EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

Withdrawal assessment report for Versiguard SARS CoV2 (EMEA/V/C/005988/0000)

Vaccine common name: SARS-CoV2 vaccine (recombinant protein)

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

The applicant Zoetis Belgium submitted on 16 December 2021 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Versiguard SARS CoV2, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 15 July 2021 as Versiguard SARS CoV2 has been developed by recombinant DNA technology.

The applicant applied for the following indication: Active immunisation of mink from 6 weeks of age to reduce infection and virus shedding following infection with SARS CoV2.
Onset of immunity: 3 weeks after completion of the basic vaccination scheme.
Duration of immunity: Duration of immunity has not been demonstrated but annual re-vaccination is recommended.
The vaccine has also been shown safe in a wide range of susceptible zoo species.

The current proposal for the indication is: Active immunisation of mink from 6 months of age onwards to reduce infection in the respiratory tract and the central nervous system and virus shedding following infection with SARS CoV2.

The active substance of Versiguard SARS CoV2 is a suspension of the extracellular pre-fusion SARS CoV-2 trimer spike protein derived from the Wuhan-1 strain S protein and contains multiple tagged on histidine residues to facilitate purification and detection of the antigen. The target species is mink. The product is intended for administration by subcutaneous use.

The applicant claims that Versiguard SARS CoV2 contains a recombinant cell expressed and purified version of the trimeric spike protein of SARS CoV2 with a histidine tag that is intended for use in veterinary species. There are no vaccines for SARS CoV2 currently authorised for use in animals in the EU therefore the applicant considered the antigen a new active substance. The CVMP considers that the extracellular pre-fusion SARS CoV-2 trimer spike protein derived from the Wuhan-1 strain S protein and containing multiple tagged on histidine residues is a new active substance, as claimed by Zoetis Belgium.

Versiguard SARS CoV2 suspension for injection contains recombinant SARS CoV2 spike protein and is presented in cardboard boxes containing:

- 1 bottle of 10 ml (10 doses)
- 1 bottle of 250 ml (250 doses)
- 1 bottle of 500 ml (500 doses)

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 15 September 2022, during the clock-stop, Zoetis Belgium communicated the withdrawal of the marketing authorisation application to the Agency.

Scientific advice

Not applicable.

MUMS/limited market status

The applicant requested eligibility of this application for MUMS/limited market by the CVMP on 15

June 2021, and the Committee confirmed on 15 July 2021 that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) data requirements would be applied when assessing the application for the requested indication in mink. MUMS/limited market status was granted as mink is considered a minor species.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system which fulfils the requirements of Directive 2001/82/EC was provided. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse event occurring either in the Union or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the active substance takes place in the United States.

Manufacture of the finished product and the primary packaging takes place either in the EEA or the United States.

Batch release takes place in the EEA.

The EU manufacturing site has been recently inspected by the correspondent national competent authority and the American one by the UK competent authority and were both found to be GMP-compliant with regard to the applicable manufacturing activities.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the active substance and of the finished product manufacturing sites has been satisfactorily established and is in line with legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Versiguard SARS-CoV-2 is a subunit vaccine containing the spike protein from the SARS-CoV-2 virus. The spike protein sequence is based on the original SARS-CoV-2 Wuhan-1 strain of the virus.

Qualitative and quantitative particulars of the constituents

Qualitative particulars

The vaccine is presented as a clear to opalescent suspension for injection without visible particles. The product is manufactured in presentations of 10 ml, 250 ml and 500 ml.

Vaccine composition

Ingredients	Function	Reference to standard
Active ingredient:		
Recombinant SARS-CoV-2 spike protein	Antigen	In house
Adjuvants		
Well-known adjuvant	Adjuvant	In house
Novel adjuvant	Adjuvant	In house
Excipients		
Cholesterol-ethanol	Stabiliser	In house
Phosphate buffer	Buffer	In house

The qualitative and quantitative particulars of the vaccine suspension are described adequately.

Quantitative particulars

The applicant provides a table indicating batches used in clinical trials. No batch protocols for these batches are provided.

The applicant states that batches used in the safety and efficacy trials were manufactured following a process representative to the one described in Part 2.B. of the dossier. However, there were several differences in the composition.

Experimental batches were formulated with spike protein based on the measurement of protein concentration determined by bicinchoninic acid (BCA) assay instead of biolayer interferometry (BLI) assay and antigen batches containing gentamicin and phosphate buffer containing purified water instead of water for injection.

Furthermore, some of the batches used in the safety and efficacy trials were blended with an antigen batch which was prepared using a commercial human embryonic cell line, which were transfected with a different plasmid (COVID19_Spike_F-His) based on the Wuhan-1 sequence. For the manufacturing of the commercial product, a different stably transfected cell line will be used for antigen production.

To demonstrate antigenic equivalence of the expressed protein from both transfected cell lines, an in-vitro equivalence study was performed.
It is concluded by the applicant that the proteins expressed in all cell lines are of sufficient

equivalence concerning antigenicity and oligomeric similarity. However, the Western blot analysis submitted does not allow appropriate assessment and the applicant is requested to provide additional information.
Nevertheless, if the validation of the BLI test method is not yet available, it cannot be assessed if the test method is fit for purpose and a final conclusion cannot be drawn until the respective validation report is available.

Moreover, for the batches used in the clinical studies, the protein concentration was determined employing the BCA test method, whilst the routine potency test method in commercial production will be the BLI assay. Therefore, both methods should be compared, and the results provided to ensure that the methods can be considered comparable. This is considered as a major objection.

Container and closure

The product consists of one container with the ready-to-use vaccine suspension.
The solution is applied to the animal via a syringe.

The product is filled in 10 ml, 250 ml or 500 ml high density polyethylene (HDPE) bottles, closed with bromobutyl- or chlorobutyl rubber stoppers and sealed with aluminium caps.

The primary packaging materials for the product Versiguard SARS Cov-2 are considered compliant with the respective requirements in the Ph. Eur. and USP and their sterilisation is adequate. However, some certificates and information regarding use of alternative resins for the containers are missing.

Product development

The vaccine antigen is a recombinant SARS-CoV-2 F spike protein. As excipients cholesterol-ethanol and phosphate buffer are included.

The vaccine holds an emergency use authorisation in the US and a special use permit in Finland, for use in mink.

The product is based on the Wuhan-1 sequence of SARS CoV-2. The applicant is requested to confirm that this sequence is still relevant regarding currently circulating variants of SARS CoV-2, especially in mink populations in Europe.

The protein is expressed in a cell culture system, in serum-free media employing characterised master cell seed and working cell seeds and is manufactured via standard bioreactor culture techniques.

A plasmid containing relevant genetic information of the SARS CoV-2 was constructed. The genetic sequence was modified to ensure the correct conformation of the final product. For construction of the stable cell line, the expression vector is recombined site-specific into artificial chromosomes in the host cell line.

Transfected cells were sorted and then imaged to ensure single-cell, clonal origin of the master cell bank (MCB). Clones were assessed for stability and protein yield.
Finally, clone 118 was chosen as acceptably stable and consistently productive. It was banked as pre-MCB and used for production of the MCB.

The applicant uses the same protein expression system for production of other products developed

by the company.

The adjuvant combination is also used by the applicant in a cattle product.

One of the adjuvants is a well-known adjuvant used in veterinary and human vaccines.

The other adjuvant is a novel product in veterinary vaccines. It is used in bacterial and viral vaccines as it elicits potent cell-mediated and humoral immune responses to antigens in humans and animals, while it is also mostly considered as well tolerated and safe.
However, adequate data on this novel adjuvant are missing from the dossier and should be provided.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation.
The list of excipients is included in section 6.1 of the SPC.

