VP4 spike; cleavage of this viral protein is necessary for infection.

Quality control testing of the active substance is performed at specified stages on the monovalent vaccine bulks. The testing scheme confirms the absence of extraneous agents, verifies potency and identity, and provides a measure of quality and process consistency.

The potency of the product was measured initially by a plaque assay. The applicant then developed the M-QPA. The M-QPA is the key potency assay for the active substance/finished product. Briefly, Vero cells are incubated with dilutions of the unknown potency pentavalent vaccine and at the same time duplicate Vero cells are incubated with a pentavalent virus standard. The PCR method uses primers and probes specific to the VP7 gene (genome segment 9) of each rotavirus reassortant strain. The probes and primers for each strain do not cross react with other strains of rotavirus reassortants.
The M-QPA assay result is related to the pentavalent standard which in turn were originally evaluated by the plaque assay.

The assays used in the testing of the active substance were adequately validated.

The control rotavirus pentavalent vaccine standard for the M-QPA assay is proposed as a reference standard. This is a final filled container batch manufactured as part of clinical lots manufacture. This lot was filled into glass vials and frozen and stored at -60°C. Future reference standards will be evaluated against the pentavalent "gold standard," which was formulated with process validation bulk retains and stored in liquid nitrogen (vapor phase). The "gold standard" was manufactured by the same process intended for marketed product. The potency of the "gold standard" was calibrated to a vaccine

lot used in the end-expiry potency study (Protocol 007) in order to establish a bridge between the release potency and the expiry potency of marketed product.

- ***Stability***

Formal stability studies for three vaccine bulk intermediates (HVF, FVF, and RVF) held at –80 to -60 °C were initiated in 2001 and 2002. All stability study lots were prepared at manufacturing scale in the production facility to be used for licensed product. The stability studies were conducted using three lots of vaccine bulk for each of the five reassortants and for each type of bulk intermediate. Stability results for these lots of vaccine bulk are available through the 36-month time point for HVF, FVF and RVF; at these time points, all results met the stability specifications. The duration of the stability studies at –80 to –60 °C is 5 years for HVF, 11 years for FVF, and 5 years for RVF.

Data will continue to be collected and analyzed until the end of the stability studies in all cases. Annual stability data in support of the bulk intermediate shelf-life will be provided.

Medicinal Product

The formulation for RotaTeq consists of stabilizer solution containing sucrose, sodium citrate, sodium phosphate, sodium hydroxide, and polysorbate-80.redispensed virus fluids (G1, G2, G3, G4, and P1), and Rotavirus Diluent, added in appropriate proportions to meet target potencies.

- ***Pharmaceutical Development***

Formulation development

Several formulations were used during the development of RotaTeq. The initial vaccine formulation was stored frozen and required the subject to be pre-fed to neutralize gastric acid prior to oral administration. Further development resulted in a formulation, which enabled a 2–8 °C stable, buffered, fully liquid rotavirus vaccine without the need for reconstitution. Two different dose volumes of the latter formulation and one dose volume of a related vaccine formulation (with higher buffer levels) were evaluated in Phase I/II clinical trials administered under Protocols 001–005. These studies ultimately supported use of a formulation, which is composed of stabilizer (that contains sucrose, sodium phosphate, sodium citrate, and polysorbate 80), the five reassortant vaccine bulks plus the volume of Rotavirus Diluent. Sodium hydroxide is used to adjust the pH. This formulation was evaluated in Phase III clinical trials administered under Protocols 006, 007, and 009, and is the formulation used for marketed product.

Manufacturing process development

Minor modifications have been made after each formulation change and prior to the transition to full-scale manufacturing. The differences between the clinical lots made at pilot and manufacturing scale include changes in composition (increase in polysorbate 80) and packaging material (change from glass vials to LDPE oral dosing tubes) that were required as the project progressed through development.
Process validation lots (used as clinical consistency lots under Protocol 009 and for re-supply of Protocol 006) were manufactured in the Merck manufacturing facility by the process that will be used in production of marketed product. All other lots for clinical trials were produced at pilot scale.

- ***Manufacture of the Product***

All finished product manufacturing operations are performed at Merck & Co., Inc, West Point, Pennsylvania, USA. Testing upon importation is performed by MSD B.V, Haarlem, The Netherlands.

A calculated amount of Rotavirus Diluent is added to the stabilizer solution and the contents are mixed to prepare the formulation buffer. The quantity to be added is determined from the potencies of the individual RVFs that will be used in formulation.

The formulation buffer is mixed to ensure homogeneity, and then sterile filtered into a sterile receiving tank. The five reassortant vaccine bulks, plus the Rotavirus Diluent are then added to the formulation buffer.

The RVF are connected to the formulation buffer tank using aseptic connections, added to the formulation buffer, and mixed to prepare the FFB. After mixing, samples of the FFB are collected for sterility testing.

Filling and sealing of the oral dosing tubes (ODTs) take place in an aseptic processing suite under Class 100, laminar-flow conditions. The ODTs passed physical and microbial challenge tests designed to test the integrity of the heat sealed closure.

All product-contact equipment is presterilized using validated processes. The oral dosing tubes are sterilized by gamma irradiation. The Class 100 manufacturing environment is monitored for nonviable particulates and viable organisms. Surfaces, air, and personnel are tested for viable organisms.

Marketed containers with approved labelling are stored at 2–8 °C until release testing is completed, then the vaccine is shipped to market. Validated, insulated containers that are designed specifically for vaccine shipments are used for shipping vaccine at all stages of distribution.

The consistency of product manufacture for formulation and filling was demonstrated through process validation using a series of three lots. All CPPs and CQAs were met; the process validation demonstrated that RotaTeq can be consistently formulated and filled.

- ***Control of excipients***

The components of the stabilizer solution are compliant with existing compendial monographs. None of the raw materials included in the stabilizer solution are animal-derived.

Rotavirus Diluent contains a single component of animal origin, cholesterol, which is derived from sheep wool grease, and is considered in compliance with the *Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products*, (EMEA/410/01 rev 2).

- ***Product Specification***

The final filled container is tested for identity, sterility, potency, appearance, dose uniformity, average deliverable volume, pH, residual Vero cell DNA. The potency specifications at release are G1 $\geq 2.91 \times 10^6$ IU/dose, G2 $\geq 4.11 \times 10^6$ IU/dose, G3 $\geq 3.25 \times 10^6$ IU/dose, G4 $\geq 5.18 \times 10^6$ IU/dose and P1 $\geq 3.75 \times 10^6$ IU/dose. The minimum potency demonstrated to be efficacious in clinical trials, final product stability losses, and assay variability, are used to derive a lower release specification, guaranteeing (with 95% confidence) minimum potency of each rotavirus reassortant type through the shelf-life of the product (24 months).

Batch analysis confirms that the drug product can be produced consistently and within specification.

The reference standard used in the M-QPA is a pentavalent final filled container. The potency of the reference standard was established through extensive replicate testing in the M-QPA, against a reference standard set that consisted of five monovalent bulk preparations – one for each reassortant

G1, G2, G3, G4, and P1. The potencies of the five monovalent reference standards had been previously established through repeat testing over a 6-month period using a plaque titration method. Future reference standards will be evaluated against the pentavalent "gold standard," which was formulated with process validation bulk retains and stored in liquid nitrogen (vapor phase). The *"gold* standard" was manufactured by the same process intended for marketed product.

- ***Adventitious agents and TSE***

Adventitious agents

Cell substrates, virus seeds, and animal-derived raw materials used during manufacture of RotaTeq are tested using validated methods to ensure that extraneous agents are not present in the drug product. The seed viruses are derived from Vero cell systems under conditions similar to those used in monovalent vaccine bulk production. Raw materials of animal origin are present in the culture media, cell substrates, and virus seeds used to produce the drug substance, final formulated bulks (FFB), and the drug product. The animal-derived raw materials include: fetal bovine serum (EDQM certificates of suitability have been provided), porcine pancreatic trypsin solutions, cholesterol, polysorbate-80 (PS-80), and amino acids. Bovine-derived PS-80 was used to manufacture seeds and early clinical lots; however, plant-derived PS-80 is currently used in the manufacturing process.

With regard to human-derived raw materials, three amino acids derived from human hair are used in the tissue culture medium (LPKM-3, Williams' E Medium) for manufacture of RotaTeq. These amino acids are highly-processed biochemicals that are commonly present in culture media used for manufacturing of live virus vaccine as well as other cell culture and fermentation processes used in the biotechnology industry.

The applicant has put adequate measures (EVCP for pre-master seeds, gamma irradiation of bovine serum and porcine pancreatic trypsin) in place to ensure absence of adventitious agents for the above materials and the virus removal or inactivation steps have been validated.

The raw materials and excipients of animal origin used in the manufacturing process for rotavirus vaccine bulks are in compliance with the requirements set forth in the European Agency for the Evaluation of Medicinal Products (EMEA) guideline, *"Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products"* (EMEA 410/01 rev.2) (EMEA TSE Note for Guidance). Details have been given to verify that there is no risk of TSE transmission due to the use of EGF and insulin.

