

Review Memo - CMC section - Rotarix

- **MEMORANDUM**

DATE: December 19th, 2007

FROM: Dino Feigelstock

SUBJECT: Review Memo for STN 125265/0 Rotarix: Human Rotavirus Vaccine Live, Oral, GlaxoSmithKline Biologicals, CMC section.

TO: Laraine Henschel, OVRP, DVRPA

THROUGH: Stephen Feinstone, Robin Levis, Anissa Cheung, Phil Krause, Jerry Weir, DVP, OVRP

cc. Loris McVittie, DVRPA

Introduction and Summary:

Brief description of product:

Rotarix is composed of a monovalent, live, attenuated rotavirus derived from the human 89-12 strain (isolated from a naturally infected child with rotavirus gastroenteritis). This strain belongs to the serotype G1P1A and genotype P[8]. Rotarix is provided as a lyophilized cake contained in a glass vial and a liquid diluent (1mL) in a prefilled oral glass applicator with a plunger stopper. The lyophilized vaccine contains amino acids, dextran, Dulbecco's Modified Eagle Medium (DMEM), sorbitol, and sucrose; the vaccine diluent contains calcium carbonate, sterile water, and xanthan. The liquid diluent contains calcium carbonate (CaCO₃), an antacid component, to protect the vaccine during passage through the stomach and prevent its inactivation due to the acidic environment of the stomach. After reconstitution, one dose of Rotarix is 1mL and contains at least 10⁶ median Cell Culture Infective Dose (CCID50) of live, attenuated HRV strain produced on Vero cells. The table below summarizes the composition of the HRV reconstituted vaccine.

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Indication:

Rotarix is indicated for the prevention of rotavirus gastroenteritis caused by G1 and non-G1 types (including G2, G3, G4, and G9). The proposed vaccination series consists of two doses administered orally. The first dose should be administered in infants 6 weeks through 14 weeks of age. There should be an interval of at least 4 weeks between the first and second dose. The 2-dose series should be completed by 24 weeks of age.

Sponsor:

Manufacturing of the drug substance is performed by:

GlaxoSmithKline Biologicals

Belgium

Testing of the drug substance is performed by:

GlaxoSmithKline Biologicals ----

Belgium

Or by:

GlaxoSmithKline Biologicals

Belgium

Cross-referenced files:

BB-IND ----

Background:

Previous clinical development of the HRV 89-12 strain (P33) was done by AVANT, a biotechnology company in the USA (previously called Virus Research Institute (VRI)). Studies conducted by AVANT demonstrated the safety, immunogenicity and efficacy of the vaccine candidate in infants. Natural infection with the 89-12 strain was shown to provide protection against subsequent illness and against reinfection in a two-year prospective study. Rotarix has been approved for use in Mexico in 2004, and subsequently in other Latin American countries and Europe.

Scientific rationale:

Oral administration of HRV vaccine induces anti-HRV antibodies production and immune memory, which confers protection of children against diarrhea, especially severe diarrhea due to rotavirus infection. Rotavirus infections induce a humoral response beginning with production of IgM antibodies followed by production of specific IgA and IgG antibodies. No correlate of protection has yet been identified. However, it is generally considered that specific serum IgA antibody is a strong marker of protection in most field studies and vaccine trials.

Novelty about product and/or its manufacture:

Rotarix is composed of a (monovalent) human attenuated rotavirus strain (derived from a human strain, "89-12", which belongs to the G1 serotype and P[8] genotype). This is different from Merck's FDA approved vaccine (RotaTeq) which is a "pentavalent" vaccine, composed of five reassortants rotavirus strains derived from a bovine strain (each of which contains a gene encoding VP4 or VP7 from human origin). Rotarix vaccine also contrasts with the RotaShield vaccine (which was withdrawn from the market in 1999); RotaShield is composed of 4 human-simian reassortants containing four serotypically distinct VP7 components.