The containers used comply with the Ph. Eur. This kind of containers is well-established for use with veterinary medicinal products.

The information provided for the generation and modification of the synthetic construct included into the vector is very brief. A complete detailed description on the construction and modifications done and the effects exerted by these modifications should be provided.

The same applies for the artificial chromosome system. A more detailed description of the system and its use for generation of the cell line should be provided.

No information or characterisation of the expressed protein is provided. The trimeric structure is mentioned, but possible non-functional aggregates or truncated degradants are not discussed. These issues should be discussed.

The composition of the adjuvant suggests that under certain conditions, it may form particles or complexes with cholesterol. Possible interactions between adjuvant components and the antigen should be discussed. Furthermore, cholesterol may be identified as part of the adjuvant and should be listed accordingly.

In general, a detailed description on how the manufacturing process has been developed, and appropriate information regarding the differences between the different manufacturing scales are missing.

Description of the manufacturing method

The production process is described and flow charts of the manufacturing process are provided.

For the manufacture of the product, cells are inoculated into cell culture vessels, and scaled up until the volume is sufficient for bioreactors. Culture is further scaled up in an intermediate bioreactor and later on passaged in the production bioreactor.

Cells are harvested and the supernatant is clarified. Bio-Layer Interferometry (BLI) assay for determination of protein content is performed. Afterwards the protein is purified. After elution, the harvest is diafiltrated and stored in sterile containers until vaccine formulation at -35°C or below. No information of the antigen bulk size is provided.

It is indicated that the antigen batches are thawed and stored at 2-8°C until formulation, however no maximum time period for storage after thawing is given. A relevant time period should be indicated.

For blending, phosphate buffer is stirred and then the antigen and the well-known adjuvant solution is added under gently mixing. The cholesterol/ethanol solution is added through shear-force equipment. Finally, the new adjuvant is added. The mixture is stirred until homogenisation is achieved. The pH value is controlled and corrected with hydrochloric acid or sodium hydroxide solution.

The final bulk is filled into sterile plastic vials (10, 250 or 500 ml) and closed. Samples are taken for quality control (QC) and the rest of the batch is stored at 2-8°C until final packaging.

The description of the manufacturing process is brief. Uncertainties on certain process steps need to be clarified. Insufficient information is given on critical process steps.

No information on the nature and model of the culture vessels and bioreactors are provided. Validation reports of cell cultivation in the respective vessels and bioreactors should be provided.

An average bulk size of 800 L is indicated, however no minimum and maximum bulk size is mentioned and should be indicated.
Furthermore, a specification of the maximum number of vials in a commercial batch should be set.

The filling volume is controlled by in-process weighing to guarantee a sufficient overage. However, no specification for the weight is indicated. A specification should be stipulated for every presentation.

Validation reports on sterility testing of the antigen fluid and the finished product are provided.

No further validation reports are provided.
Validation for the tests on absence of mycoplasma, antigen concentration and identity (BCA and BLI), and adjuvants content should be provided. This is considered as a major objection.

For manufacturing process validation, data on five pilot batches are provided with a slightly different composition than that described in the dossier: namely, additional gentamicin and purified water instead of water for injection. Furthermore, these pilot batches were formulated with different target of SARS-CoV-2 spike protein based on total protein concentration determined by BCA assay. Only limited data on finished product testing are available.

Additionally, data for at least two industrial scale antigen batches should be provided post authorisation to confirm consistency of antigen production.

Due to missing data, no final conclusion on the validation of the production process can be drawn yet. This is considered as a major objection.

Production and control of starting materials

Starting materials listed in pharmacopoeias

For all starting materials listed in a Pharmacopoeia a satisfactory certificate of analysis or in-house testing is provided.

All materials comply with the respective Ph. Eur. monographs.
No certificate of analysis is provided for purified water. It is not tested on a batch basis but kept under continuous monitoring. This is acceptable.
Furthermore, no certificate for water for injections is included in the dossier and should be provided.

The starting materials listed in a Pharmacopoeia are satisfactorily described and compliant with the

current version of the Ph. Eur. where applicable.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

SARS-CoV2 Spike F-HIS Master Cell Bank (MCB), Working Cell Bank (WCB) and end of production passage cells

The history, including origin of isolate, parent cell line information, plasmid description, transfection and cloning, as well as production and testing of the SARS CoV-2 Spike F-HIS MCB, WCB and end of production passage cells are described.

The description of the production and the starting materials of the pre-MCB, MCB, WCB and end of production cells are detailed and considered satisfactory.

MCB, WCB and end of production cells were subjected to extensive testing.

The MCB, WCB and end of production cells were confirmed by general microscopy as consistent for mononucleated cells. No abnormalities or any cytopathic effect were observed.

Purity testing of MCB, WCB and end of production cells was performed at Zoetis following Ph. Eur. 2.6.1. and USP. No bacterial or fungal growth was detected throughout the test period.

Testing for absence of mycoplasma was conducted accordance with Ph. Eur. 2.6.7. and USP. MCB, WCB and end of production cells were confirmed as free from mycoplasma contamination.

Identity and species specificity were examined with three different methods.

Confirmation of identity and specificity as production cells for SARS CoV-2 MCB, WCB and end of production cells were analysed for cell line identification. It is concluded that the results demonstrate consistency with the production cells. The provided data are satisfactory; identity of the cell line is considered demonstrated.

The test method used to confirm the identity of the SARS CoV-2 spike antigen expressed by the MCB, WCB and end of production cells is the BLI assay. The BLI assay measures the binding rate of the interaction between two proteins. Here the rate of binding of the canine antibody to the SARS CoV-2 spike protein antigen is measured. The binding rates were determined using the Octet analysis software. The binding rate of all samples was significantly higher than the negative control. Therefore, it is concluded that the presence of SARS-CoV2 spike protein antigen in the MCB, WCB and high passage cells is positively confirmed.

The data provided in the frame of cell bank testing are considered conclusive. However, a validation report for the BLI test, which is also used as potency test during finished product testing, is not yet provided. Therefore, a final conclusion cannot yet be drawn.

Cell line stability was investigated by DNA sequencing of the SARS-CoV-2 protein sequence found within the MCB, WCB and end of production cells.
Genomic DNA from MCB, WCB and end of production cells was isolated, amplified by PCR and sequenced by Sanger sequencing.

It is concluded that results indicate that all samples showed the correct PCR product size and still contain the SARS-CoV-2 spike gene as described with no detectable mutations over passages intended for vaccine production. Sequence identity over all passages was 100%. Cell line stability is

considered sufficiently demonstrated.

For karyology testing, MCB and WCB (MCB+3) were expanded a single passage in preparation. MCB+1 and MCB+4, along with actively growing cultures of MCB+15 and MCB+25, were submitted for chromosomal analysis. All four preparations showed similar morphology and chromosomal distribution. It is concluded that the cell line candidate is genetically stable.

Tumourigenicity testing and testing for residual DNA and host cell protein was not performed. It is agreed that the product is highly purified, however, no data are provided. At least some data for testing on residual host cell protein from the first commercial batches of the product should be provided.

All cell banks were produced using animal-free materials. Therefore, the risk assessment on extraneous agents (EA) testing focuses on the species of origin and intent (mink). Mink is considered as a minor species. No requirements for EA testing exist in the relevant pharmacopoeias.