- ***Stability of the Product***

Stability studies have been conducted for process validation lots and manufacturing development lots. All lots were studied at the proposed long-term storage condition for the vaccine (2–8 °C).

The stability studies for process validation lots held at 2–8 °C, 13–17 °C, and 23–27 °C support establishment of loss rates. The loss rates are used in the release model to support a shelf life of 24 months. The requested 24-month shelf life considers the total time from filling to administration of the vaccine.

Real time data from batches stored for 24 months at 2-8 °C have been submitted. A mathematical model is used to ensure (with 95% confidence) that the minimum potency for each rotavirus reassortant is present throughout the proposed shelf-life (24 months).

Discussion on chemical, pharmaceutical and biological aspects

The main quality issues were related to the potency assay (M-QPA) (comparability with the plaque assay and robustness of the assay), the limit for residual Vero cell DNA, justification of storage periods of intermediates and the proposed potency specifications and their relationship to vaccine potencies evaluated in Phase III studies. All quality issues hare been resolved and a number of commitments were made by the applicant, to provide further information post-approval.

3.3 Non-clinical aspects

GLP aspects

The repeated-dose toxicity in mice and two definitive studies on Vero cell DNA uptake in rats were conducted under GLP conditions.

Pharmacology

No non-clinical pharmacology studies have been performed. The applicant acknowledges that the EMEA "Note for guidance on preclinical pharmacological and toxicological testing of vaccines" (CPMP/SWP/465/95) and the "WHO guidelines on nonclinical evaluation of vaccines" recommend that immunogenicity studies in animal models be conducted. However, because a significant body of clinical data was generated prior to the EMEA and WHO guidances were established in 1998 and the lack of an appropriate animal model to evaluate the immunogencitiy of RotaTeq, no non-clinical pharmacology studies were performed.

Pharmacokinetics

Experimental studies to demonstrate absorption, distribution, metabolism, and excretion of the active ingredients in RotaTeq have not been performed for any of the component viruses. This is in line with Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95).

Toxicology

- Single dose toxicity

Single-dose toxicity was assessed within the repeat-dose toxicity study in mice.

- Repeat dose toxicity

A 10-week oral toxicity study in mice was performed under GLP conditions to investigate the potential toxicity arising from single and repeated oral administration of RotaTeq. During the 64-day study, mice were treated with either a single dose (given on Study Day 1) or three 0.2 mL oral doses (given on study days 1, 29, and 57) of either RotaTeq vehicle or RotaTeq 6.98 x 10^6 infectious units (based on actual potency data)

Complete necropsies, histopathology examinations, and assessments of haematology and serum biochemistry parameters were performed both 7 days after the first dose (to assess acute toxicity) and 7 days after the third dose (to assess toxicity arising from repeated dose administration).

Oral administration of RotaTeq to mice as a single or 3-dose regimen over approximately 10 weeks was well tolerated. There were no treatment-related effects on mortality, physical signs, body weight, food consumption, haematological parameters, or serum biochemical parameters. There were no treatment-related gross, histopathological or organ weight changes at either the interim (Study Day 8) or terminal (Study Day 64) necropsy.

Based on these findings, the no-effect level had a safety margin relative to the projected human dose of approximately 14-fold.

- Genotoxicity

No genotoxicity studies were conducted; this is in line with CPMP/SWP/465/95.

- Carcinogenicity

No carcinogenicity studies are required according CPMP/SWP/465/95.

- Reproduction Toxicity

The reproductive toxicity potential of RotaTeq was not evaluated. RotaTeq is a paediatric vaccine and is not indicated for use in adults. No studies were preformed as per CPMP/SWP/465/95 and Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals (ICH S6).

- Local tolerance

The local tolerance of RotaTeq was not evaluated. RotaTeq is indicated for oral use, there is very little likelihood of skin or ocular irritation in vaccine recipients. This is in line with CPMP/SWP/465/95

- Other toxicity studies

The Vero cell DNA uptake studies in rats were carried out to support the safety of the residual Vero cell DNA levels in RotaTeq, as requested in the Scientific Advice letters.

Vero cell DNA prepared in either PBS or the RotaTeq vehicle and administered as a single 125-µg *im* or oral-feeding dose to female and male rats produced no treatment-related deaths during the 8-day study. Vero cell DNA levels were measured and as expected, the DNA uptake level was markedly higher in the *im* group than in the oral group at each time point and the levels decreased with time for both routes of administration. The *im* versus oral ratio was 1.0×10^6. This ratio corresponds to an upper safety limit of about 10,000 µg (10 mg) after converting the WHO recommended limit for residual DNA from continuous cell lines in parenteral vaccines, to a corresponding limit for oral vaccines. Thus, the results of this animal study support the safe administration of this vaccine to humans, as it found the levels of residual Vero cell DNA present in RotaTeq to be acceptable.

Ecotoxicity/environmental risk assessment

An environmental risk assessment (ERA) was conducted to assess any possible risks due to normal use of RotaTeq. The ERA was conducted in accordance with the relevant Guideline on environmental risk assessment of medicinal products for human use (CHMP/SWP/4447/00/draft) and EU Directives.

Rotaviruses are ubiquitous in nature, and due to the segmented characteristics of the genome, reassortment of rotavirus genes occur naturally. The five reassortant rotavirus strains contained in RotaTeq were generated in cell culture by taking advantage of the natural property of rotaviruses to reassort.

The vaccine strains are shed in a low proportion of subjects and for a brief period of time. Therefore, there is limited opportunity to be transmitted horizontally.

In the very unlikely event that horizontal transmission would occur, the vaccine-virus strains will not be able to replicate productively in the intestinal tract of humans or most animals.

The vaccine strains are attenuated (presumably during cell culture adaptation) with respect to wild type strains.

In clinical settings where vaccines are usually administered, oral dosing tubes are disposed as infectious biohazard materials and the routine decontamination methods will result in rapid and effective inactivation.

There is no proven animal reservoir capable of sustaining transmission of these viruses and no carriers or vectors have been identified.

The vaccine viruses will not impact the flora or organisms living in sewage treatment plants or the effluent receiving streams and the reassortants are not genetically modified organisms.

3.4 Clinical aspects

Introduction

The vaccine contains five live human-bovine reassortants (HBRV).

- The bovine strain (WC3) used to make the reassortants was of G6 P7[5] type.
- The four human strains comprised G1 P1[8], G2 P2[4], G3 P1[8] and G4 P1[8].

From these parent strains, the five reassortants in the vaccine are as follows:

- Four each express one VP7 glycoprotein from the four human rotavirus strains (i.e. one of G1-G4) plus the VP4 protein (P7) from the bovine strain.
- One expresses the VP4 protein from the G1 P1[8] human rotavirus strain plus the VP7 protein (G6) from the bovine strain.

The minimal content of each strain ranges from 2.0-2.8 x 10^6 infectious units (IU).

The clinical program to support licensure consisted of 5 phase I/II studies (n=3,186) and 3 phase III trials (n=70,141).

Phase I/II studies

The five phase I/II studies (Protocols 001, 002, 003, 004, and 005) of the predecessors of the HBRV vaccine, which included vaccines of different formulations and reassortant compositions, were conducted involving 3,186 infants (2,470 vaccine recipients) and 46 adults (30 vaccine recipients).

The results of these studies were utilized to select the formulation, potency (dose), and reassortant composition of the vaccine, which was evaluated in the Phase III clinical trials. The most important data come from *Study 005*, which compared pentavalent vaccine (not final formulation) of three aggregate potencies with a quadrivalent G1-G4 vaccine, a monovalent P1 vaccine and placebo.

Phase III studies

The three phase III studies involved administration of the final formulation vaccine to 35,365 infants and placebo to 34,776 infants. The studies were performed at 396 sites in 11 countries and enrolled healthy infants 6 to 12 weeks of age. Three doses of vaccine were given at 4- to 10-week intervals. There were no restrictions on breastfeeding or on the prior or concomitant use of licensed vaccines except for oral polio virus vaccine (OPV). Data on safety, immunogenicity and efficacy were collected in all or in subsets of infants.

- The large-scale **Rotavirus Efficacy and Safety Trial, REST (Protocol 006)** evaluated the efficacy and safety of the HBRV vaccine, particularly with regard to intussusception. In total, 70,301 infants were randomised.
- The **Dose-Confirmation Efficacy Study (Protocol 007)** involving 1,312 infants evaluated the efficacy of the end-expiry potency of the HBRV vaccine in the final formulation intended for licensure.
- The **Consistency Lots Study (Protocol 009)** provided a clinical evaluation of the consistency of the manufacturing process. The immunogenicity and safety of three manufacturing lots were evaluated in 793 subjects.

All 3 studies utilized the same methods for case finding and data collection, the same case definitions for safety and efficacy endpoints, and the same immunological assays.