Previous Human Use:

Rotarix has been approved for use in Mexico in 2004, and subsequently in other Latin American countries and Europe. Eight clinical trials performed in the USA, Latin America and Finland have shown that the vaccine is safe and protected children against any and severe rotaviruss gastroenteritis.

In this BLA, results of ten randomized placebo-controlled clinical studies are provided to support licensure of the candidate HRV vaccine in the US. A total of 74,570 infant subjects participated in these 10 studies with 36,755 infant subjects receiving the HRV vaccine at the potency intended for commercial use in the US market (i.e., at least 106.0 CCID50 antigen per dose).

Chemistry, Manufacturing and Control:

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**23 PAGES
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TO BE RELEASABLE**

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Pre-clinical / Animal Studies (pharmacology/toxicology) and Laboratory Studies:

There is no universally accepted animal model for rotavirus infection and pathology induced by human strains of rotavirus. Evidence is presented justifying the selection of the young ----- rat as an appropriate susceptible animal model to study the toxicity of the GSK Bio candidate vaccine. The same model was also used to investigate the toxicological profile of the candidate rotavirus vaccine in a GLP compliant combined single and repeated-dose toxicity study. The sponsor notes that studies in rats were conducted for the purpose of toxicity studies. Although some serology was performed, it was not the aim to study the specific immune response in this model.

Results indicated no treatment-related mortality, clinical signs, ophthalmological lesions, effects on bodyweight and food intake, effect on body temperature, hematology, coagulation and clinical chemistry parameters. There were no effects on organ weights or macroscopic or histopathological changes that were considered related to treatment. No histopathological changes were seen in the intestinal villi. No pathognomonic epithelial syncytia and no intracytoplasmic eosinophilic inclusions were present in the ileum (as commonly seen during infection by Rotaviruses). Standard examination of the lymphoid organs did not reveal any changes related to the administration of the rotavirus candidate vaccine. Combined virology and serology analysis demonstrated a measurable vaccine take in 80 % of rats that received the vaccine candidate (Rix 4414 ---- mg of CaCO₃) and in 40 % of rats that received the vaccine strain alone (without antacid), suggesting the additive value of using CaCO₃ as an antacid. The coexistent proof of vaccine take, based on the induction of an anti-rotavirus antibody response and shedding of the virus in the feces, provides a good indication that the vaccine is immunogenic without any histopathological changes, clinical symptoms or diarrhoea, and without evidence of intussusception.

Apart from the main repeated dose toxicity study, it should be noted that monkey neurovirulence tests (MNVT) have been performed on Rotavirus Vaccine ----- for characterization purpose. Study results showed no unexpected clinical or histopathological evidence of involvement of the central nervous system was attributable to the inoculated ----.

The sponsor concludes that the candidate human rotavirus vaccine is immunogenic and non-toxic when tested in the young ----- rat. The vaccine is well tolerated, does not induce clinical signs, any clinical disease symptom, hyperthermia or signs of inflammation in the gastrointestinal tract or adjacent lymph nodes. Also, the final vaccine formulation does not contain any new or hazardous excipient and is therefore considered devoid of any toxicity. The coexistent proof of RIX 4414 vaccine take, based on the induction of an anti-rotavirus antibody response and shedding of the virus in the feces, provides a good indication that the vaccine is immunogenic without any histopathological changes, clinical symptoms or diarrhoea, and without evidence of intussusception.

Summary and Conclusions:

Major strengths:

For the manufacturing of the vaccine, the sponsor has taken into account FDA guidelines and several recommendations made by CBER during the last years. The sponsor has taken into account guidelines from European Regulatory agencies.

The sponsor has made extensive studies to ensure that cell banks and virus seeds have no contamination with extraneous infectious and non-infectious agents.

The sponsor has adapted the cell substrate (VERO cells) to grow in -----

Therefore, the vaccine is grown in ----- medium.

