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Patient Health Protection

Assessment report for RotaTeq

Common name: rotavirus vaccine, live, oral

Procedure number: EMEA/H/C/669/A-20/0025

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

1. 1. Background information on the procedure

The European Medicines Agency (EMA) was made aware on 21 April 2010 by the marketing authorisation holder (MAH) of RotaTeq, Sanofi Pasteur MSD, SNC of information concerning the presence of DNA of a viral strain of porcine circovirus (PCV) in its live attenuated rotavirus vaccine. These results were preliminary and warranted confirmation. On 3 May 2010, the MAH provided additional information indicating that the preliminary tests suggested the presence of low levels of DNA fragments derived from PCV-1 and PCV-2 in drug substance and drug product. The detection of PCV was not part of the product specifications routine screening and was done using an analytical detection method which was not part of the current control method used; this was initially reported in a research publication[1], although RotaTeq tested negative in the referred publication.

On 6 May 2010 the Agency informed the European Commission that further in-depth analysis of this issue was appropriate.

In view of the above the European Commission initiated on 10 May 2010 a procedure under Article 20 of Regulation (EC) No 726/2004. The European Commission requested the CHMP to assess the impact of the above findings on the quality of RotaTeq, and to give its opinion on measures necessary to ensure the quality of this product, and on whether the marketing authorisation for this product should be maintained, varied, suspended or withdrawn.

The Vaccine and Biologics Working Parties were consulted for the assessment of this procedure, as appropriate.

2. Scientific discussion

RotaTeq (rotavirus vaccine, live) is a live attenuated vaccine indicated for the active immunisation of infants from the age of 6 weeks for prevention of gastro-enteritis due to rotavirus infection; RotaTeq is administered orally. RotaTeq's safety profile has been established based on placebo controlled clinical trials data, including monitoring of non-serious and serious adverse events. More than 72,000 subjects have been included in clinical trials, of which at least 37,000 subjects received RotaTeq.
The initial evidence provided by the MAH indicated that RotaTeq vaccine had tested positive for PCV DNA, with DNA levels near the limit of detection (LOD) of the assay. Preliminary data suggested that low levels of DNA fragments derived from PCV-1 and PCV-2 were present in samples of the vaccine drug substance and drug product. PCV-1 and PCV-2 are small (<20 nm), non-enveloped, single-stranded DNA viruses of the Circoviridae family. Mammalian circovirus include only these two closely related species, PCV-1 and PCV-2, infecting pigs. PCV type 1 is widespread in swine and the virus has not been linked to any animal or human disease. PCV type 2 is widespread in swine and the virus has not been linked to any human disease. PCV-2 is the essential infectious agent of post-weaning multi-systemic wasting syndrome (PMWS) in pigs. PCVs are highly prevalent in healthy pigs, thus human dietary and respiratory exposure to this virus is likely to be common through pork consumption and/or inhalation of particles from pig faeces in the swine industry.

In order to investigate the unexpected presence of PCV in RotaTeq, several experiments were initiated. The evaluation focused mainly on investigating the root cause, determining the nature and the content in PCV-1/PCV-2 DNA or viral particles and their infectivity, identifying in which batches PCV had been detected, and if those had been used for vaccination, seroconversion data was to be provided. Efforts also included establishing plans to remove PCV from the vaccine, as applicable.

2.1. Assessment of the impact of the PCV finding on RotaTeq

2.1.1. PCV detection and potential root cause

PCV-1 and PCV-2 presence was investigated in several stages of development, and pilot, clinical and manufacturing lots were selected for testing. Vaccine bulk lots manufactured at the pilot scale in support of the Phase III Rotavirus Efficacy and Safety Trials (REST, study/protocol 006[2]), and at the

[1] Viral Nucleic Acids in Live-Attenuated Vaccines: Detection of Minority Variants and an Adventitious Virus. Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S and Delwart EL. *J Virol. 2010 Jun;84(12).* http://jvi.asm.org/cgi/content/short/84/12/6033

[2] Study 006, REST, (Rotavirus Efficacy and Safety Trial) evaluated the efficacy and safety of Rotateq, particularly with regard to intussusception.

commercial facility starting with process validation lots manufactured in 2001 and continuing through to lots manufactured in 2010 were examined. Clinical lots associated with REST (006), clinical lots associated with process validation and clinical consistency (study 009[3]) and clinical lots associated with other studies (011, 015, 021, 027, 028 and 029[4]) were selected to cover the broadest array of clinical experience and manufacturing.