One reagent containing bovine serum albumin (BSA) was used during the cloning process of the pre-cell bank material.
It is not considered relevant for the risk of transmissible spongiform encephalopathy (TSE) due to the minimal volumes of BSA containing raw materials used and the dilution effect. Furthermore, BSA used was sourced from an EDQM certified vendor with materials from the USA. A respective certificate of suitability issued by EDQM ensuring TSE safety of BSA, was provided.

Testing for retroviral contamination of all cell bank seeds was performed. The test method used was "Quantitative product enhanced reverse transcriptase (Q-PERT). The test identified the absence of replication-competent retrovirus. The respective validation report is provided. The applicant discusses the results and comes to the conclusion that no replication competent retrovirus is present in the seeds. The testing for retroviral particles and discussion of results provided is considered sufficient.

An in-vivo test for the presence of viral contaminants was performed on MCB, WCB and end of production cells. Testing was performed for the relevant viruses. Sera from all animals were negative for the presence of antibodies to the tested agents.

Furthermore, samples of MCB, WCB and end of production cells were evaluated by co-culturing on indicator cell lines. For the MCB and WCB samples were found negative for any of the tested pathogens specific for the species of origin. However, for the samples of the end of production cells the majority of tests are still ongoing and should be provided as soon as they are available.

Additional to EA testing for relevant extraneous agents from the species of origin and mink, extraneous agents tests for canine, feline, bovine, porcine and equine agents were performed. All available testing results for extraneous agents from different species for MCB, WCB and end of production cells are provided. The respective certificates of analysis and descriptions of methods are provided. No extraneous agents from canine, feline, bovine, porcine and equine species were detected in the samples.

The TSE risk assessment on the MCB concludes that SARS CoV-2 Spike F-HIS Master Cell Bank is very unlikely to present any risk of TSE contamination. The CVMP agrees with this conclusion.

Description of the preparation and testing of the MCB, WCB and end of production cells is considered as mostly satisfactory. However, some data on the extraneous agents testing for the species of end of production cells are missing and should be provided as soon as they are available. Furthermore, the validation report for the BLI test used for identity testing of the expressed protein is not yet available. Therefore, a final conclusion on this point cannot be drawn yet.

Cholesterol

Cholesterol is used as component of the cholesterol-ethanol solution. The raw material is produced from the wool grease of sheep and complies with Ph. Eur. 0993. A risk assessment on the omission of EA testing is provided. The applicant concludes that the presence of EA can be excluded based on the drastic treatment applied to the product during manufacture, the production process of the vaccine and the sterilization of the cholesterol-ethanol solution. The omission of the EA testing for cholesterol is acceptable.

Information on all suppliers of cholesterol is missing.

Well-Known adjuvant component

Satisfactory in-house specifications and a certificate of analysis is provided.

Starting materials of non-biological origin

For all starting materials of non-biological origin as listed in the dossier a satisfactory certificate of analysis and/or in-house testing is provided.

The starting materials of non-biological origin are considered as satisfactorily described.

In-house preparation of media and solutions consisting of several components

During production of the vaccine, several media and buffers are used.
Detailed information on qualitative and quantitative composition, methods of preparation, sterilisation and storage of media and solutions are provided for the in-house prepared media and solutions.
Suppliers are listed as applicable and the medium or solution is referred to its respective certificate of analysis.

The information on in-house prepared media and solutions is considered satisfactory.

TSE risk assessment

The information provided on TSE compliance is satisfactory.

The applicant provides an additional document for a more detailed TSE risk assessment on cholesterol, as this starting material originates from the wool grease of sheep. Quality statements and/or certificates of suitability for all listed suppliers are provided and satisfactory.

The applicant concludes that the overall risk of using cholesterol supplied by the stated companies is negligible and that considerable support is given in minimising the risk of transmitting animal spongiform encephalopathies of this product.

The TSE risk assessment is considered satisfactory.

Control tests during the manufacturing process

The following in-process controls are carried out on the final antigen: antigen concentration determination - BLI assay using anti-SARS-CoV-2 monoclonal antibodies, sterility testing (membrane filtration technique) and test of absence of mycoplasma (culture method or RT-PCR).

For the in-process control tests, the method, frequency and timing of testing, function of the test and acceptance criterion are presented as well as the respective description of the test method. However, no validation data, except for sterility testing, are available. The applicant is asked to discuss possible impurities (independent of residual host cell protein and residual DNA) coming from the production process and provide information on reference standards used. Clarification is needed on the appropriateness of the BLI assay antigen concentration determination. Comparability between the BLI assay and the BCA method used during product development should be demonstrated. This is considered a major objection.

Control tests on the finished product

Finished product controls performed on the bulk vaccine are: appearance, pH, potency and identity, adjuvant components content and sterility. Brief descriptions of the test methods are provided but some details regarding appearance control are missing.

The filled product will be controlled by in-process weighing at regular intervals throughout the filling operation. A test on extractable volume is not foreseen, this should be justified.

However, no validation data for all control tests, except for sterility testing, are available.

The potency test is performed by BLI assay to determine the concentration of the SARS-CoV-2 recombinant spike protein by using an anti-SARS-CoV-2 mAb. The binding rate of antigen to the antibody is concentration-dependent. Binding results in a change in the wavelength, which will be monitored. The concentration of the test sample is automatically determined by interpolation from a standard curve and dilution factor. The average value of the dilutions is reported. A specification at release and an end of shelf-life (EOSL) specification are defined. The lower limit of the range was set based on antigen input of batches used in laboratory efficacy studies. The limit at release considers potential loss during storage to ensure that the minimum efficacious dose established during development is maintained throughout the shelf-life. The range between release and end of shelf life and the consequently accepted heavy loss of antigen must be justified. In general, due to missing stability data, it is not demonstrated that the release specification is sufficient to ensure an EOSL specification. Furthermore, no validation data are available for the BLI. Further, a conclusion cannot be drawn about its suitability to detect sub-potent batches.
This is considered as a major objection.

The well-known adjuvant component content of the vaccine is determined using size exclusion chromatography. An adjuvant standard is prepared and tested in parallel to allow the construction of a calibration curve and the concentration of adjuvant component in the sample is determined by interpolation. A specification is laid down in the dossier. No validation data are available. Furthermore, results on the well-known adjuvant component content in vaccine batches are not provided.

To quantify the new adjuvant component content in the SARS COV-2 vaccine human embryonic cell line -Blue hTLR9 cells are stimulated with the new adjuvant component and the secretion of Secreted Embryonic Alkaline Phosphatase (SEAP) into the cell culture supernatant is induced. Using a detection medium, a color change is observed in the presence of the alkaline phosphatase activity. A specification is laid down in the dossier. No validation data are available. Furthermore, results on the new adjuvant component content in vaccine batches are not provided.

No suitability is demonstrated for the control tests to determine the potency and identity, and adjuvant components content, as no validation data are available. Further, no information is given

about standard material, positive control, etc. This issue is considered as a major concern.

Sterility testing is performed as described in Ph. Eur. 2.6.1 using the technique of membrane filtration. Validation data are provided in Part 2.B of the dossier. The suitability of the method has been satisfactorily shown in the presence and in the absence of samples of SARS-CoV-2 spike protein antigen and of the finished product.

A justification for omission of testing of the finished product for freedom from extraneous agents in line with Ph. Eur. 0062 should be provided.

The expressed SARS CoV-2 Spike protein exists in an oligomeric state (trimer). Information regarding the protein profile is poor. A stable trimeric structure of the vaccine pre-fusion S protein is important to generate protective antibodies that neutralise the viral fusion protein and thereby prevent viral entry into the host's cells. The applicant should provide adequate information on the nature of protective (neutralizing) epitope(s) and the epitope recognised by the mAb as well as on the stability of the folded protein and its processing into oligomers. In the absence of adequate data, an appropriate finished product test may be necessary.