Overview Phase III trials

Study ID	No. of centres / location	Design	Formulation Study Posology	Study Objective Study population	Subjects by arm entered; completed.	Duration
Protocol 006 Rotavirus Safety and Efficacy Trial (REST)	Multicentre (n=356) US, EU, South Am, Taiwan (2001-04)	Randomised, double-blind, placebo-controlled	Pentavalent G1,G2,G3,G4,P1 67.2 - 124 x10^6 IU /dose 3 doses x 2 ml 4-10 w intervals	**Safety** (IS) *Subsets:* Efficacy Imunogenicity Detailed Safety Concomitant use Healthy infants 6-12 weeks	**Total n= 69,274** Vaccine: 34,644 Placebo: 34,630 *Completed vaccination* 29,667 / 29,598 *Evaluated for primary efficacy:* 2207/2305	42 day FU Two seasons
Protocol 007 Efficacy - at expiry potency	Multicentre (n=30) US, Finland (2002-04)	Randomised, double-blind, placebo-controlled	Pentavalent G1,G2,G3,G4,P1 expiry dose ~1.1 x10^7IU/dose 3 doses x 2 ml 4-10 w intervals	**Efficacy,** safety, immunogenicity at expiry potency Healthy infants 6-12 weeks	**Total n=1312** Vaccine: 651 Placebo: 661 *Completed vaccination* 593/608 *Evaluated for primary efficacy:* 551/564	42 day FU 1 season
Protocol 009 Consistency lots study	Multicentre (n=10) US (2003-04)	Randomised (2:2:2:1), double-blind, placebo-controlled	Pentavalent G1,G2,G3,G4,P1 Lot1; 8.81x10^7IU Lot2: 8.01x10^7IU Lot3: 6.91x10^7IU 3 doses x 2 ml 4-10 w intervals	**Immunogenicity** Consistency of 3 lots Healthy infants 6-12 weeks	**Total n=793** Lot 1: 226, Lot 2: 225 Lot 3: 229, Placebo: 113 *Completed study*: 201/208/200/97	42 day FU
				Total:	**Vaccine: 35,365 Placebo: 34,776**	

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 were not performed since these were not applicable. (Note for guidance on clinical evaluation of new vaccines CPMP/EWP/463/97).

Pharmacodynamics

The pharmacodynamics of the vaccine relate to its interaction with the immune system. Therefore, this section concentrates on the immunogenicity data and the documentation of faecal shedding of vaccine and wild-type viruses from the dose-finding study (005), which was not performed with the final formulation, and the large safety and efficacy study (006) in which the final formulation was evaluated.

- Antibody assays were performed on sera and faeces at one of two US centralised laboratories. A validated EIA was used for determination of serum neutralising antibody (SNA). This EIA had been shown to correlate with PRN_{50} data. The ED_{40} values were used for determining titres.
- Faecal shedding was evaluated by plaque assay of stool specimens followed by electropherotyping. Wild-type human rotavirus is not cytopathic in cell culture so the plaque assay should detect only live WC3-reassortant rotavirus.
- Stool samples collected during acute gastroenteritis (AGE) were screened by EIA for detection of rotavirus antigen. Stools that were EIA positive but negative in the plaque assay were deemed in all protocols to represent wild type rotavirus. However, this assumption was later re-examined (see under Efficacy) since it became clear that the EIA was also picking up vaccine virus strains that occasionally did not grow in cell culture. EIA positive stools were

subjected to a novel in-house PCR/sequencing assay that was initially applied to the VP7 gene to determine G serotypes. During the assessment process, the applicant also supplied the results of an in-house PCR applied to VP4 to determine P genotypes of a selection of viruses obtained during studies 006 and 007.

Study 005 was a double blind dose-finding study with a primary endpoint of efficacy that ran from 1998-2001 in Finland (for details see below). Healthy infants aged 2-8 months received three doses of one of the five candidate vaccines or placebo (as in the table below) with 4-8 weeks between each dose.

Group	Clinical Material	Lot Number	Dosage	Aggregate (PFU/Dose)
1	Live rotavirus vaccine (G1, G2, G3, G4, P1)	V260 OSO 003 E001	1.0 mL	2.69×10^7
2	Live rotavirus vaccine (G1, G2, G3, G4, P1)	V260 OSO 003 C001	1.0 mL	7.92×10^6
3	Live rotavirus vaccine (G1, G2, G3, G4, P1)	V260 OSO 003 B001	1.0 mL	2.41×10^6
4	Live rotavirus vaccine (G1, G2, G3, G4)	V260 OSO 002 C001	1.0 mL	2.90×10^7
5	Live rotavirus vaccine (P1)	V260 OSO 004 E001	1.0 mL	9.24×10^6
6	Placebo	PV260 OSO 002 A001	1.0 mL	0

The existence of a dose-response relationship between pentavalent vaccine potency and ≥ 3-fold rises in G1 SNA was explored for first year data from the PP population using logistic regression modelling. A statistically significant dose-response relationship was detected among the pentavalent vaccine groups.

Based on per-protocol (PP) cases of rotavirus AGE occurring during the first rotavirus season in the PP protocol population, logistic regression models showed a significant inverse relationship between rate of rotavirus AGE and the post-dose 3 SNA titres against G1, G2, G3, P1A and G6 SNA titres as well as with proportions with ≥ 3-fold rises in SNA titres to G1 and G2. The findings need to be viewed with caution due to multiplicity and the fact that the great majority of the rotavirus AGE cases were caused by the G1 serotype. However, it was found that post-dose 3 G1 SNA of 51 dilution units was predictive of protection with > 70% sensitivity and that the odds of contracting rotavirus AGE would be 4.54 times higher for a subject with a post-dose 3 level ≤ 51 compared to a subject with a titre >51 dilution units.

The percentage that shed vaccine virus 3 to 5 days post-dose 1 ranged from 1.4 to 6.3% across the vaccine groups. Typing of the shed strains after the first dose of vaccine showed that the majority were G1 or P1 reassortants. After the second dose, one infant shed G1, two shed P1 and one shed G1 with bovine 3. Only one infant shed G3 virus after the third dose.

In Study 006, the immunogenicity sub-study included the first 300 enrolled at selected sites in Finland (used for the primary analysis of immunogenicity) and the US, the first 300 randomised from the Navajo and White Mountain Apache Nations and a U.S. Concomitant Use Cohort of approximately 1,490 infants who were given COMVAX, INFANRIX, IPOL and PREVENAR on the same days as doses of RotaTeq. Schedules varied considerably across sites. Faecal shedding of vaccine-virus strains in stools collected 4-6 days after each vaccination was assessed in the first 150 randomised in each of Finland and the US. The results for SNA responses to G1 among Finnish infants after three doses are shown below.

SNA against G1 in Finnish infants (PP) [samples collected 9-33 days post-dose*]*

	RotaTeq	Placebo
Number with data available for analysis GMT pre-dose 1 95% CI	139 17.3 (14.2, 21.1)	133 19.9 (16.4, 24.3)
Number tested with data available for analysis GMT post-dose 3 95% CI	119 272.7 (220.1, 337.8)	90 10.7 (8.3, 13.8)
Number with data available for analysis Number (%) with ≥ 3-fold rise 95% confidence interval p-value for percent ≥ 42%	117 95 (81.2) (72.9, 87.8) <0.001	89 8 (9.0) (4.0, 16.9)

The responses of these Finnish infants to the other G serotypes in the vaccine and to P1 showed marked differences in parameters between the vaccine and placebo groups. The magnitude of the responses in the RotaTeq group was lowest for G2 and G3 serotypes.

The analyses regarding the immunogenicity of concomitantly administered vaccines in the US Concomitant Use Cohort suggested that there was unlikely to be any clinically important immune interference.

In study 006, there were 12.7% of the infants in the RotaTeq group and none in theplacebo group that shed a vaccine virus 4 to 6 days after the first dose and no shedding was found in either group after doses 2 or 3. The strains shed were either from the vaccine or reassortants of vaccine strains.The number and percent that shed vaccine virus strains at any time following each vaccination (i.e. from samples taken 4 to 6 days after each dose and from samples collected as a result of a potential AGE) are shown below for studies 006 and 007 combined. Almost all instances occurred within one week of vaccination.

Study 009

The primary objective of this study was to demonstrate consistency of the antibody responses to 3 lots of the HBRV vaccine produced in the final manufacturing facility. The study was performed at 10 US study sites. A total of 793 subjects were enrolled and randomised (2:2:2:1) to 1 of 3 lots of HBRV vaccine at potency within the range of final potencies ($\geq 8.94 \times 10^6$ IU/dose to max 1.05×10^8 IU/dose) or placebo. The potency for each dose was targeted for the middle of the range and actual potencies ranged from 6.91×10^7 IU/dose to 8.81×10^7 IU/dose.

The primary immunogenicity analysis was based on post-dose 3 SNA GMTs against rotavirus serotypes G1, G2, G3, G4, and P1 and a requirement that the two-sided 90% confidence interval on the ratio of each pair of GMTs excluded a difference of 2-fold or more, for each of the serology components measured). This criterion was satisfied. With respect to the proportions of subjects with ≥3-fold titre rise of SNA antibodies there were some differences between lots, but due to the limited number of samples tested no conclusions could be drawn.

Clinical efficacy

Data on protective efficacy with pentavalent formulations were obtained from studies 005, 006 and 007. The final formulation was used in the latter two studies as follows:

- In **006**, 33 lots of RotaTeq were used with aggregate potencies from 6.7-12.4 x 10^7 IU/dose.
- In **007**, two lots of RotaTeq were used with aggregate potencies of 1.07 and 1.13 x 10^7 IU/dose.