Quantitative PCR (qPCR)-based assays were used to screen for the presence of relatively small fragments (less than 100 nucleotide [nt]) of PCV-1 and PCV-2 DNA in RotaTeq, vaccine bulk lots (the individual drug substance lots used to formulate RotaTeq), cell banks, viral seeds, and porcine trypsin used as manufacturing inputs. Samples in which small fragments of PCV DNA were detected by qPCR were further tested in the appropriate long PCR assay to ascertain whether it was likely that large intact fragments of genome, and potentially full intact genomes, might be present at detectable levels (endpoint PCR method). Certain starting materials, given their criticality, and an early set of bulks, were assessed using the endpoint PCR method despite the fact that qPCR results were below the characterised limit of detection of the qPCR method. DNase treatment to degrade cellular DNA was used, as applicable.

QPCR results

DNase-treated Rotavirus seeds and Vero cell banks, master cell banks, working cell bank, master seeds and stock seeds were tested for both PCV-1 and PCV-2. Only trace signals of PCV-1 DNA (below the LOD) were sporadically detected, and thus the starting materials were considered negative for PCV-1 DNA. For PCV-2, despite the fact that detected amplification signals were below the assay LOD (with the exception of the untreated G4 master seed), cell banks and seeds testing were advanced to the endpoint PCR testing to determine if longer sequences of PCV-2 DNA were present. Intact longer PCV-2 target sequences were not detected in cell banks or seeds by endpoint PCR testing, whether samples were tested with or without DNase treatment. The starting materials were considered negative for long PCV-2 target sequences.

RotaTeq is manufactured using raw materials of animal origin (i.e. trypsin). The MAH investigated the potential root cause of PCV DNA and tested several bulk lots (produced from 2006 to 2010) and the corresponding trypsin lot used for its manufacture.
PCV-1 DNA was not detected above the LOD in any of the bulk lots tested. Low levels of small fragments of PCV-2 DNA were consistently detected by qPCR testing of the vaccine bulk lots. To evaluate if infectious virus were present, vaccine bulk lots that were positive for small fragments of PCV-2 DNA were tested for longer PCV-2 DNA fragments. Two of the five lots tested were determined to be positive for PCV-2 DNA. All five lots were further assessed by infectivity testing (see 2.1.4 Infectivity assays). Trypsin was also tested for longer PCV-2 DNA fragments.The trypsin lot found to be positive for small fragments of PCV-2 was included in further infectivity testing, although it was determined to be negative for longer fragments of PCV-2 DNA. Mass balance calculations and the results above suggested irradiated trypsin as the source of PCV DNA detected. Results of trypsin analysis were consistent with the conclusion that there are no infectious PCV virus in these material, and thus no particles are replicating in the manufacturing process for RotaTeq. Nevertheless, infectivity tests were conducted to confirm these results.

Two suppliers of trypsin were used in the lifecycle of the product; the clinical bulk lots manufactured at pilot scale using one of the suppliers of trypsin were the only set of bulk lots in which neither PCV-1 DNA nor PCV-2 DNA was detected.
Notwithstanding this fact, current testing guidelines have been followed and the quality of porcine trypsin used today by the MAH in commercial vaccine production is guaranteed by strict sourcing conditions and a testing program for potential adventitious contaminations.