The sponsor applied several in process controls during the manufacturing of the vaccine to ensure its safety.

The sponsor performed extensive testing at several steps of vaccine production to ensure sterility and absence of contamination with extraneous infectious and non-infectious agents.

The bulk vaccine, the bulk ----- and the ----- solution are ----- on --- μ m membranes.

The proposed testing for Lot Release of final containers (rotavirus) includes: Description, identity, sterility, moisture content, -----, potency and loss of potency after -- days at ---°C.

The proposed testing for Lot Release of final containers (diluent) includes: Description, identity of -----, identity -----, volume, and calcium carbonate content.

The vaccine is administered orally and contains no adjuvant.

The vaccine strain is an attenuated HRV strain that has been ----- times.

Although the sponsor couldn't determine the determinants for attenuation, ----- to be specific for the vaccine strain (-----). The sponsor shows genetic stability of the vaccine strain during the steps involved in vaccine manufacture ----- . The sponsor also claims genetic stability of the vaccine after passing through the gut, by analyzing virus recovered from the feces of children inoculated with the vaccine (the vaccine doesn't revert to wild type during passage in the gut of individuals).

Animal studies (using young ----- rats) have shown that the candidate human rotavirus vaccine is immunogenic and non-toxic. The vaccine is well tolerated, does not induce clinical signs, any clinical disease symptom, hyperthermia or signs of inflammation in the gastrointestinal tract or adjacent lymph nodes. No evidence of histopathological changes, clinical symptoms, diarrhea, and intussusception was found.

The final vaccine formulation does not contain any new or hazardous excipient (see next point below) and is therefore considered devoid of any toxicity.

Regarding ----- content, it is -----/dose in commercial bulks. This amount of ----- is -----/dose recommended by the WHO for parenteral vaccines, and -----/does, the value for the RotaTeq vaccine (Merck & Co., Inc) recently approved by the FDA.

Rotarix has been approved for use in Mexico in 2004 and other Latin American countries.

Subsequently, in January 2006, The European Marketing Authorization from the European Commission approved its use in the European Union.

Major weaknesses:

No "major" weakness were identified.

One point to consider is the inclusion of ----- during rotavirus vaccine bulk production ----- . CBER has requested data showing clearance of -----, and the sponsor states that clearance has been demonstrated to be ----- . So, there is ----- in the final product. If there is no previous use of ----- in FDA approved products, we may want to ask the sponsor to provide data showing that ----- is not toxic for animals/humans.

There is ----- Rotavirus titer ----- per vaccine dose.

Problematic areas

Points worthy of discussing with the review team during decision meetings.

Recommendation on hold / no hold.

Comments for the Review Team (I raised these comments during the mid-cycle review meeting that took place on 11.15.07. The recommendations suggested by the review team are summarized):

The sponsor propose that the final formulation should contain at least 6.0 log₁₀ CCID₅₀ (cell culture infectious dose 50%) of virus. This lower limit in active ingredient was set based upon efficacy results obtained during clinical development of the *Rotarix* vaccine. The sponsor states that phase II clinical data have shown clear evidence that two doses of the HRV vaccine at a viral concentration of 10^{5.2} ffu and of 10^{5.8} ffu (clinical lots DRVC010A48 and DRVC004A46 respectively) provided high protection against any and severe rotavirus disease.

1. Why were those specific lots chosen to select the final dose? Phase 3 lots (and other phase 2 lots) have a higher titer.
2. Clinical lot DRVC004A46, selected by the sponsor, has a \log_{10} CCID50/dose of 6.6, higher than the proposed 6.0.

In addition, there is ----- specification for potency.

2) 3.2.S.4.2, page 4 out of 7. Identity of rotavirus and attenuation.

A point of consideration is that there is no test to ensure that the virus in the vaccine is attenuated (and didn't revert to wild type). The basis for not testing (for attenuation) is the following: -----

[illegible]

-----for ----- at the GMP inspection conducted at -----
----- The ---- has further concurred with the opinion of the -----.