As stated by the applicant tests for residual host cell protein (HCP) and residual DNA were not considered necessary, as this is a highly purified antigen as compared to a conventional vaccine. A safety risk is not expected. Nevertheless, batch data for HCP and host cell DNA should be provided for at least five antigen batches in order to justify the omission of HCP and DNA testing. Descriptions of the respective test methods applied for these two parameters should be provided as well.

Although it is stated in the dossier that several manufacturing steps are implemented to remove/reduce impurities such as host cell DNA, host cell protein or antifoam, no data are available which demonstrate appropriate removal of process- and product-related impurities. The applicant is asked to discuss possible impurities (with the exception of residual host cell protein HCP and residual DNA) coming from the production process. For impurities which are not completely removed by the manufacturing process, respective test methods should be validated and established with appropriate limits/ranges. These limits/ranges should be included in the list of IPCs and the manufacturing flow chart and process description updated accordingly.

It should be pointed out, that none of the presented finished product batches used for consistency or stability data, have been produced and/or tested in accordance with the current dossier's specifications. Control testing was not complete, especially in view of the adjuvants included in the formulation. A justification of why the cholesterol/ethanol solution is not included within the final product specifications should be given.

Batch-to-batch consistency

Antigen Production

Consistency of antigen production has been evaluated on three consecutive antigen batches, which were produced as described in Part 2.B. of the dossier and tested in accordance with the methods described in Part 2.D. (antigen concentration determination - BLI assay, sterility, test of absence of mycoplasma). For two batches, manufacturing data and the results of the in-process tests are provided. For the third batch, the in-process test results are still not available. The volume of the production vessel described here is about 200 l. According to the dossier, intermediate bioreactors and production bioreactors have maximums of 1000 and 2000 l, respectively. The provided data are in line with the MUMS/limited market guideline, but to verify consistency data two industrial scale

runs should be provided. This can be done by a post-authorisation measure.

Further, three R&D antigen batches were examined to generate consistency data for the production process. These batches were prepared with gentamicin and tested for sterility and absence of mycoplasmas with equivalent R&D methods. In-process data for one batch are not available. It should be discussed if the deviating production steps and composition (e.g. gentamicin is added) have an impact on the consistency of the antigen. A power outage has been reported during manufacturing of one of these batches; relevant information thereof should be submitted. In the tables provided, some parameters are missing (absence of several tests).

The relevant batch production protocols and control protocols are not available and should be provided.

A final conclusion regarding consistency of the manufacturing process of the antigen cannot be drawn, as the relevant test methods for in-process controls are not validated (except sterility).

Finished Product

To demonstrate consistency of the finished product manufacturing, the applicant has provided data of five pilot batches manufactured with R&D antigen batches at commercial scale. All batches comply with control specifications (sterility, absence of mycoplasmas, antigen concentration by BLI assay and BCA assay) and results were equivalent for all batches.
However, the formulation of these pilot vaccine batches differs in the parameters described in Parts 2.B and 2.D of the dossier. The applicant is asked to discuss the possible impact on product consistency.

No data are available for the adjuvant components. Respective data for consistency purposes should be provided.

Further, no data are available regarding protein profile, tests for residual host cell protein (HCP) and residual DNA.

Appearance control is not included in testing of the consistency batches; this should be justified.

The applicant stated that further consistency batches are planned to be produced. Specific plans should be specified and a timeline should be provided.

Overall, no final conclusion can be drawn regarding consistency of the final product in the production process.

Stability

Stability for active ingredient:

The applicant provides preliminary stability data on three R&D batches. The only parameter is the antigen concentration, measured with BLI assay. Data are currently available for 6 months. As this assay is not validated, no final conclusion can be drawn.

The applicant plans to initiate a stability programme with the consistency antigen batches. The following parameters will be monitored: Antigen concentration by BLI assay, sterility (Ph. Eur.), mycoplasma testing (Ph. Eur.), native PAGE Western blot, Host Cell Protein, residual DNA.

Regarding the stability programme, the applicant is asked to detail the antigen batches used and provide an appropriate timetable as well as specify the containers used for the antigen stability studies.

The blended antigen bulk may be kept for up to 7 days; respective data should be provided.

The proposed shelf-life for the antigen of 39 months stored at -35°C or below cannot be supported, as no sufficient data are available.

Stability for finished product:

The applicant provides preliminary stability data on R&D batches prepared with antigen batches containing gentamicin and formulated with spike protein based on the measurement of protein concentration determined by BCA assay instead of BLI assay. To demonstrate stability, the smallest (10 ml) and largest container (500 ml) sizes were chosen to cover all sizes. This approach is acceptable. The vaccine batches were stored at +2 to +8°C and samples taken at 0 months after storage and thereafter at regular intervals and tested for potency by BLI assay only using the method described in Part 2.E of the dossier.
Bromobutyl rubber stoppers are used for the 10 and 500 ml presentation, and for the 250 ml presentation a chlorobutyl rubber stopper is used. Despite the difference in rubber stoppers, the applicant considers this approach as representative. This should be justified by the applicant, especially as the certificates of analysis for the bromobutyl stoppers are missing.

It is noted that the 500 ml presentation and the 10 ml presentation were manufactured in different production sites. Further, the protein content of these presentations, measured by BLI assay, differs although all were formulated with the same target concentration of SARS- CoV-2 spike protein based on BCA assay. Based on these divergent results, the comparability of BCA and BLI assay should be demonstrated by the applicant. The only parameter that was determined in this study is the protein content until six months. After six months, a decrease in protein content is clearly visible. Furthermore, the total protein content of one batch from one of the manufacturing sites does not meet the requirements for a standard vaccine to be used for verification of safety. This should be explained.
Therefore, the applicant is asked to justify the difference in protein concentrations in the finished product manufactured in the two manufacturing sites, and should confirm that the manufacturing process can be considered comparable at the different manufacturing sites of the presentations.

Due to the lack of further data for the finished product, no conclusion can be drawn regarding stability and shelf-life.
The proposed shelf-life for the finished product of 12 months when stored and transported refrigerated at +2 to +8°C is currently not supported.

As post-approval commitment, the applicant plans to initiate a 27-month stability programme on at least one industrial scale batch manufactured with the recently produced consistency antigen batches in Part 2.F in accordance with the proposed manufacturing method. The following parameters will be monitored: Potency by BLI assay, Western Blot, sterility (Ph. Eur.), mycoplasma (Ph. Eur.), appearance, pH value and adjuvant components content.

Regarding the stability programme, the applicant is asked to provide an appropriate timetable.

No batch, used for generating stability data, has been produced and/or tested in accordance with the current dossier. Due to the lack of valid batch testing and stability data the proposed specifications regarding the release titre cannot be evaluated.

Stability data on the broached vial stored are not provided. The applicant is requested to provide appropriate data for in-use-stability.

Overall conclusions on quality

Versiguard SARS-CoV-2 is a subunit vaccine containing the spike protein from the SARS-CoV-2 virus. The spike protein sequence is based on the original SARS-CoV-2 Wuhan-1 strain of the virus.

The recombinant F spike protein vaccine is adjuvanted and intended for the active immunisation of mink against SARS-CoV-2 (COVID-19).

The qualitative and quantitative particulars of the vaccine suspension are described adequately.