Fourteen of the fifteen lots manufactured in support of study 009 (and for which the same trypsin lot was used) were tested using PCV-specific qPCR (retain samples for one of the fifteen lots could not be located). Clinical bulk lots that were positive for small fragments of PCV-2 DNA by qPCR were further

[3] Study 009 provided a clinical evaluation of the consistency of the manufacturing process. The immunogenicity and safety of three manufacturing lots were evaluated.
[4] Study 011 addressed the safety and immunogenicity of RotaTeq in children born to HIV-positive mothers.
Study 015 looked to the safety and efficacy of RotaTeq in developing countries in Africa and Asia.
Study 021 was a paediatric study addressing safety, tolerability and immunogenicity of vaccination with RotaTeq in healthy infants in India.
Study 027 addressed safety and immunogenicity of RotaTeq in elderly subjects.
Study 028 was on the safety and tolerability of RotaTeq in chinese healthy adults, children and infants.
Study 029 addressed the safety and efficacy of RotaTeq in healthy Japanese infants.

evaluated for longer fragments in the PCR assay for PCV-2. PCV-1 DNA was not detected in any of the fourteen lots tested by PCV-specific qPCR. PCV-2 DNA was consistently detected in all fourteen lots tested. Four lots tested positive for longer sequences of PCV-2 DNA. These were further evaluated through infectivity assays (see 2.1.4 Infectivity assay).
Seven of the twenty lots manufactured in support of the clinical studies 011, 015, 021, 027, 028 and 029 were tested using PCV-specific qPCR. This selection was based on the fact that these seven lots represented trypsin lots utilised in the manufacturing process. Vaccine bulk lots that were positive for small fragments of PCV-2 DNA by qPCR were further evaluated in the PCR assay for PCV-2. PCV-1 DNA was not detected in any of the seven lots tested by PCV-specific qPCR. PCV-2 DNA was consistently detected in all seven lots tested. Two of the seven bulk lots tested were determined to be positive for the longer PCV-2 sequences. These were further evaluated through infectivity assays (see 2.1.4 Infectivity assay).

2.1.2. Serology and stool samples

Seroconversion data from vaccinated subjects were analysed. Results from testing of sera from 79 clinical study 009 subjects who received placebo (13) or RotaTeq (66) for which the corresponding vaccine bulks tested positive for PCV-2 DNA by the longer fragment endpoint PCR were presented. The pre-dose 1 (PD1; N=79) and post-dose 3 (PD3; N=79) sera were tested for antibody to PCV-2. The assay did not detect antibody against PCV-2 in these 158 samples.

No serology testing for PCV-1 was performed since negative PCV-1 DNA qPCR results were obtained on testing of clinical trial vaccine bulk lots.
Samples came from study 009 and included 66 paired sera from RotaTeq recipients but it was not clear if these represented a subset of available paired sera from subjects who received RotaTeq manufactured using the positive clinical bulks and, if so, how they were selected for the test. The MAH committed to provide the necessary clarifications for sera selection for antibody testing.
It was noted that stool samples are not available from study 009. However, the MAH committed to provide the results of a random selection of stool samples from another study (number to be justified). Albeit the results on absence of presence of PCV-2 virus in RotaTeq, these data will be examined for completeness.

2.1.3. Natural exposure to PCV

A literature review of the epidemiology of PCV was provided.
Most prevalence studies have been conducted in veterinary settings. Published data quantifying the prevalence of PCV in humans is limited. Since both PCV species are highly prevalent in healthy pigs, human dietary and respiratory exposure to this virus is common through pork consumption or inhalation of particles from pig faeces by workers in the swine industry. Metagenomics have been used to identify PCV DNA sequences in stool samples from humans in Pakistan, Nigeria, Tunisia, and the United States (US) and from wild chimpanzees. There was evidence of PCVs and other members of the Circoviridae family in the human stools; circoviridae detection in the stools of US adults was limited to PCVs which were also found in most US pork products.
Literature results suggest that detection of PCV DNA in stool may reflect dietary consumption of PCV-infected pork. In contrast, several studies of blood or tissue samples have not detected evidence of prior systemic exposure, even in persons who work in the swine husbandry industry.
There seems to be little or no evidence for human tropism or pathogenesis of PCV-1 or PCV-2.
Within-gut exposure of humans to PCV-1 and PCV-2 may occur depending most likely on diet but the lack of seropositivity reported suggests that significant systemic exposure probably does not occur.
There was no evidence uncovered from the literature review that points to a clear potential for risk of any oral administration of whole PCVs or of PCV-derived nucleic acids to infants.

