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Reflection paper on the use of heat treatment to inactivate endogenous retroviruses in live immunological veterinary medicinal products (IVMPs)

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* This reflection paper replaces the Reflection paper on the use of heat treatment to inactivate endogenous retroviruses in live immunological veterinary medicinal products (EMA/CVMP/IWP/37924/2014). The current revision consists of administrative changes made in order to align the reflection paper to Regulation (EU) 2019/6. As no changes were made to the scientific content, no concept paper and no public consultation were deemed necessary.

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Executive summary

This reflection paper outlines the data requirements to be submitted by the marketing authorisation holder (MAH) to introduce a heat treatment step to inactivate endogenous retroviruses in the active substance during the production of live viral immunological veterinary medicinal products (IVMPs) and to show the absence of negative impact of this treatment on the IVMP.

1. Introduction

Following the 2010 Journal of Virology publication [1] reporting the detection of retrovirus RD114 in some live attenuated feline and canine vaccines commercially available in the EU and Japan, a scientific assessment conducted by EMA/CVMP (EMA/CVMP/300321/2010) concluded that the presence of replicative retrovirus RD114 in existing feline and canine vaccines due to the use of feline origin starting materials (such as CrFK feline kidney cells, [2]) in their manufacturing processes could not be ruled out.

The EMA/CVMP assessment considered that immediate regulatory action was not necessary, based on the history of use of these vaccines in the EU to date (e.g. millions of doses administered over a number of decades). The safety profile of authorised feline and canine vaccines was not considered to have changed; rather, the issue related to improvements in detection methods for retroviruses (e.g. use of molecular technologies). The benefit of the continued use of available vaccines against cat and dog diseases was considered to clearly outweigh the risk linked to the potential presence of replicative retrovirus RD114.

However, the IWP also considered a risk management strategy for already existing veterinary vaccines in relation to RD114 and identified some actions in order to have on the market only feline vaccines that have retroviral RD114 levels within an established acceptance limit, and, for canine vaccines, only products that are free of replicative retrovirus RD114.

One of the actions concerns adjustments to the manufacturing processes so that the retroviral RD114 content of the currently authorised vaccines is within the acceptance limit (e.g. use of inactivation steps/replacement of contaminated starting materials -e.g. seed materials, etc.). This document outlines the requirements with regard to the inactivation process that may be used to inactivate RD114. The requirements in this reflection paper are also considered to be applicable to the use of heat treatment to inactivate endogenous retroviruses in general, in live viral vaccines.

2. Scope

The presence of RD114 retrovirus in vaccines is considered to be related to the use of starting materials of feline origin (e.g. cells, viruses) in the manufacturing processes of feline and canine vaccines.

A meeting was convened on 8 February 2013 with industry to discuss and understand practical possibilities and proposals on how to reduce and/or to clear finished products of replicative competent RD114 virus and the timing of introduction of any such proposals. One of the methods discussed at the meeting was the use of the heat treatment to inactivate RD114 virus in live active substances.

Therefore, this document considers the possible use of heat treatment during production or at the active substance level for the inactivation of replicative RD114 virus and, more generally, endogenous retroviruses in live vaccines.

This document outlines the requirements that need to be fulfilled to show that there is no negative impact of this treatment on the vaccine.

Before process changes, such as the introduction of a heat treatment step to remove/inactivate retroviruses can be accepted, a standardised and validated detection assay needs to be developed, and the acceptable limit for the relevant retroviral agent must be established (the term "limit" takes into account the detection limit of the assay as well as the levels of the retroviral agent in current vaccines, which to date are not considered to be associated with a significant risk).

3. Background

According to Annex II, Part IIIb.2C2.1. of Regulation (EU) 2019/6, 'Seed materials, including cell seeds and raw serum for anti-serum production shall be tested for identity and the absence of extraneous agents shall be demonstrated according to the European Pharmacopoeia (…). If the presence of extraneous agents is detected or suspected, the corresponding material shall be discarded or processed to reduce the risk of presence with a validated treatment. If after treatment presence is detected or suspected, the corresponding material shall be used only when further processing of the product ensures their elimination and/or inactivation; elimination and/or inactivation of such extraneous agents shall be demonstrated.'