The antigen blended in some of the batches used in clinical trials was expressed in an experimental human embryonic cell line instead of the cell line to be used in routine production. To demonstrate antigenic equivalence of the expressed protein from both transfected cell lines, an in-vitro equivalence study was performed. The applicant concludes that equivalence between proteins in both cell lines was demonstrated. However, while the obtained results were similar, validation of the BLI test method is not yet available and the older batches were tested with the BCA test. Therefore, no final conclusion on the comparability of the expressed proteins can currently be drawn.

The description of the product development gives a brief overview on the development, manufacture and testing of the product. However, the information provided for the generation and modification of the synthetic construct included into the vector is very brief and further information needs to be provided. No information or characterisation of the expressed protein is provided. The trimeric structure is mentioned, but possible non-functional aggregates or truncated degradants are not discussed. The composition of the adjuvant suggests that under certain conditions, the well-known adjuvant component and cholesterol may form particles or complexes. The applicant is asked to discuss possible interactions between adjuvant components and the antigen.

Flowcharts of the manufacturing process are provided. The description of the manufacturing process is brief. No information on the nature and model of the culture vessels and bioreactors are provided. Validation reports of cell cultivation in the respective vessels and bioreactors should be provided. Some further details on the manufacturing process are missing and should be provided.

Validation reports on sterility testing of the antigen fluid and the finished product are presented. No further validation reports are included. The missing validation reports need to be provided. No final conclusion on the validation of the production process can be drawn yet.

For all starting materials listed in a Pharmacopoeia a satisfactory certificate of analysis or in-house testing is provided. All materials comply with the respective Ph. Eur. monographs.

The description of the preparation and testing of the cell seed banks used for production of the vaccine (MCB, WCB, end of production cells) is provided, including information on the starting materials used in the process and considered satisfactory.

The description of the production and starting materials of the MCB, WCB and end of production cells is described in detail and considered satisfactory.
MCB, WCB and end of production cells were subjected to extensive testing. Certificates and description of test methods are provided. Tests on general microscopy, purity, detection of mycoplasma, identity, species specificity, karyology and extraneous agents testing were performed and are mostly satisfactory. Some tests on extraneous agents are currently ongoing. A final conclusion on the testing of the cell banks can only be drawn after the requested missing data are provided.

A TSE risk assessment on the MCB is provided and it is concluded that the MCB is very unlikely to present any risk of TSE contamination.

For all starting materials of non-biological origin, a satisfactory certificate of analysis and/or in-house testing is provided, except for water for injections for which a respective certificate should be submitted

Detailed qualitative and quantitative composition, methods of preparation, sterilisation and storage of media and solutions are provided for the in-house prepared media and solutions.

A detailed TSE risk assessment is provided. Quality statements and/or certificates of suitability for all listed suppliers are provided and satisfactory.

The in-process tests performed to control the critical steps in the manufacturing process are described. However, no validation studies have been provided for these key tests, which is considered as a major concern. Further, possible impurities (with the exception of residual host cell protein (HCP) and residual DNA coming from the production process were not reflected. This issue and concerns regarding standard materials need to be discussed.

The finished product controls performed are described and specifications are proposed. However, no validation studies have been provided which substantiate the suitability of the tests and the specifications set. This is considered as a major concern.

Based on the provided data no conclusion can be drawn on the consistency of the antigen and finished product manufacturing process.

The proposed shelf-life for the antigen of 39 months stored at -35°C or below and of 12 months for the finished product at +2 to +8°C cannot be supported, as no sufficient data are available.

No batch data has been provided for a batch produced and tested in accordance with the current dossier.

The major objections on quality comprise questions on the missing validation of the BLI test and several other in-process and finished product controls as well as on the identity of the protective epitopes and the stability of the folded protein.

As batches used in the clinical trials were experimentally produced in human embryonic cell line instead of the cells which will be used in routine production, and the validation of the potency test is currently missing, it can currently not be concluded that the batches used in the study are representative of the commercial vaccine. Therefore, a major objection on this matter was included. Furthermore, a major objection on the process validation, namely missing description of critical process steps of the manufacture was included.

Based on the review of the data on quality, manufacture and control of Versiguard SARS-CoV-2, the quality of the product is currently considered not acceptable until the outstanding issues have been resolved satisfactorily.

Part 3 – Safety

Introduction and general requirements

Versiguard SARS CoV2 is an adjuvanted vaccine containing a purified recombinant fragment of the SARS CoV2 spike protein. It is intended for mink (*Neovison vison*) and deemed safe for some zoological species by the applicant.

A synthetic spike with the S1 and S2 domain of the SARS CoV2 Wuhan strain is stabilised in a pre-

fusion conformation. This spike is embedded with adjuvants and excipients.

The vaccine was initially intended to be administered subcutaneously to minks aged from 6 weeks onwards.

Versiguard SARS CoV2 has already been used in the USA in 2021 and has a national authorisation for use in Finland. The CVMP classified it as MUMS/limited market. In line with this classification, the applicant performed preclinical studies which were not GLP-compliant, albeit their performance to GLP standard was attempted as far as possible. No field studies in the EU were undertaken, while one US field study was performed. Observational safety results obtained from zoo animals were reported.

Different batches which were manufactured in two different eukaryotic cell lines were used in the two safety studies performed in minks. The batches used for preclinical and US field studies were manufactured using human embryonic cell line, while the final product is drawn from a different cell line and was used in the laboratory challenge study in minks. The applicant has provided quality data to show that these two types of batches are equivalent. However, additional data are requested to verify this.

The basis for the assessment was the requirements for immunological veterinary medicinal products as described in the Directive 2001/82/EC (amended by Directive 2004/28/EC and Directive 2009/9/EC). Another guidance on safety of the vaccines was:

- Ph. Eur. 0062: Vaccines for Veterinary Use
- CVMP/IWP/043/97-Note for guidance on the use of adjuvanted veterinary vaccines
- Ph. Eur. 5.2.6: Evaluation of safety of veterinary vaccines and immunosera
- VICH GL44: Guideline on target animal safety for veterinary live and inactivated vaccines
- OECD Principles of Good Laboratory Practice (as revised in 1997) [ENV/MC/CHEM (98) 17], 26-Jan-1998
- EMEA/CVMP/IWP/54533/2006: User safety for immunological veterinary medicinal products
- EMA/CVMP/038/97: EU Requirements for Batches with Maximum and Minimum Titre or Batch - Potency for Developmental Safety and Efficacy Studies
- EMA/CVMP/IWP/206555/2010: Requirements for the production and control of immunological veterinary medicinal products
- EMA/CVMP/IWP/123243/2006: Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market

The vaccination was monitored by several serological tests (lateral flow assay [LFA], serum neutralization lentiviral assay, virus neutralization test and in-house ELISA).

The serological methods were validated with dog and cat sera by comparison with seroneutralisation results. Presumably positive and negative sera from SARS CoV2-free and experimentally infected dogs, pre-SARS CoV2 (2016–2017) field sera as well as sera from dogs experimentally challenged with *Bordetella spp.*, canine influenza and canine parainfluenza were used. For cats, presumably positive and negative sera from SARS CoV2-free and experimentally infected cats, pre-SARS CoV2 (2017–2018) field sera as well as sera from cats experimentally challenged with FeLV, FIV, FHV1, calicivirus, panleukopenia virus, *Toxoplasma gondii* and feline coronavirus were included.

Operator repeatability, robustness, matrix effects (whole blood vs plasma) for the LFA were shown to be adequate. The specificity of the LFA was higher than 90%, except for feline coronaviruses (80% for 45 tested samples). The sensitivity of the LFA was better than for seroneutralisation tests.