2.1.4. Infectivity assays

PK-15 and Vero Cells studies
Infectivity assays were performed mainly in lots that were positive for longer PCV-2 fragments.
The set of five vaccine bulk lots and a trypsin lot were evaluated via in vitro infectivity testing using PK-15 (porcine kidney) and Vero cells.
While PCV-1 and PCV-2 controls grew vigorously, confirming that the PK-15 cell line was permissive for PCV propagation, testing of the five vaccine bulk lots and one trypsin lot yielded negative infectivity results. PK-15 cells, but not VERO cells used in this study were susceptible to infection by PCV-1 and

PCV-2. PCV-1 and PCV-2 positive controls showed viral infection by increase in PCV DNA in culture supernatants over at least 3 culture passages, presumably representing an increasing fraction of cells being infected and producing virus. Cytopathic effects (CPE) were seen in PK-15 cells inoculated with PCV-2 but not PCV-1. Negative controls showed neither detectable PCV DNA nor CPE.

Inoculation of PK-15 and VERO cells with neutralised vaccine bulk samples, and PK-15 cells with ultracentrifuged pellet fraction from trypsin, did not result in detectable PCV DNA at any one time-point beyond the similar sporadic events noted in the negative controls, nor any increase in signal (lower CTs) across time-points in 28-day, 8 passage study.

The four study 009 and the two studies 011, 015, 021, 027, 028 and 029 clinical bulk lots that were positive in the endpoint PCR testing were also further evaluated by a 28-day in vitro infectivity assay (qPCR-based detection in actively growing PK15 cells). All clinical bulks tested were negative for infectious PCV-1 and PCV-2 through day six of the assay. The positive controls amplified in culture across these three time-points (day 0, 3 and 6).

Although all results for were negative through 6 days of infectivity testing, the in vitro infectivity testing will be monitored through the remaining 22 days of culture for the sake of completeness. The MAH committed to providing the final results for all ongoing studies.

Human cell line studies
The results of the tests provided above indicated that no infectious particles of either PCV-1 or PCV-2 virus are present in RotaTeq, or in the cell banks, viral seeds, or in the porcine trypsin used during the manufacturing process, thus no further studies in other cell lines than those presented were required. Furthermore, information in the literature suggests that PCV is not transmitted from pigs to humans, and does not infect humans. Previous studies demonstrated that although PCV gene expression and replication took place in human cells, the infection is non-productive (*Hattermann et al*, 2004). Although human stool samples contain PCV at a low frequency due to consumption of pork products, screening of plasma or serum from blood donors or animal health workers were negative for antibodies to either PCV-1 or PCV-2 (*Ellis et al*, 2000).

2.2. Measures to ensure RotaTeq quality

PCV free vaccine
Results provided demonstrated that RotaTeq, including its cell banks, viral seeds or the porcine trypsin used as manufacturing input, does not contain infectious PCV-1 or PCV-2 particles. Small PCV-2 DNA fragments are present in RotaTeq. The MAH will take the necessary measures to continue to ensure that the vaccine is manufactured free of PCV.

2.3. Product Information

No changes to the product information were considered necessary by the CHMP.