All the studies utilised the same methods for case finding and data collection, the same case definitions for safety and efficacy endpoints, and the same immunological assays. Because of the large

sample size required for P006 (REST) for the intussusception evaluation, efficacy against rotavirus acute gastroenteritis (AGE) was assessed in a nested sub-study.

Whenever an infant had symptoms consistent with an AGE, the parent/legal guardian was instructed to collect two stool samples as soon as possible and within 14 days following the onset of symptoms and optimally within 48 hours of the onset of symptoms. The per protocol (PP) case definition for rotavirus AGE required infants to meet the following criteria:

- ≥ 3 watery or looser-than-normal stools within a 24-hour period and/or forceful vomiting
- Rotavirus detected in a stool specimen taken within 14 days after the onset of symptoms.

Only G1-, G2-, G3-, or G4-specific rotavirus AGE occurring ≥14 days after the third dose of vaccine or placebo during the first rotavirus season were included in the primary efficacy analyses. Thus, the primary efficacy analyses in each study were based on a definition of ***true vaccine failures.*** The severity of AGE was scored based on the same schema in all studies that classified a score of 1 to 8 points = mild, 9-16 = moderate and >16 points = severe.

- Dose response study

Study 005

The study was conducted at a single centre in Finland and was double blind, randomised and placebo-controlled. Subjects were randomised to 1 of 6 treatment groups to receive either vaccine of varying potencies and compositions or placebo. Healthy infants 2 to 8 months of age received three 1-mL doses of vaccine/placebo with an interval of 4-8 weeks. All subjects were pre-fed by breastfeeding or with infant formula 30 minutes prior to vaccine administration. If infant refused pre-feeding, an antacid was given. There was no restriction on the administration of the usual paediatric vaccines with the exception of oral polio vaccine (OPV).

Statistical methods

The primary null hypothesis in this 3-year Finnish study was that the efficacy of each of the high-dose, middle-dose and low-dose pentavalent vaccines and the monovalent (P1) vaccine against all G1-4 cases of rotavirus GE occurring ≥14 days post-dose 3 during a single season would be <0%.

Results

Through the first rotavirus season postvaccination, the primary analysis gave point estimates for efficacy of 68%, 74% and 57% for pentavalent vaccine of decreasing potency against G1-G4 infections as shown below. Efficacy estimates through two seasons for the middle-dose and low-dose pentavalent vaccines were lower but estimates for the high-dose pentavalent and the monovalent vaccines were higher than in the first season.

Efficacy in the PP population in the first rotavirus season

Group	**1**	**2**	**3**	**4**	**5**	**Placebo**
Contributing to analysis	276	237	252	201	268	262
Rotavirus AGE PP definition	12	8	14	7	20	33
Efficacy estimate (%) and confidence interval	**68.0** (31.1, 86.4)	**74.3** (37.9, 91.0)	**57.6** (11.8, 80.9)	**74.0** (40.3, 90.3)	**43.4** (-1.7, 69.2)	---
p-Value for efficacy >0	<0.001	<0.001	0.004	<0.001	0.029	---
RR burden of illness (%) and 95% CI						
For first episode scores	75.6 (50.7, 87.9)	82.9 (59.9, 92.7)	71.1 (43.0, 85.4)	84.4 (61.1, 93.7)	53.1 (15.4, 74.0)	
For worst episode scores	76.2 (52.0, 88.2)	81.2 (57.5, 91.7)	71.8 (44.5, 85.7)	84.8 (62.1, 93.9)	51.3 (12.3, 73.0)	------
Rotavirus AGE PP definition score > 8						
For first episode scores	10	5	8	5	14	28
For worst episode scores	10	6	8	5	15	29
Efficacy estimate (%) and 95% CI						
For first episode scores	**68.4** (33.1, 86.3)	**81.0** (50.2, 94.3)	**71.4** (35.6, 88.7)	**78.0** (42.2, 93.4)	**53.3** (8.3, 77.3)	---
For worst episode scores	**69.6** (35.8, 86.8)	**78.0** (46.2, 92.5)	**72.5** (38.2, 89.1)	**78.8** (44.6, 93.6)	**51.7** (6.9, 75.9)	---

Groups 1, 2 and 3 received pentavalent vaccine at 2.4 x 10^6, 7.9 x 10^6 and 2.7 x 10^7 pfu/dose, respectively
Groups 4 received quadrivalent vaccine at 2.9 x 10^7 pfu/dose
Group 5 received monovalent P1 vaccine at 9.2 x 10^6 pfu/dose.

The analysis of efficacy against all serotypes in the PP population using the PP case definition included 41 additional rotavirus AGE cases (14 were G9, 1 was G8 and 26 could not be typed). Efficacy estimates for all serotypes were generally slightly lower (ranging from 41.6 to 70.5% across all vaccine groups) as compared with the primary analysis and the lower 95% were 32% and 43% for groups 1 and 2 but 20% for the lowest potency pentavalent vaccine, 24% for the quadrivalent vaccine and only 3% for the P1 monovalent vaccine.

- Main study

Study 006 (the Rotavirus [vaccine] Efficacy and Safety Study - REST study) was a double blind study conducted at 356 investigative sites worldwide from 2001-2004. Countries included were Belgium, Costa Rica, Finland, Germany, Guatemala, Italy, Jamaica, Mexico, Puerto Rico, Sweden, Taiwan and the United States. The Navajo and White Mountain Apache Nations, where G3 has historically been predominant, were considered as a demographic entity within the US.

METHODS

Study Participants and Treatments

Eligible infants were aged 6-12 weeks at the time of the first dose and three 2 mL volume doses of vaccine or placebo were to be administered with 28-70 days between doses. The study was run over three rotavirus seasons. If enrolled early enough into the study, infants were followed for a second rotavirus season.

The per-protocol population was primary and included subjects who received the 3 scheduled doses and adhered to the study protocol.

The modified intention-to-treat (mITT) population included any subject with valid efficacy data, including protocol violators.

Per protocol (PP) case definition for rotavirus AGE required infants to meet both of the following criteria:

- Three or more watery or looser-than-normal stools within a 24-hour period and/or forceful vomiting
- Rotavirus detected by EIA in a stool specimen taken within 14 days after the onset of symptoms. All rotavirus-positive stools underwent serotype identification by PCR that focused on VP7 (G protein) and identification by VP4 (P protein) was not performed. As necessary, a protocol amendment was employed so that the PCR results were used in the primary analysis due to the improved specificity over EIA for serotype identification.

Objectives

There were two co-primary objectives:

(1) To evaluate the **efficacy** of a 3-dose regimen of RotaTeq against **rotavirus disease caused by serotypes G1, G2, G3, and G4** occurring at least 14 days following the third dose

(2) To assess the **safety** of RotaTeq with respect to **intussusception** within 42 days of any dose of vaccine/placebo (see next section on this endpoint).

Outcomes/endpoints

Case ascertainment for AGE was by means of:

- Active surveillance that consisted of telephone contacts or home visits every 2 weeks during the rotavirus season to remind the parent/legal guardian to report all cases of AGE to study staff, to complete the worksheets and collect stool samples for rotavirus testing.
- Passive surveillance that called for the parent/legal guardian to report cases of AGE at any time after the first vaccination regardless if it was during the rotavirus season.

In the event that a subject had multiple episodes that met a case definition, the subject was counted only once for the efficacy analyses and the efficacy analyses was based on the date of the first episode.

Statistical methods and Sample size

The primary null hypothesis referred to efficacy against all G1-, G2-, G3-, or G4-specific cases of rotavirus AGE occurring through the first rotavirus season that begins 14 or more days post-dose 3. Assuming 2295 evaluable subjects in each group, and assuming the true rotavirus attack rate was 10% and RotaTeq had a true efficacy of 60%, there was approximately 98% power to declare that RotaTeq was efficacious.

RESULTS

The US sites (including Jamaica and Puerto Rico) contributed 50% of the total enrolled into the entire study (Safety Cohort) while Finland contributed 33%. There were 5,686 infants randomised into the Efficacy Cohort, of which 5128 (90.2%) received three doses and were followed up for 42 days post-dose 3.

Baseline data

Baseline data for the Efficacy Cohort were as follows:

	RotaTeq		Placebo	
Randomised (N):	2841		2845	
	n	(%)	n	(%)
Gender				
Male	1462	(51.5)	1467	(51.6)
Female	1379	(48.5)	1378	(48.4)
Age (weeks)				
5 And Under	1	(0.0)	1	(0.0)
6 to 12	2832	(99.7)	2827	(99.4)
Over 12	8	(0.3)	17	(0.6)

Numbers analysed

There were 112 in the vaccine group but only 59 in the placebo group who were excluded from the primary analysis due to protocol-defined wild type rotavirus before 14 days post-dose 3. Similar imbalances were noted in studies 005 and 007. These infants had stools that were EIA positive but negative on plaque assay, which implied that no vaccine strains were present and that they had wild-type infections. However, the results of VP7 analyses in studies 006 and 007 indicated that there had been EIA detection of non-viable vaccine strains in many of the infants in the vaccine group. Indeed, of those infants for whom a serotype could be identified, only 24/86 in the vaccine group but all those tested in the placebo group had a wild-type virus in stool collected during an episode of AGE that occurred before 14 days post-dose 3.