The SARS-CoV2 RT-qPCR assay was validated with mink rectal and oral swabs spiked with the

SARS-CoV2 RNA sequence "GE". The limit of detection (LOD) was established at 2.5 x 10^3 copies GE/ml for oral swab samples and 5 x 10^3 copies GE/ml for rectal swab samples. The specificity of the RT-PCR has not been assessed. And this assay cannot differentiate between replicative and non-replicative virus. The applicant should clarify whether the recombinant spike used in the serological tests was identical to the one included in the vaccine (i.e. the prefusion spike protein).
Regarding safety of the new adjuvant component, while information was provided, detailed data are missing from the dossier and should be provided especially on the impact of the new adjuvant component in the target species, including component class, signalling pathways, possible autoimmune reactions, risk of DNA integration in the genome and carcinogenicity should be submitted.
The applicant should also justify that the validation of all the tests were not provided (ELISA, Lentiviral Assay), that no reference materials were included in the samples to set thresholds of the assays and to link the results each other and that all these flaws do not undermine their results.
Regarding the specificity of the tests, and because mink may be infected with alphacoronaviruses (Vlasova 2011), the applicant should clarify how important are the cross-reactions with alphacoronaviruses in their tests taking into account that a 80% specificity was found with PIF sera.
The applicant should clarify and justify the positive threshold of the serological tests (lateral flow assay, lentivirus assay, virus neutralisation test) and detection assays which have been considered in all the studies. The applicant should justify the specificity of the SARS-CoV-2 RT-PCR.

Study	***Remarks***
Lab safety in minks	Human embryonic cell line
Field safety in minks	Human embryonic cell line
Lab efficacy in minks	*Lowest Ag content (10 µg) & 2 manufacturing processes on pre-MCB (purified or clarified)*
Field safety in minks	*Pre-MCB*
Lab efficacy in dogs	Human embryonic cell line *Modified adjuvant system*
Lab efficacy in cats	Human embryonic cell line *Modified adjuvant system*
Lab efficacy in cats	Human embryonic cell line *Modified adjuvant system*
Field study Zoo animals	Pre-MCB

Laboratory tests

In the preclinical studies, the recruited minks were non-SPF sourced from an accredited breeding facility. Vaccinations and all the procedures where minks were manipulated were performed in sedated animals. The applicant should justify that the sedation has not confounded the monitoring of any immediate general adverse reaction (within 1 day after vaccination) The study animals' ages were constrained by the date when the studies were undertaken because minks have one litter a year between April and mid-June and therefore they were recruited at least at 6 months of age in the safety study and at 9.5 months of age in the efficacy study.

Three safety studies were carried out in dogs and cats with the same antigen but with a different adjuvant system and total protein concentration.

Besides a dedicated safety study in minks, safety information was also drawn from a preclinical

efficacy study (lowest antigen content) in minks as well as a study performed in dogs and two studies performed in cats. Information was also drawn from the emergency use of Versiguard SARS CoV2 in minks in commercial farms and in zoo animals.

Safety of the administration of one dose and an overdose

In a study, the safety of a vaccine dosed at the antigenic upper limit was investigated after two injections 3 weeks apart to 6-month-old American minks (8 in each group) with 1 dose or a double dose. In the control group, 4 animals were administered the adjuvant only. The clinical signs of the minks were monitored daily up to 3 weeks after the 2nd injection.

The correct administration of the vaccine was monitored by dosing the antibody with a lateral flow test. The vaccine was well injected since all the animals were seropositive 3 weeks after the first administration, while the control group remained seronegative.

Lots of animals had temperature (T> 40.3 °C) before injection both in the control and the vaccine groups. The stress of the animal handling may have confounded the monitoring of the body temperature as well as the adjuvant which was injected as the control product and it was not possible to determine whether the vaccine actually caused hyperthermia, albeit this temperature increase was only of moderate nature. The bodyweight (bw) increased in all animals but one (in the 1x group). Neither injection site reactions nor general clinical signs including depression/lethargy, cough, vomiting, diarrhoea, nasal and ocular discharge were noticed.

In a study, the safety and the efficacy of a vaccine dosed at the antigenic lower limit per dose and with either a clarification or the purification step was investigated after two injections 3 weeks apart to 9.5-month-old American minks in two groups of vaccinated animals (8 in each group). The 8 minks of the control group were administered saline. The clinical signs of minks and their bw were monitored daily up to 11 days after the 2nd injection. Neither local nor general reactions were detected and their weight gain was not modified. One control mink died 1 day after the 2nd injection probably due to the anesthesia (no lesions were found). More details on this study can be found in part IV of this report.

Safety of the repeated administration of one dose

No repeated administration study was performed. In accordance with the guideline EMA/CVMP/IWP/123243/2006 on data requirements for immunological veterinary medicinal products intended for MUMS/limited market, this is considered acceptable.

Examination of reproductive performance

No studies on effects of the vaccine on reproductive performance were conducted and an appropriate warning was included in section 4.7 of the SPC. In accordance with the guideline EMA/CVMP/IWP/123243/2006 on data requirements for immunological veterinary medicinal products intended for MUMS/limited market, this is considered acceptable.

Examination of immunological functions

No study on the effect of the vaccine on immunological functions was conducted. This is acceptable, as they are not considered necessary for this type of vaccine.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with the CVMP "Guideline on user safety for immunological veterinary medicinal products" (EMEA/CVMP/IWP/54533/2006).

The active substance is a purified and inactive modified viral protein which is thus not infectious. The well-known adjuvant component has a longstanding use in vaccines. With regard to the amount of the new adjuvant component in each dose, it meets the safety criteria of substances considered as not falling within the scope of Regulation (EC) No. 470/2009. Cholesterol, ethanol and phosphate buffers are used in many human and veterinary nutritional, medicinal and cosmetic products and are not considered to be a safety risk to users. No components of the vaccine are expected to be carcinogenic at the dosage they were used.

The adverse reactions which might be expected to emanate from the product are allergic reactions, which can occur after self-injection or splash in the eye, as well as local irritation after skin contact.

Since the vaccine should be administered by health care professionals, appropriate warnings have been added to the section 4.5. of the SPC as follows:

"Hypersensitivity reactions, including anaphylaxis, could potentially occur in the case of accidental self-injection. Accidental self-injection may result in an immune response to SARS CoV2 spike protein. It is not expected for this to cause any adverse effects, however, repeated self-administration may increase the risk of hypersensitivity reactions. In case of accidental self-injection, seek medical advice immediately and show the package leaflet or the label to the physician."

Study of residues

This section is not applicable as the product is not intended for a food-producing species.

Interactions

No information has been submitted and that is noted in the section 4.8. of the SPC.

Field studies[1]

The safety of the vaccine was investigated in an US non-controlled field trial where 90 minks aged from 8–20 months, mostly females, originating from 3 farms were vaccinated according to the proposed schedule (two 1 mL subcutaneous doses 21 days (+/- 2 days) apart). Minks with an history of anaphylactic reactions or any vaccine reactions were not included in the trial. Except for one mink, which was found dead 12 days after the second administration with a pyelonephritis, neither general nor local reactions were detected.

In the US a 9 CFR 103.3 permit was granted by the USDA-CVB in 2Q21 which allowed a large-scale role out of the vaccine across a substantial number of animals throughout 69 US mink farms. Pharmacovigilance data from 5 batches of the final formulation (MCS-derived antigen) of the vaccine (3,089,977 doses) were collected, of which 2,542,597 doses had been administered to 6–8-week-old kits. The fatality rate reported by the 69 farms is considered to be normal and very few

[1] If relevant for safety.

adverse events were attributed to the vaccination.