3. Overall discussion and benefit/risk assessment

New information became available regarding the unexpected presence of DNA sequences of non-pathogenic viral strains of porcine circoviruses (PCV-1 and PCV-2) in RotaTeq. This DNA had been detected in preliminary tests using an analytical detection method which was not part of the approved control method used. PCV-1 and PCV-2 are small (<20 nm), non-enveloped, single-stranded DNA viruses of the Circoviridae family. PCV-1 is not known to be pathogenic for humans or animals. PCV-2 is not known to be pathogenic for humans but is known to cause disease in pigs.

Experiments were performed in several stages of RotaTeq manufacturing process. Quantitative PCR (qPCR)-based assays were used to screen for the presence of relatively small fragments of PCV DNA in RotaTeq, vaccine bulk lots (the individual drug substance lots used to formulate RotaTeq), cell banks, viral seeds, and porcine trypsin used as manufacturing inputs. Endpoint PCR assay was used to detect long PCV DNA sequences that, if present, could suggest the presence of intact PCV genomes. Cell culture infectivity testing using permissive cell lines was then initiated, in particular in those samples that tested positive for long PCV DNA sequences.
The analyses submitted confirmed the absence of detectable PCV-1 DNA, indicating that this virus is not present in the vaccine. Low level of small PCV-2 DNA fragments were consistently detected by

qPCR in different stages of the manufacturing process. The amount of PCV-2 DNA found in the vaccine bulk lots can be accounted for by the PCV-2 DNA present in the trypsin (the sole raw material of porcine origin) used in one of the steps of the vaccine manufacturing process.
No infectious viral particles of either PCV-1 or PCV-2 were present in any of cell banks, viral seeds, clinical bulk lots or vaccine bulk lots tested, or in the porcine trypsin used during manufacture. Endpoint PCR and available infectivity testing results confirmed that infectious PCV-2 virus is not present. Nevertheless, ongoing infectivity testing will be finalised and results submitted for assessment.

Seroconversion data from vaccinated subjects was analysed. No seroconversion was observed in all subjects tested. Further clarifications on the process of sera selection for antibody testing will be provided. Results of the testing of a random selection of stool samples will be provided for completeness.

RotaTeq is indicated for the active immunisation of infants from the age of 6 weeks for prevention of gastro-enteritis due to rotavirus infection; the vaccine is effective in preventing rotavirus infections which are responsible for half a million deaths each year, mostly in developing countries. Its safety profile has been established based on placebo controlled clinical trials data, including monitoring of non-serious and serious adverse events. More than 72,000 subjects have been included in clinical trials, of which at least 37,000 subjects received RotaTeq. There are no safety signals indicating that the presence of PCV-2 DNA fragments has a negative effect on the efficacy or on the safety of the vaccine.

In conclusion, there are no infectious viral particles of PCV-1 or PCV-2 present in the vaccine. The presence of small fragments of PCV-2 DNA does not raise any safety concern; its origin has been linked to the use of trypsin, a material of porcine origin. The MAH will take the necessary measures to continue to ensure that the vaccine is produced free of PCV. In this regard, the MAH committed to provide the necessary information regarding the measures put in place to further minimise the risk of PCV virus entering the manufacturing process. The CHMP endorsed these efforts.

Benefit/risk balance
Taken all the above into account, the benefit risk balance for RotaTeq remains favourable.

4. Overall conclusion

Having considered the overall data provided by the MAH in writing on the quality and in relation to the detection of PCV in RotaTeq the CHMP concluded that the benefit risk balance of RotaTeq remains positive.

Therefore the CHMP recommended the maintenance of the marketing authorisation.

5. Conclusion and grounds for the recommendation

The Committee considered the procedure under Article 20 of Regulation (EC) No 726/2004 for RotaTeq initiated by the European Commission.

The Committee considered all available data submitted by the MAH on the quality and in relation to the detection of PCV DNA in RotaTeq.

The Committee concluded that the benefit still outweighs the risks in the currently authorised therapeutic indication for RotaTeq.

The Committee concluded that no changes to the product information are required and that the MAH should continue to ensure that the vaccine is produced free of PCV.