	RotaTeq	Placebo
Subjects vaccinated in the Efficacy Cohort	2834	2839
Subjects included in primary efficacy analysis	**2207**	**2305**
Subjects excluded from primary efficacy analysis	627	534
Protocol violations	**295**	**271**
Temperature excursion among administered vials	15	10
Less than 3 vaccinations or less than 28 days between vaccinations	276	256
Prematurely unblinded	0	1
Temperature excursion among administered vials and less than 3 vaccinations or less than 28 days between doses	1	2
Cross-treated	0	1
Less than 3 vaccinations or less than 28 days between vaccinations and prematurely unblinded	2	0
Cross-treated and prematurely unblinded	1	1
No follow-up	11	6
Non-evaluable according to per-protocol case definition:	**321**	**257**
Wild-type positive stool antigen EIA prior to 14 days Post-dose 3	112	59
Incomplete clinical and/or laboratory results or stool sample out of day range	209	198

Outcomes and estimation

In the primary analysis of efficacy, protective efficacy was estimated at 74%, with a lower 95% CI of 67%. In the analysis of efficacy based on cases with severity scores > 8, protective efficacy for first and worst episode scores in the PP population using the PP case definition was 82% with a lower CI at

75%. For efficacy against episodes with severity scores >16, the point estimate was 98% with a lower 95% CI of 88%. Very similar results were obtained in the mITT population using the PP case definition.

The data for all rotavirus AGE (i.e. regardless of G serotype) that met the PP case definition and occurred at least 14 days after the third dose in the PP population during the first season post-vaccination gave a point estimate of 71.8% (95% CI: 64.5, 77.8). Most of the non-vaccine G serotypes were non-typable. Efficacy against rotavirus AGE that occurred through the first and second rotavirus seasons gave a point estimate of 71.3% (95% CI: 64.7%, 76.9%). A similar analysis that looked at efficacy only in the second rotavirus season gave a point estimate of 62.6% (95% CI: 44.3%, 75.4%). Protective efficacy among the non-native US infants was lower than that for the US Concomitant Use Cohort. An analysis of efficacy by race among US infants (not including native Americans) suggested that the point estimate was 60% in whites, with 95% CI of -7.7, 87.2. However, this was based on 410 and 465 infants per group and only 6 and 16 cases of rotavirus AGE, respectively. There was no gender effect on efficacy. The analysis by gestational age at birth included 153 of the 204 enrolled with gestational ages of 30-35 weeks at the time of birth. The point estimates for protective efficacy were 74.2% for full term infants and 70.3% for those born before 36 weeks, with lower 95% CI at 66.8% and -15.4%.

Sensitivity analyses were provided that counted all post-dose 1 rotavirus AGE. Results for prevention of AGE due to vaccine serotypes or any serotype were consistent with the above analyses for rotavirus AGE from 14 days post-dose 3. In addition, an ITT analysis in which all AGE from dose 1 was counted and infants with missing data were assumed to have rotavirus AGE gave a point estimate of 25% and a lower 95% CI of 16%.

The table below provides a breakdown of the primary analysis data by G serotype. For the non-G1 serotypes included in the vaccine, the numbers were very small and the 95% CI were very wide, spanning zero for G3 and G4.

Efficacy in the PP population using the PP case definition

	RotaTeq	Placebo
Subjects vaccinated	2834	2839
Protocol violators	295	271
Subjects with no follow-up	11	6
Classified as rotavirus gastroenteritis cases per per-protocol case definition		
G1 serotype	72	286
G2 serotype	6	17
G3 serotype	1	6
G4 serotype	3	6
Efficacy estimate (%) and 95% confidence interval:		
G1 serotype	74.9 (67.3, 80.9)	---
G2 serotype	63.4 (2.6, 88.2)	---
G3 serotype	82.7 (-42.6, 99.6)	---
G4 serotype	48.1 (-143.2, 91.6)	---

The applicant's RT-PCR and sequence-based assay for VP4 (P) typing was used to test 80 clinical samples collected during studies 006 + 007 and identified as G2, G3, G4 and G9 from both treatment groups. Also tested was a subset of samples identified as G1 and randomly selected by season.

Two P-types were identified:

- P1a[8] was associated with all G1, G3, G4, G9 and G12
- P1b[4] was found with all G2 strains

The primary analysis of efficacy was supplemented during the assessment process by data on hospitalisations and emergency room visits in the much larger total study population. This analysis yielded much larger numbers of PP rotavirus AGE for which the serotype was determined. The next table summarises the findings for cases from 14 days post-dose 3. For the four G serotypes in the vaccine, point estimates exceed 85% and the lower 95% are over about 50% except for G2, for which the lower 95% CI is negative. The data below indicated efficacy against G9P[8] rotavirus and the findings suggested that the P1[8] reassortant in the vaccine may be contributing to or wholly providing the protection observed against G9.

Serotype-specific efficacy against hospitalisation and ER visits for rotavirus AGE

	Number of Cases			
Serotype	RotaTeq N=34,035	Placebo N=34,003	Efficacy Estimate (%)	95% Confidence Interval
G1	16	316	94.9	91.3, 97.0
G2	1	8	87.6	-5.3, 98.5
G3	1	15	93.4	49.4, 99.1
G4	2	18	89.1	52.0, 97.5
G9	0	13	100.0	40.2, 100.0
Per-protocol population and case definition				

With somewhat conflicting data regarding efficacy against G2P[4] in the two analyses shown above, and uncertainty of the real effect due to the small numbers of cases, the applicant provided a further analysis of healthcare contacts for PP rotavirus AGE cases from the day of first vaccination. The data shown below demonstrate that the lower 95% CI for efficacy against G2 in this analysis was above zero.

Hospitalisations and ER Visits for Rotavirus AGE in the mITT population (PP Case definition from day of first vaccination)

	Number of Cases			
Serotype	RotaTeq N=34,035	Placebo N=34,003	Rate Reduction (%)	95% Confidence Interval
G1-4	38	466	91.9	88.0, 94.5
G1	32	414	92.3	88.2, 95.0
G2	1	12	91.7	34,7, 99.0
G3	3	20	85.1	49.6, 95.6
G4	2	20	90.1	57.2, 97.7
G9	2	25	92.1	66.1, 98.2

On the basis of these data and those of the primary analysis it was concluded that efficacy had been demonstrated against G1, G3, G4 and G9 but only when these serotypes were associated with the P1[8} genotype. Efficacy against G2P[4] was less certain but it was considered that there was sufficient evidence to mention this type in the indication provided that section 5.1 of the SPC showed the data clearly and explained that overall efficacy might be less since there would be no contribution from the immune response elicited by the P1[8] component of the vaccine.

- Clinical studies in special populations

N /A

- Supportive study

Study 007

This was specifically designed to assess the efficacy of RotaTeq at end of shelf life potency. .

The primary analysis of efficacy was based on the PP population. The mITT population included all infants with valid efficacy data (including protocol violators).

The statistical primary null hypothesis was that the efficacy of RotaTeq at expiry potency against all G1-, G2-, G3-, or G4-specific cases of rotavirus AGE occurring at least 14 days post-dose 3 through one rotavirus season would be ≤ 0%. This hypothesis was tested using an exact binomial procedure based on the proportion of subjects with rotavirus AGE. Assuming 437 evaluable subjects in each group, and assuming the true rotavirus attack rate was 10% and RotaTeq had a true efficacy of 60% (one-sided $\alpha = 0.025$), there was 90% power to declare that RotaTeq at expiry potency was efficacious. The protocol was amended to increase the study numbers and to allow enrolment over two seasons to achieve the target

Results

The primary efficacy estimate was 72.5% (lower 95% confidence interval 50.6%). In this analysis, 66/69 rotavirus AGE cases were of G1 serotype while the other three were G3. For severe cases (i.e. score >16; using either the first episode or the worst score episode), the point estimate for efficacy was 100% (95% CI 13, 100). In the efficacy analysis using the ITT case definition in the mITT population the point estimate for efficacy was 45.2% with a lower 95% CI at 23.5. For infants enrolled within the USA, the efficacy estimate (based on the PP case definition in the PP population) in white Caucasians was 85.4% (95% CI -9.3%, 99.7%) while that in Hispanics was 100% (95% CI -386.7%, 100.0%). Within the homogeneous infants enrolled in Finland, the point estimate was 70.3% (95% CI 44.9%, 85.0%. An analysis of PP cases in the PP population by gender gave a point estimate for males of 72.0% (95% CI 37.0%, 89.0%) compared to 73.1% for females (95% CI 36.4%, 90.1%).

- Analysis performed across trials (pooled analyses and meta-analysis)

N / A

- Discussion on clinical efficacy

It was considered that efficacy was demonstrated against G1 and that, using the hospitalisation and ER room visits data, efficacy was demonstrated also for G3, G4 and G9 but only when these serotypes were associated with the P[8] genotype. Efficacy against G2P[4] was less certain but it was considered that there was sufficient evidence to mention this type in the indication.