These pharmacovigilance data have prompted the applicant to claim a vaccination schedule from 6 weeks of age onward, with respect to the potential benefits of the vaccinating kits.

Since zoo animals can be susceptible to SARS CoV2, 2 batches of the vaccine were provided to 209 zoos or similar organisations. More than 17,000 doses were administered to mammals, especially to very susceptible species such as non-human primates, mustelids, viverrids and big cats. No significant adverse events attributable to the vaccine itself were reported from the 3,600 individual use reports received; the adverse events were attributed rather to the vaccination procedure.

Environmental risk assessment

The applicant has presented an environmental risk assessment which has been conducted in accordance with the CVMP "Note for guidance: Environmental Risk Assessment for Immunological Veterinary Medicinal Products"(EMEA/CVMP/074/95). The vaccine does not contain any components raising toxic or pathogenic concerns. Since no hazards have been identified, the risk for the environment is considered to be effectively null and, thus, the environmental risk assessment may stop in phase 1.

Overall conclusions on the safety documentation

Versiguard SARS CoV2 is a subunit vaccine containing an adjuvanted purified protein. Further information on the statement that the new adjuvant component is rapidly and extensively absorbed from the injection site and is metabolised into chain-shortened nucleic acids and excreted mainly in the urine should be provided, including the possibility of autoimmune reactions (autoimmune myocarditis, arthritis, and flu-like symptoms have been observed in experimental animal models – according to publications provided by applicant), the component class, the risk of incorporation of this DNA into the genome and carcinogenicity. The safety of the vaccine produced in human embryonic cell line was investigated in conventional minks older than 6 months in a preclinical study at double dose and in a field clinical study, in addition to having been tested in preclinical studies using dog and cats. No product-related systemic reactions were reported in these studies. Furthermore, no adverse effects were extracted from the available pharmacovigilance data collected during emergency use programmes in more than 1 million farmed minks, in which most of the vaccinated animals were 6 weeks old, as well as in a wide variety of zoo animals. However, the pharmacovigilance data in minks and zoo animals should be updated to support use of the vaccine in 6 weeks old minks to justify further that vaccination of minks from the age of 6-8 weeks onwards is safe and does not weaken the benefit-risk balance of Versiguard SARS CoV2.

The risk to user is minimal because the vaccine is to be administered only by trained personnel under controlled conditions, in addition to the vaccine components not being a health risk for humans. Minor injuries associated with needle stick or accidental injection may however occur during the vaccination phase. Additionally, there is the risk of a hypersensitivity reactions in humans. Appropriate warnings to that respect have been included in section 4.5 of the SPC.

The risk to the environment is considered negligible due to the use under controlled conditions and the inertness of the vaccine components.

The investigation of safety of the human embryonic cell line-manufactured vaccine does not support the vaccine safety for minks, because the standard production will be carried out on routine production cells. Only the laboratory study in minks performed with vaccine batches containing

routine production cell line-derived antigen reliably reflects the safety of Versiguard SARS CoV2. There is an only study (in the field (vaccine batches containing routine production cell line-derived antigen were also used in the US emergency use field safety p3c-mink-103-3-safety study and in the study in zoo animals). The results of the laboratory safety data in routine production cells in non-mink species (study in cats) cannot be considered supportive because other adjuvants system (group T02 and T04) or total protein contain (groups T03 and T05-6) were used. The equivalence of human embryonic cell line and routine production cell line expression system should be more extensively discussed and demonstrated.

No laboratory study has been performed on minks aged 6 weeks but on minks aged 6 months and older and so this age should be considered in the SPC, section 4.2.

Part 4 – Efficacy

Introduction and general requirements

Versiguard SARS CoV2 efficacy has been investigated mainly in mink, hence the classification of the dossier as MUMS/limited market, but also in pets. Since the vaccine has already been used in the USA in 2021, and has a national permit for use in Finland, pharmacovigilance data have been included, inter alia as support for its use in zoo animals.

The vaccine was initially intended for use in mink from 6 weeks of age onwards to reduce virus shedding and virus infection of target tissues. One millilitre of the vaccine should be administered subcutaneously in two doses (3 weeks apart).

The vaccine is a suspension and a dose of vaccine contains purified recombinant fragment of the SARS CoV2 spike protein (S) and is adjuvanted. The batches used for preclinical efficacy studies in dogs and cats and US field studies were manufactured with human embryonic cell line while the final product is drawn from a transfected different cell line and was used in the laboratory challenge study in minks. The applicant has provided quality data to show that these two types of batches would be equivalent.
Minks are highly susceptible to SARS CoV2 infection and exhibit clinical signs and pathogenesis similar to human beings so that they have been considered to play an important role in the epidemiology of the human pandemic (propagation of the virus, emergence of new variants, …). Their vaccination could provide a tool for preventing clinical disease and reducing the transmission of the virus.
The applicant initially proposed the following indication to the SPC section 4.2:
For the active immunisation of mink from 6 weeks of age to reduce infection and viral shedding following infection with SARS CoV 2
Onset of immunity: 3 weeks after completion of the second dose of the basic vaccination scheme
Duration of immunity: Duration of immunity has not been established but annual revaccination is recommended, if required.

The choice of the vaccine strain has been justified in study. One of its objectives was to investigate the antibody cross neutralisation to Covid variant Delta (B.1.617.2) and other variants of interest by a serum neutralisation assay using pseudoviruses constructed with a lentivirus expressing SARS CoV-2 spike protein. The reported neutralisation titres against pseudovirus particles expressing the spike protein from either the original Wuhan virus or the Delta variant were very similar. These results suggest that vaccines based on the original spike sequence can induce a robust immune

response that can neutralize the Delta variant, if it becomes problematic in animals.

The basis for the assessment was the requirements for immunological veterinary medicinal products as described in the Directive 2001/82/EC (amended by Directive 2004/28/EC and Directive 2009/9/EC). Another guidance on safety of the vaccines was:
- Ph. Eur. 0062: Vaccines for Veterinary Use
- Ph. Eur. 5.2.7: Evaluation of efficacy of veterinary vaccines and immunosera
- CVMP/VICH/595/98: Guideline on Good Clinical Practices
- EMA/CVMP/038/97: EU Requirements for Batches with Maximum and Minimum Titre or Batch - Potency for Developmental Safety and Efficacy Studies
- EMA/CVMP/IWP/206555/2010: Requirements for the production and control of immunological veterinary medicinal products
- EMA/CVMP/IWP/123243/2006: Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market

- EMEA/CVMP/IWP/439467/2007: Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals
- EMEA/CVMP/852/99: Note for Guidance on field trials with veterinary vaccines
- EMEA/CVMP/682/99: Note for Guidance on duration of protection achieved by veterinary vaccines

Challenge model:

The applicant resorted to an experimental challenge model (Schlottau, 2020) which showed that intranasal instillation of 0.25 mL of 10^5 $TCID_{50}$ SARS-CoV-2 per animal provided 89% of infection. A SARS-CoV-2 strain isolated in October 5, 2020, from a Danish human exposed to infected minks was chosen as the challenge strain for mink. This strain was used in the challenge study in mink.

Supportive data about cats were obtained with an experimental model developed at Kansas State University where the animals were challenged with the SARS-CoV-2 USA-WA1/2020 strain from the Biodefense and Emerging Infection Research Resources Repository (lineage A).

Efficacy parameters and tests:

The same methods as those used for safety demonstration were applied in the efficacy studies. For their validation refer to part 3.