3) Sterility testing has been conducted on a significant number of commercial lots (at least --
) up to validation of the ----- release.

4) The method for sterility testing requires a -----, which entails a significant risk of false negativity. The implementation of ----- release would be highly beneficial to guarantee the diluent sterility. Furthermore, there is no requirement for compliance with the ----- sterility testing in case of oral route of vaccine administration. It seems to me that the sponsor claim is acceptable.

5) 3.2.P.7 Container Closure System - Diluent

Some parts of the closure system (syringe plunger rods, backstops for syringes, a transfer adapter consisting of two plastic parts molded together: a rigid ----- vial adapter whose spike will pierce the vial stopper, and a terminal ----- Elastomer) are not sterilized. The sponsor states that "This material is not sterilized, considering the oral route of the vaccine administration and the short time of contact of the vaccine with the device." It seems to me that the sponsor's claim is acceptable.

There are no other questions or issues regarding the diluent sterility that would prevent licensure.

6) The sponsor -----rotavirus titer ----- per vaccine dose.

Review Team discussion and recommendation: the issue will be further investigated.

Questions for the sponsor (the below questions were raised during the December 07 pre-approval inspection; I provide the answers given by the sponsor below each question):

Final container specifications:

Please ----- rotavirus titer per vaccine dose in final container.

The sponsor stated that other live attenuated vaccines have ----- specifications, included some recognized to be associated with neurovirulence. The sponsor also stated that it is not in the interest of the company to produce a vaccine with ---- potency(-----
-----).

It should be noted that Rotateq (the Merck's FDA approved Rotavirus live attenuated vaccine) has -----.

I don't consider that the answer of the sponsor is satisfactory.

Production of -----:

Please provide a plan to demonstrate that the genomic sequence of -----
of RIX4414 vaccine is the same as the one used for the clinical trials.

The sponsor states that they have ----- for the next -----). When a -
----- and ----- will be prepared, the FDA will be notified.

I consider that the answer of the sponsor is satisfactory.

3.2.S.2.3. 1.1 page 6 of 74

The vaccine is produced in VERO cells that have been adapted to grow in ----- medium. The sponsor offers a clear characterization of the cells and medium. The sponsor states that before the use of VERO cells "The original isolate was passaged 26 times in Primary African Green Monkey Kidney cells (AGMK) in order to prepare seed material." "The vaccine developed by AVANT was further prepared by passage on an AGMK cell line to the seventh passage i.e. passage level P33" and then "GSK Biologicals' HRV vaccine was developed from the P33 virus material." (-----).

Please describe the primary AGMK cells and the AGMK cell line used to propagate the original isolate. Please describe the culture conditions, including composition and origin of media, used to propagate the above mentioned cells.

The issue was clarified by the sponsor during the inspection. The sponsor provided a document stating that they don't have information on the primary cells used for the first passages conducted by AVANT. With respect to the AGMK cell line used from P26 to P33, the cell line was developed by ----- at ----- . The sponsor states that this cell line was approved by the FDA under BB-IND ---- and clinical studies have been conducted by AVANT under BB-IND ---- with a total of --- recipients of 89-12 strain at passage 33.

3.2.S.2.3, 4.2 page 73 of 74. Ingredients not described in a pharmacopoeia

Please provide data (origin, source, composition, purity, etc) for the ----- inhibitor (used to -----), --- (-----) and -----.

The issue was clarified by the sponsor during the inspection. The sponsor provided documents indicating the characteristics of the above mentioned substances.

3.2.S.2.3, Tables 9 and 10, page 23 of 74.

Please clarify if all the tests are performed using adequate positive and negative controls.

The issue was clarified by the sponsor during the inspection. The sponsor stated that in all tests, positive and negative controls are performed.

3.2.S.2.3, Table 9, page 25 of 74. Test for extraneous agents.