It was considered a deficiency of the dossier that only 3-dose regimens had been studied. It was also difficult to examine any effect of schedule since RotaTeq was administered along with a variety of infant primary series.

However, the majority of infants in the Efficacy Cohort received the third dose of vaccine between 19-22 weeks of age. Therefore, taken together with the analysis of efficacy according to whether doses were given </> 42 days apart, it was concluded that giving all three doses by 22 weeks would provide suitable efficacy. In addition, there would be a potential advantage in terms of prevention of infection and of minimising the number of cases of intussusception that could occur in temporal relationship with vaccine doses in recommending that all three doses should preferably be given by 22 weeks and, based on the median ages that all three doses should be given by 26 weeks. This is reflected in the final SPC recommendations.

The individual reassortant potencies (IU/dose) across the 33 final container lots supplied to the 006 study were as follows:

	G1	G2	G3	G4	P1
Lowest potency per 2 mL dose:	1.1×10^7	1.1×10^7	7.5×10^6	1.2×10^7	1.0×10^7
Highest potency per 2 mL dose:	2.3×10^7	4.2×10^7	2.9×10^7	3.3×10^7	2.8×10^7

These data raised a potential issue since the applicant's proposed potencies for each reassortant at release were lower at G1 $\geq 2.91 \times 10^6$ IU/dose, G2 $\geq 4.11 \times 10^6$ IU/dose, G3 $\geq 3.25 \times 10^6$ IU/dose, G4 $\geq 5.18 \times 10^6$ IU/dose and P1 $\geq 3.75 \times 10^6$ IU/dose. However, taking into account the estimates of efficacy obtained with formulations of varying potencies by reassortant in studies 005 and 007 it was accepted that vaccine of the specified release potency should provide efficacy similar to that observed in 006. However, the applicant will monitor effectiveness in the post-licensure period.

Clinical safety

- Risk of vaccine-associated intussusception was determined in *study 006*

To address the primary safety endpoint, the study employed a group-sequential design. Initially, 60,000 infants were to be enrolled, receive three doses of assigned treatment and complete 42 days of safety follow-up after the final vaccination. After this point was reached, the DSMB was to unblind the treatment arm of positively-adjudicated intussusception cases and assess whether the pre-defined statistical criteria for the primary safety hypothesis were met. If the criteria were not met with 60,000 infants, then an additional group of 10,000 was to be enrolled and then further groups of 10,000 were to be added until the pre-defined statistical criteria were met or until the total had reached 100,000.

The case definition for intussusception included the occurrence of the following within 365 days of the first dose of allotted treatment:

- Radiographic or surgical confirmation of the diagnosis of intussusception or
- Evidence of intussusception at autopsy.

The applicant's SOP regarding detection, adjudication and reporting of cases of intussusception stated that Infants were to have at least one of the following imaging findings:

Radiographic	**Description**
Exam	
1.Barium or air contrast enema	Intra-luminal soft tissue mass, which is described as the coiled-spring sign, meniscus sign, and/or target sign.
2.Ultrasound (US)	Intra-luminal soft tissue mass, which is described as a donut or pseudokidney appearance, multiple concentric, ring sign, and/or crescent-in-donut sign.

And/or one of the following surgical findings:

1 Idiopathic intussusception with no anatomic lead point:
a) Visualization of the telescoped bowel, treated with manual reduction.
b) Visualization of the telescoped bowel, treated with resection.
c) Spontaneous reduction of intussusception.

2 Intussusception associated with anatomic lead point:
If there is a lead point found, describe the lead point in detail

and / or autopsy confirmation.

Case ascertainment involved contacting parents/legal guardians on approximately Days 7, 14 and 42 after each vaccination with RotaTeq or placebo to ask about all SAEs, including intussusception. The sponsor obtained relevant medical records and reported each case to a blinded independent Safety Endpoint Adjudication Committee (SEAC), which reviewed and adjudicated all potential cases of intussusception as they occurred by examining the relevant medical records and diagnostic tests in a blinded fashion. The DSMB unblinded the treatment arm of the SEAC's positively-adjudicated

(confirmed) cases of intussusception and made recommendations for continuing the study based on pre-defined safety boundaries that were designed such that the study would be stopped early if the relative risk of intussusception in any of two overlapping day ranges (1 to 7 and 1 to 42 days after any vaccination) was statistically significantly increased among recipients of RotaTeq *vs* placebo recipients.

The primary null hypothesis was that RotaTeq would increase the risk of intussusception relative to placebo within 42 days of any dose. Assuming an underlying incidence of 1/2000 per annum, a 102-day follow-up period, and that the true relative risk was 1.0, there was approximately 94% power to declare that the relative risk was ≤10.0. The primary safety analysis was based on all subjects who received at least one dose of RotaTeq or placebo.

Of the 56,310 infants that completed the safety follow-up either for 365 days following the first dose or until the study site end-of-study date 46,372 (82.4%) were actually followed for 365 days and 9,938 (17.6%) reached the end-of-study date before Day 365. The baseline characteristics of the Safety Cohort were as follows:

	RotaTeq	Placebo
Randomised (N):	34644	34630
	n (%)	n (%)
Gender		
Male	17586 (50.8)	17529 (50.6)
Female	17058 (49.2)	17101 (49.4)
Age (weeks)		
5 And Under	1 (0.0)	4 (0.0)
6 to 12	34551 (99.7)	34527 (99.7)
Over 12	92 (0.3)	99 (0.3)
Mean	9.8	9.8
Race		
White	23772 (68.6)	23788 (68.7)
Hispanic American	4963 (14.3)	4911 (14.2)
Black	2908 (8.4)	2941 (8.5)
Multiracial	1815 (5.2)	1817 (5.2)
Asian	536 (1.5)	552 (1.6)
Native American	531 (1.5)	514 (1.5)
Other	119 (0.3)	107 (0.3)

There were 30 infants with positively-adjudicated (confirmed) intussusception that occurred at any time throughout the study. Twelve of these had received RotaTeq and 18 received placebo. Three cases were reported after completion of 365 days follow-up post Visit 1 and all three occurred in placebo recipients.

The data for the planned primary evaluation are shown below. There was no pattern of clustering of cases in the early period after each dose with either vaccine or placebo. Ask rapporteur

Confirmed cases of intussusception within 42 days following any vaccination)

	RotaTeq	Placebo
Number vaccinated†	34002	33969
Number with complete follow-up‡	28038	27965
Confirmed cases of intussusception		
Post-dose 1	0	1
Post-dose 2	4	1
Post-dose 3	2	3
Group-sequential adjusted estimate of relative risk and group-sequential adjusted 95% confidence interval‡	1.6 (0.4, 6.4)	---
Group-sequential adjusted p-Value for relative risk ≤10.0	0.006	---
† Excludes subjects who were cross-treated. There were no confirmed cases of intussusception among cross-treated subjects. ‡ Based on latest day of safety follow-up		

The timing of cases in relation to dosing and the unadjusted estimates for relative risk were as follows:

- **Within 7 days** following vaccination, there was one infant in the RotaTeq group but no placebo group infant who had a positively-adjudicated (confirmed) case of intussusception. The case occurred two days following the second dose.

- **Within 14 days** of vaccination, there was one confirmed case in each treatment group. These cases occurred at two days following the second dose of RotaTeq (as above) and at 10 days following the third dose of placebo.

- **Within 60 days** of vaccination, there were eight cases in the RotaTeq group and six in the placebo group with an unadjusted relative risk for the vaccine group of 1.3 and 95% CI at 0.4, 4.6. Numbers were 1, 5 and 2 cases after sequential vaccine doses and 1, 2 and 3 after sequential doses of placebo.

- There were 28 infants who had a positively-adjudicated (confirmed) case of intussusception **within 365 days** following vaccination Visit 1 (13 in the RotaTeq group and 15 in the placebo group). The addition of these extra four and nine cases in respective groups, all of which occurred after the third dose had been given, gives an estimate of relative risk of 0.8 and 95% CI at 0.3, 1.8. These cases included 16 males and 11 females.

The applicant also provided estimates for absolute risk for the various post-dose windows as follows

	RotaTeq	Placebo	Risk Difference and 95% CI§
Confirmed cases of IS/Subjects with complete follow-up †‡			
Within 7 Days of Any Dose	1 / 29638	0 / 29561	0.3 (-1.0, 2.0)
Within 14 Days of Any Dose	1 / 29624	1 / 29547	0.0 (-1.6, 1.6)
Within 42 Days of Any Dose	6 / 28038	5 / 27965	0.4 (-2.4, 3.2)
Within 60 Days of Any Dose	8 / 26153	6 / 26082	0.8 (-2.3, 4.1)
† Excludes subjects who were cross-treated.			
‡ Based on latest day of safety follow-up.			
§ Per 10,000 subjects.			

The ages at the time of diagnosis of intussusception of the 13 and 15 infants captured up to 365 days post-dosing showed a similar spread. Among the RotaTeq and placebo group cases that had received three doses before onset of intussusception, the third dose had been given no later than 30 weeks (7.5 months) of age.