4. Validation of the heat treatment

Objective of the study:

Define the parameters of the heat treatment step and provide evidence demonstrating that this treatment will effectively inactivate replicative endogenous retroviruses.

Design of the study:

The active substance to be heat treated is spiked with a known amount of the live endogenous retrovirus (e.g. RD114) or another relevant virus. The active substance should be produced in accordance with the manufacturing process described in the marketing authorisation dossier. Once the heat treatment has been applied, an assay for the amount of residual spiked retrovirus is performed. The amount of retrovirus added to the active substance should be sufficiently high in order to determine the ability of the heat treatment to effectively inactivate the virus and to allow calculation of the reduction factor.

A validated quantitative infectivity assay should be used to determine the retrovirus titre before and after the treatment.

Limits:

The method of quantification of the retrovirus should have adequate sensitivity and reproducibility and should be performed with a sufficient number of repeated measurements to ensure statistical accuracy of the results.

5. Possible impact of the heat treatment on the finished product

It is assumed that the heat treatment is to be applied to the live active substance of the vaccine during manufacture. The aim is to inactivate completely the replicative retrovirus with minimal impact on the live active substance.

The heat treatment may potentially have different consequences:

- it may be necessary to increase the antigen content of the active substance in the finished product as part of the live active substance may be inactivated during the heat treatment. In order to comply with the specifications, the applicant may need to adjust the formulation to ensure the same content of live active substance as in vaccine batches produced without the additional heat treatment. In practice, this may result in a higher overall antigen content (consisting of a mixture of live and inactivated antigens of the same active substance).
- it may lead to the selection of live virus variants (quasispecies) that are more resistant to heat, potentially with different properties which then will be largely present in the finished product.

6. Requirements to demonstrate the absence of negative impact

If the heat treatment is introduced into the production process, the marketing authorisation holder must demonstrate that this treatment has no negative impact on the quality, safety and efficacy of the finished product.

In order to demonstrate the absence of negative impact of the heat treatment, the following points have to be considered:

- with regard to the quality of the finished product, except for the heat treatment and the possible adjustment of the content of the live active substances in order to still comply with the authorised specifications, the manufacturing process of the vaccine should not be modified unless adequately justified and supported by appropriate data. The results of the titration of the live active substance in the finished product shall remain within the range established for the untreated vaccine.
- with regard to the safety of the vaccine, the safety of an overdose of the heat-treated live active substance must be demonstrated under laboratory conditions in compliance with the requirements of Regulation (EU) 2019/6. Where the concerned active substance is part of a combined vaccine(s), and the heat treatment does not require an increase of its antigen content, it is sufficient to demonstrate the safety of a monovalent vaccine containing the heat-treated live active substance under assessment. The results obtained should confirm that the vaccine containing the heat-treated live active substance is safe for use.
- where the in vitro and manufacturing data generated to support the heat inactivation of the retrovirus indicate that the heat treatment does not adversely affect the viability of the live microorganism in the vaccine, and no adjustment of the manufacturing specifications is required, confirmation of efficacy of the heat-treated active substance in the target species is not necessary. Where there is any indication that the efficacy of the concerned active substance may be impacted, the efficacy of the vaccine containing the heat-treated active substance has to be tested under laboratory conditions according to the requirements of the immunogenicity test described in the relevant Ph. Eur. monograph. The results should comply with the threshold defined in the corresponding Ph. Eur. monograph.

If no Ph. Eur. monograph exists, the immunogenicity test should comply with the requirements of the Ph. Eur. general monograph "*Vaccines for veterinary use*" and with the Ph. Eur. chapter 5.2.7 "*Evaluation of efficacy of veterinary vaccines and immunosera*". The test should be the same as that performed to demonstrate the efficacy of the active substance of the original vaccine and the results obtained should be similar.

References

[1] Miyazawa T et al. Isolation of an infectious endogenous retrovirus in a proportion of live attenuated vaccines for pets. J. Virol. 2010 Apr;84(7):3690-4.

[2] Baumann J et al. CrFK Feline Kidney Cells Produce an RD114-Like Endogenous Virus That Can Package Murine Leukemia Virus-Based Vectors. J. Virol., 1998 Sep;72(9):7685-7687.