It is stated (tests 2.6) that "-- ml of ----- product are inoculated on Vero cells".

Please explain how the product is -----.

It is stated that there should be absence of ----- effect at the ----- period (-- days). Please explain why the rotavirus present in the ----- doesn't produce ----- (if the product is -----, it is difficult to ensure a ----- which will not produce ----- after -- days).

The issue was clarified by the sponsor during the inspection. The sponsor stated that in the test for extraneous agents, the -- ml of ----- product is not treated with -----; therefore the rotavirus present in the ----- is not able to -----

3.2.S.2.5, 3.1.2, page 12 of 15. Acceptance criteria of -----

It is stated that "potency results should be consistent (trend analysis)".

Please clarify the term "trend analysis". Please provide quantitative criteria for the consistency.

During the inspection, the sponsor agreed to provide a statistic analysis.

3.2.S.3.1, pages 5-9 out of 15, sequence data:

Sequence was obtained ----- the rotavirus genome by -----

Please explain the measures taken to avoid (---) -----.

Please provide evidence that there was no ----- during the ---, and the obtained sequences correspond to the corresponding viruses (especially for the ----- sequences)

During the inspection, the sponsor mentioned the ----- used during ---. The sponsor stated that there is no ----- during the "--" reaction, and I strongly recommended the sponsor to add this control in future experiments.

3.2.S.3.1, 2.3.2, page 10:

Please provide results and conclusions from the sequence of the -----, especially the comparison between RIX4414 vaccine virus --- and virus ---.

The issue was clarified by the sponsor during the inspection. The sponsor provided information regarding the comparisons of --- and ---.

3.2.S.3.1, page 11 out of 15, figure 4:

Please explain the missing ---- in -----

The issue was clarified by the sponsor during the inspection. The sponsor showed photos of different ----.

3.2.S.3.2, 2.1, from page 1 out of 35. -----

You state that ----- content has been assessed using the ----- system. According to the submission, the system can't efficiently detect ----- of a length of ----- . In addition, ----- are detected with just --- efficacy. Finally, small ----- are eliminated by the -----, so there is no assessment of those ----- . Please explain how the total amount of ----- is assessed in the vaccine.

The issue was clarified by the sponsor during the inspection. The sponsor stated that the objective of the test is to detect -----, and those are well detected.

3.2.S.4.2, 2.4.2, page 6 of 7. "CCID50" method

Please provide a detailed description of the CCID50 method (how many repetitions per dilutions, what is considered a positive well, how many cells in a well should be rotavirus positive for the well to be considered positive, which antibody dilutions are used, how is --- stored, etc).

The issue was clarified by the sponsor during the inspection. The sponsor provided the SOP for the CCID50 method.

3.2.P.3.5 page 1 of 9. Testing each batch of diluent.

It is stated that each batch of diluent is tested for appearance, identity -----, identity ---- ----- volume, and calcium carbonate content.

We believe that the reconstitution test is critical to validate new batches of diluent. Please explain why the reconstitution test is not part of the testing of each batch of diluent.

During the inspection, the sponsor stated that given the consistency of the product, they don't consider that the test is necessary. In addition, the sponsor stated that it is not that easy to perform the potency test with the diluent, given its -----.

3.2.P.8.3 Stability Data - HRV

It is stated that "tests and methods applied in stability follow-up of HRV vaccine component are those applied for control testing of final vaccine lots at release. Specifications are the same as proposed for routine release of commercial vaccine lots". The tests shown in tables 3, 4 and 5 for stability results are not the same to the "Proposed tests and specifications for diluent to be used for vaccine reconstitution" (see 3.2.P.5.1, Table 1). "Identity -----", "Identity -----", "Volume", and Calcium carbonate content by -----" are missing from stability studies. Please clarify.

The issue was clarified by the sponsor during the inspection. The sponsor recognized that there was a mistake in the submission, and they wanted to express that the specification were the same, but not the tests performed.