The delay between last dose given and onset varied from approximately 2 days to 6 months in the RotaTeq group although all except one case occurred at least 2 weeks post-dose as already mentioned above. In the placebo group, the range of times to onset ranged from 10 days to about 9 months. Among infants that developed intussusception only after three doses of vaccine or placebo had been administered, the times of onset after the last dose ranged from approximately 5 weeks to 6 months in the RotaTeq group and from 5 weeks to 9 months in the placebo group.

In addition, there were 85 infants who were medically evaluated for intussusception but were determined by the SEAC not to meet the per-protocol case definition. Of these 85 cases, 76 (89%) had a radiographic procedure which definitively ruled out IS while 8 (9%) were clinically assessed as having diagnoses other than intussusception. One negatively-adjudicated subject (in the placebo group) had IS diagnosed by the investigator based on ultrasound performed in the office but this was not confirmed by a repeat ultrasound performed in the hospital. The diagnoses for the AE that triggered concern regarding potential intussusception included conditions such as abdominal distension and colic, urinary tract infections, milk allergies, viral gastroenteritis, haematochezia and vomiting.

- Patient exposure

For the three studies (006, 007 and 009) that used the final formulation of the vaccine numbers exposed were as tabulated below.

All randomised in studies 006 [REST], 007, and 009

	RotaTeq		Placebo		Total	
	n	(%)	n	(%)	n	(%)
Randomised (N):	35975[§]		35404[%]		71379	
Vaccinated At:						
Visit 1	35365	(98.3)	34776	(98.2)	70141	(98.3)
Visit 2	32305	(89.8)	31797	(89.8)	64102	(89.8)
Visit 3	30872	(85.8)	30303	(85.6)	61175	(85.7)
Completed the Third Study Vaccination and the 42-FU[¶]:	30847	(85.7)	30269	(85.5)	61116	(85.6)
Discontinued Before Third Dose or the 42-Day Safety FU Due To:	2967	(8.2)	2945	(8.3)	5912	(8.3)
Clinical adverse experience	224	(0.6)	212	(0.6)	436	(0.6)
Deviation from protocol	991	(2.8)	1037	(2.9)	2028	(2.8)
Refused further participation	225	(0.6)	228	(0.6)	453	(0.6)
Lost to follow-up[#]	80	(0.2)	100	(0.3)	180	(0.3)
Moved	210	(0.6)	195	(0.6)	405	(0.6)
Other	1237	(3.4)	1173	(3.3)	2410	(3.4)

- Adverse events

For infants in the pre-defined Detailed Safety Cohort of study 006 parents/legal guardians were asked to record any AEs that occurred within 42 days following any vaccination. Other participants in this study were followed only for SAEs.

Within 42 days following any vaccination in the Detailed Safety Cohort of study 006 and all infants in 007 and 009, in the RotaTeq group 5273 (86.1%) infants and 4805 (86.6%) in the placebo group reported at least one AE.

AEs in the Detailed Safety Cohort Protocols 006 [REST], 007, and 009

	RotaTeq (N=6157)				Placebo (N=5595)			
	All AEs		VR		All AEs		VR	
	n	(%)	n	(%)	n	(%)	n	(%)
Subjects in analysis population†	6131				5562			
Subjects without follow-up	8				12			
Subjects with follow-up	6123				5550			
Number (%) of subjects with one or more adverse experiences	5273	(86.1)			4805	(86.6)		
Number (%) of subjects with no adverse experience	850	(13.9)			745	(13.4)		
Ear And Labyrinth Disorders	**104**	**(1.7)**	**1**	**(0.0)**	**73**	**(1.3)**		
Eye Disorders	**462**	**(7.5)**	**8**	**(0.1)**	**446**	**(8.0)**	**3**	**(0.1)**
Gastrointestinal Disorders	**2648**	**(43.2)**	**1697**	**(27.7)**	**2312**	**(41.7)**	**1423**	**(25.6)**
Diarrhoea	1382	(22.6)	1002	(16.4)	1075	(19.4)	751	(13.5)
Vomiting	928	(15.2)	621	(10.1)	752	(13.5)	452	(8.1)
General Dis/Admin Site Conditions	**2832**	**(46.3)**	**1293**	**(21.1)**	**2580**	**(46.5)**	**1058**	**(19.1)**
Pyrexia	2602	(42.5)	1274	(20.8)	2373	(42.8)	1036	(18.7)
Infections And Infestations	**3328**	**(54.4)**	**438**	**(7.2)**	**3052**	**(55.0)**	**401**	**(7.2)**
Bronchiolitis	319	(5.2)	3	(0.0)	267	(4.8)	2	(0.0)
Bronchitis	101	(1.6)			84	(1.5)	1	(0.0)
Candida nappy rash	56	(0.9)	1	(0.0)	57	(1.0)		
Croup infectious	63	(1.0)	1	(0.0)	53	(1.0)		
Gastroenteritis	571	(9.3)	279	(4.6)	574	(10.3)	289	(5.2)
Upper respiratory tract infection	1580	(25.8)	59	(1.0)	1448	(26.1)	43	(0.8)
Psychiatric Disorders	**1512**	**(24.7)**	**717**	**(11.7)**	**1396**	**(25.2)**	**676**	**(12.2)**
Agitation	325	(5.3)	12	(0.2)	281	(5.1)	9	(0.2)
Crying	222	(3.6)	101	(1.6)	243	(4.4)	107	(1.9)
Irritability	1162	(19.0)	620	(10.1)	1050	(18.9)	584	(10.5)
Restlessness	82	(1.3)	29	(0.5)	80	(1.4)	29	(0.5)
Respiratory, Thoracic And Mediastinal Disorders	**1501**	**(24.5)**	**103**	**(1.7)**	**1316**	**(23.7)**	**79**	**(1.4)**
Skin / Subcutaneous Tissue Dis	**704**	**(11.5)**	**67**	**(1.1)**	**592**	**(10.7)**	**54**	**(1.0)**

† Excludes subjects who were cross-treated in Protocol 006 (REST) and Protocol 009 and 1 subject from Protocol 007 who received 4 doses of RotaTeq

Among the 11,673 infants followed for all AEs, the three most frequently reported were pyrexia, upper respiratory tract infection, diarrhoea and irritability. These all occurred at numerically similar rates in vaccine and placebo groups. However, the applicant reported that the incidences of diarrhoea (22.6% *vs* 19.4%), vomiting (15.2% *vs* 13.5%), nasopharyngitis (6.9% *vs* 5.9%) and otitis media (14.4% *vs* 12.9%) were statistically higher in the group that received RotaTeq.

In the Detailed Safety Cohort, parents/legal guardians were also to record the daily number of episodes of vomiting, diarrhoea and elevated temperature (≥38.1˚C) and irritability for the first 7 days following any vaccination. Temperature was taken rectally 4 to 6 hours after each dose and then daily at approximately the same time each day for 7 days. Based on the comparisons made below, RotaTeq was associated with higher rates of diarrhoea after the first, second and summed vaccinations and with a higher rate of vomiting after the first and summed doses compared to placebo.

AEs of special interest within 7 days of vaccination (Detailed Safety Cohort from 006 [REST], 007, and 009)

	RotaTeq			Placebo			Risk Difference (RotaTeq-Placebo) (95% CI)	p-Value (2-sided)
	n	m	%	n	m	%		
Diarrhoea								
Post-dose 1	577	6123	9.4%	445	5550	8.0%	1.4% (0.4 , 2.4)	0.007
Post-dose 2	458	5671	8.1%	300	5140	5.8%	2.2% (1.3, 3.2)	0.000
Post-dose 3	317	5434	5.8%	248	4927	5.0%	0.8% (-0.1 , 1.7)	0.074
Post Any Dose	1028	6123	16.8%	761	5550	13.7%	3.1% (1.8 , 4.4)	0.000
Elevated Temperature								
Post-dose 1	959	5613	17.1%	820	5073	16.2%	0.9% (-0.5 , 2.3)	0.202
Post-dose 2	1037	5196	20.0%	910	4712	19.3%	0.6% (-0.9 , 2.2)	0.420
Post-dose 3	874	4822	18.1%	761	4340	17.5%	0.6% (-1.0 , 2.2)	0.461
Post Any Dose	2022	5747	35.2%	1766	5205	33.9%	1.3% (-0.5 , 3.0)	0.168
Irritability								
Post-dose 1	433	6123	7.1%	395	5550	7.1%	-0.0% (-1.0 , 0.9)	0.925
Post-dose 2	339	5671	6.0%	334	5140	6.5%	-0.5% (-1.4 , 0.4)	0.264
Post-dose 3	232	5434	4.3%	221	4927	4.5%	-0.2% (-1.0 , 0.6)	0.592
Post Any Dose	785	6123	12.8%	719	5550	13.0%	-0.1% (-1.4 , 1.1)	0.829
Vomiting								
Post-dose 1	410	6123	6.7%	300	5550	5.4%	1.3% (0.4 , 2.2)	0.004
Post-dose 2	287	5671	5.1%	228	5140	4.4%	0.6% (-0.2 , 1.4)	0.129
Post-dose 3	194	5434	3.6%	159	4927	3.2%	0.3% (-0.4 , 1.0)	0.338
Post Any Dose	713	6123	11.6%	546	5550	9.8%	1.8% (0.7 , 2.9)	0.002

- Serious adverse event/deaths/other significant events

Overall, 1723 infants in these studies had at least one SAE, with rates of 834 (2.4%) for RotaTeq and 889 (2.6%) for placebo. There were 123 infants with at least one vaccine-related SAE in these three studies, of which 47 (0.1%) received RotaTeq and 76 (0.2%) received placebo.

The most frequent SAEs within 42 days following any vaccination visit were infections and infestations (in 1.7% and 1.9% per group), with bronchiolitis as the commonest diagnosis followed by gastroenteritis. The three most frequent vaccine-related SAEs were gastroenteritis, pyrexia, and dehydration. Of these, gastroenteritis considered vaccine-related occurred in 16 in the RotaTeq group and 32 subjects in the placebo group. Gastro-oesophageal reflux disease (GERD) was reported as a SAE more frequently in the RotaTeq group (25 *vs* 17 cases in the placebo group; risk difference = +2.2/10,000). It was considered that GERD and other serious gastrointestinal illnesses should feature in the Risk Management Plan.

There were 26 deaths reported in studies 006, 007 and 009 that occurred within 42 days following any vaccination, of which 15 occurred in the RotaTeq group. For 52 deaths that occurred at any time during the Phase III studies 25 received RotaTeq. The most commonly reported cause of death at any time was SIDS (8 RotaTeq and 9 placebo). One death occurred in an infant diagnosed with intussusception on Day 98 after the third dose of RotaTeq that required surgical resection and was followed by post-operative sepsis considered probably not related to vaccine by the investigator. All deaths were considered probably or definitely not related to vaccination/placebo.

- Discontinuation due to adverse events

There were 205 subjects in 006, 007 and 009 that discontinued from these studies due to AEs, of which 111 received RotaTeq and 94 received placebo. There were 149 infants who discontinued from studies 006, 007 and 009 due to a SAE, of which 80 (0.2%) received RotaTeq and 69 (0.2%) received placebo. In addition, 29 discontinued due to a vaccine-related SAE, including 13 that received RotaTeq and 16 that received placebo

- Safety related to drug-drug interactions and other interactions

There were 6790 infants in study 006 that received a hexavalent vaccine concomitantly (>99% on same day) with RotaTeq or placebo. Of these, 95 (2.8%) in the group that received RotaTeq and 102 (3.0%) in the placebo group had a SAE within 42 days of any dose. The most common SAEs were infections and infestations. In general, types and rates of SAEs were similar between vaccine and placebo groups.

In the US Concomitant Use Cohort of 1358 infants who were followed for AEs, 545 (82.3%) in the RotaTeq group and 606 (87.1%) in the placebo group reported at least one AE, of which the most frequent were pyrexia (46.5% and 49.1%), upper respiratory tract infection (23.6% and 27.2%) and diarrhoea (13.9% and 18.1%).

In the German Detailed Safety Cohort (637 who received a concomitant hexavalent vaccine), AE rates were 286 (88.3%) in the RotaTeq group and 274 (87.5%) for placebo. Pyrexia occurred in 42.3% and 43.8%, diarrhoea in 28.7% and 26.8% and vomiting in 23.5% and 23.3%. Other AEs occurred at lower rates with no notable differences between treatment groups.

- Post marketing experience

Not applicable

- Discussion on clinical safety

Regarding the risk of intussusception, even a study of this size, which is really as large as any Company could realistically be expected to undertake pre-licensure, cannot rule out the possibility of an excess risk for intussusception associated with the vaccine. However, the only way to arrive at a better assessment will be to study safety and efficacy in very large numbers of infants in the post-licensure period and to conduct very frequent and detailed reviews of the findings. Close collaboration will be needed between the Company, the CHMP and Public Health Authorities to ensure that every possible measure is taken to detect potential problems as early as possible.

The occurrence of mild AGE in vaccine recipients was thought to be most likely a side effect of shedding vaccine strains although there are no data to rule out the possibility that these infants were co-infected with another pathogen. The proposal is based on the fact that the WC3 bovine rotavirus is thought to undergo a process of abortive replication in the human host, which could result in some mild AGE symptoms. It was considered highly unlikely that the mild AGE observed in these infants was due to development of virulent reassortants of vaccine and wild type strains because:

- Shedding studies showed that this occurs only in a small proportion of infants, in low quantities and predominantly during the week after the first dose so there is very limited opportunity for reassortment to occur between live vaccine and wild type strains
- A reassortant would be very unlikely to have the virulence of the wild-type human strain. Since the vaccine reassortants contain genes of bovine origin except for 1 or 2 human genes multiple reassortments would have to occur with exchange of bovine segments for human segments before a rotavirus with the virulence of a human strain would result.

A slightly higher number of CNS events occurred in the RotaTeq group although rates for those without an identified aetiology were similar to the placebo group. Although the current data do not show any clear evidence of an association between RotaTeq and the potential extra-intestinal manifestations of wild-type rotavirus that have been reported in the literature, monitoring for such events has been highlighted in the Risk Management Plan.

Gastro-oesophageal reflux disease (GERD) was reported as a SAE more frequently in the RotaTeq group (25 *vs* 17 cases in the placebo group; risk difference = +2.2/10,000). It was considered that GERD and other serious gastrointestinal illnesses should feature in the Risk Management Plan

3.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfilled the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan.

Table Summary of the risk management plan

Safety issue	Proposed pharmacovigilance activities
Intussusception	Passive reporting and US post marketing safety surveillance study Collaboration with ESPED on the epidemiology of intussusception in Germany
Vaccine effectiveness and strain replacement	Rotavirus Surveillance in Europe: determining the diversity of co-circulating rotavirus strains in consecutive rotavirus seasons
Risk of transmission from vaccinees to close contacts and the potential for new reassortants to emerge	Passive reporting
Potential for extra-intestinal manifestations of rotavirus infection (including neurotropic activity and hepatotoxicity	Passive reporting and US post marketing safety surveillance study
Administration to HIV-infected infants that will also serve to assess the risk to such individuals of any accidental transmission of vaccine virus from other vaccinated infants.	Clinical study in Thailand and South Africa (protocol 011)
Potential gastro-oesophageal reflux disease (GERD)	Passive reporting and US postmarketing safety surveillance study
Hypersensitivity reactions	Passive reporting and US postmarketing safety surveillance study

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

3.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

During the evaluation of RotaTeq, a number of issues were identified. The main issues related to the potency assay (M-QPA) (comparability with the plaque assay and robustness of the assay), the limit for residual Vero cell DNA, justification of storage periods of intermediates and the proposed potency specifications being lower than those used in the main efficacy study 006. Satisfactory answers have been provided to resolve these concerns.

Other minor concerns have been adequately addressed; however, a number of follow-up measures are defined to provide further information post-approval. In conclusion, all quality issues are resolved.

Non-clinical pharmacology and toxicology

The limited programme of non-clinical studies in support of this vaccine generally fulfilled the recommendations in the Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95).

Oral administration of RotaTeq to mice as a single or 3-dose regimen over approximately 10 weeks was well tolerated. There were no treatment-related effects on mortality, physical signs, body weight, food consumption, haematological parameters, or serum biochemical parameters. There were no treatment-related gross, histopathological or organ weight changes at either the interim (Study Day 8) or terminal (Study Day 64) necropsy

The Vero cell DNA uptake studies in rats were carried out to support the safety of the residual Vero cell DNA levels in RotaTeq, as requested in the Scientific Advice letters. The presented data do support the conclusion that the uptake of DNA from an orally administered dose of RotaTeq containing 100 µg of residual DNA is lower than from a parenteral vaccine containing DNA at the upper limit according to the WHO recommendations.

Efficacy

Efficacy was demonstrated against G1 in the primary analysis. Efficacy against G3, G4 and G9 was based on data on hospitalisations and ER visits for rotavirus AGE. However, these claims for efficacy were restricted to strains with the P[8] genotype. Efficacy against G2P[4] was less certain but it was considered that there was sufficient evidence to mention this type in the indication. .

Safety

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

Regarding the risk of intussusception, even a study of this size, which is really as large as any Company could realistically be expected to undertake pre-licensure, cannot rule out the possibility of an excess risk for intussusception associated with the vaccine. However, the only way to arrive at a better assessment will be to study safety and efficacy in very large numbers of infants in the post-licensure period and to conduct very frequent and detailed reviews of the findings. Close collaboration will be needed between the Company, the CHMP and Public Health Authorities to ensure that every possible measure is taken to detect potential problems as early as possible.

- User consultation

The applicant performed readability testing ("user consultation") and a satisfactory report has been provided.

Risk-benefit assessment

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 risk-benefit balance of RotaTeq indicated for
"the active immunisation of infants from the age of 6 weeks for prevention of gastroenteritis due to rotavirus infection. In clinical trials, efficacy was demonstrated against gastroenteritis due to rotavirus of serotypes G1P1[8], G2P[4], G3P1[8], G4P1[8], and G9P1[8]" was favourable and therefore recommended the granting of the marketing authorisation.