Efficacy documentation

Beside a challenge study in the target species, immunisation studies in commercial minks and two studies in cats and one in dogs as well as data from a laboratory challenge in cats contributed to draw the efficacy profile of Versiguard SARS CoV2.

Study	*Remarks*
Lab safety in minks	Human embryonic cell line
Field safety in minks	Human embryonic cell line
Lab efficacy in minks	*Lowest Ag content (10µg) & 2 manufacturing processes on pre-MCB (purified or clarified)*
Field safety in minks	*pre-MCB*
Lab efficacy in dogs	Human embryonic cell line *Modified adjuvant system*

Study	Remarks
Lab efficacy in cats	Human embryonic cell line *Modified adjuvant system*
Lab efficacy in cats *Lab challenge in cats*	Human embryonic cell line *Modified adjuvant system*
Field study Zoo animals	pre-MCB

Laboratory trials

Dose determination

While two antigen content concentrations have been used in the studies and both conferred protection to the minks, no specific dose-determination study was undertaken. The minimum protective dose has been addressed as part of the onset of immunity data package and this is considered acceptable.

Onset of immunity

The applicant has demonstrated an onset of immunity by a challenge of minks 3 weeks after the completion of their immunisation scheme.
In this study, the safety and the efficacy of a vaccine dosed at the antigenic lower limit per dose and with either a clarification or the purification step, was investigated after two injections 3 weeks apart of 9.5-month-old American minks (8 in each group). The difference between purified and clarified antigen used in the final formulation of the vaccine should be explained. Eight minks in the control group were administered with saline. On Day 32, twenty three animals were moved to a biosafety level (BSL)-3 laboratory and challenged on Day 42 with SARS CoV-2 through intranasal instillation. The animals were followed for health observations and sample collection (nasal washing and blood samples) before euthanasia on day 46 (4 animals per group) and day 63 (4 animals per group except in control group, 3). The primary efficacy endpoints were virus excretion (nasal washings) measured by RT-PCR during the challenge phase, virus load in target tissues (central and peripheral nervous system, respiratory and digestive tract, spleen, muscle) measured by RT-PCR, gross observations at necropsy and histopathology. The seroconversion, monitored by lateral flow assay, ELISA and virus neutralisation assays as well as clinical signs were chosen as secondary efficacy parameters.
The control minks remained seronegative before the challenge and SARS CoV-2 nucleic acid was detected mainly in the respiratory tract and the central nervous system 4 days after challenge. In other internal organs (kidney, liver, spleen, muscle, myocardium) and more surprisingly in intestine, when virus was found, its load was very low.
In vaccinates, SARS CoV-2 nucleic acid amount in the nasal washing was lower than in controls from 4 days after challenge onwards but no significant decrease of the total excreted load (AUC) was measured over the 3 weeks. The difference in duration of virus shedding between vaccinated and control groups was not evaluated.
In the organs, the SARS CoV-2 nucleic acid was lowered by around 1 $\log_{10}$ in the central nervous system and merely abolished in the lung of the vaccinates by day 4 after the challenge. Three weeks after, SARS CoV-2 nucleic acid was still detected in the lower respiratory tract of 1/3 control while it remained in the upper respiratory tract (2/4), olfactory bulb and cerebrum (1/4) and spleen (1/4) of animals vaccinated with purified antigen (group T2).
Pathological results are still missing.

Few clinical signs attributable to the challenge (depression, anorexia, low appetite) were reported in the vaccinated minks and none in the controls.
Seroconversion was observed after the first vaccine administration in all the vaccinates but one, in the clarified group, which finally seroconverted at challenge. Vaccinates' seropositivity at challenge was corroborated by ELISA and virus neutralisation tests.
In brief, this study supports the indication that vaccination decreases nasal viral shedding in the late stage of the infection by decreasing the viral load in lungs and the dissemination of the virus in the central nervous system.
To demonstrate the triggering of the immune system by vaccination with the vaccine or the antigen blended with other adjuvants, the applicant has provided serological data of minks, cats and dogs.

Serology in the target species, mink

In a study, the safety and the serological response associated to a vaccine dosed at the antigenic upper limit was investigated after two injections 3 weeks apart of 6-month-old American minks (8 in each group) with one dose or a double dose. In the control group, 4 animals were administered with the adjuvant only. The vaccinates became seropositive 3 weeks after the first injection while the control group remained seronegative throughout the study with the lateral flow test. Both virus neutralisation test and lentiviral assay showed neutralising antibodies 3 weeks after the second injection (geometric mean of the titre: 279).

Complementary data from other species

In a study, fifteen Beagle dogs aged from 6 to 10 months (8 females and 7 males) were randomly allocated into three treatment groups (5 animals each) and administered either with the vaccine at its highest antigenic payload adjuvanted with the routine vaccine adjuvants or with the antigen adjuvanted with 1% aluminum-based adjuvant, or with 1% aluminum-based adjuvant only (controls). The injections were scheduled according to the proposed scheme.
Conversely to control animals, all vaccinated dogs seroconverted after the second injection and the titres were not significantly different between the two groups, while numerically higher in the vaccine group.
In a study, the serological response of 6-10 months cats vaccinated with the antigen at its highest antigenic payload blended with a cholesterol-based adjuvant (without the new adjuvant component) was compared to the response to vaccination with the antigen at its highest antigenic payload blended with RT adjuvant (including the new adjuvant). Both vaccines elicited high titres of seroneutralising antibodies by day 20 and 42 after vaccination.

In study B880W-US-20-119 the serological response of cats vaccinated with the antigen blended with a cholesterol-based adjuvant was compared to another vaccine, a replication-deficient simian adenovirus expressing the SARS-CoV-2 spike protein. Both vaccines elicited high titres of seroneutralising antibodies by day 20 and 42 after vaccination (around 1300 and 2048 respectively).

In a study, four SPF cats (10-13 months old), vaccinated in the course of a study with the antigen at its highest antigenic payload adjuvanted with cholesterol-based , were challenged around 6 weeks afterwards with 10^6 $TCID_{50}$ SARS-CoV-2 alpha strain by both nasal and oral route.
The four control cats which received saline injections excreted the virus in all their emunctories (saliva, rhinorrhea, feces) after challenge and seroconverted by 2 weeks but no clinical signs were noted, except one cat with a sporadic fever. Mild lesions attributable to the infection were reported 2 weeks after infection. The vaccinates had lower nasal and oral excretion both in duration,

quantities and area under the curve (AUC) and their fecal shedding was decreased on day 2. But they experienced the same level of lesions (lung atelectasis 2/4, congestion and discoloration 4/4).

In conclusion the studies have demonstrated that vaccination of 9.5-month-old conventional American minks lowers some parameters of the infection, viral load in respiratory tract and central nervous system tissues and nasal shedding of virus, and is associated with seroconversion with neutralising antibodies. The rise of neutralising antibodies against the trimeric spike protein was corroborated in other species (dogs and cats) and with other adjuvants.

Duration of immunity

No data about the duration of immunity were provided. This is acceptable for a MUMS/limited market product which is intended for an animal whose production lifetime does not usually expand beyond one year and that has been adequately indicated in the SPC, according to the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/IWP/123243/2006-Rev.3). However, in accordance with this guideline, some data on whether the vaccination provides immunity over the production lifetime of the minks should be collected.

Maternally derived antibodies (MDA)

No data about MDA were provided and an appropriate warning is included in the SPC. This is acceptable for a MUMS/limited market indication for a vaccine, according to the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/IWP/123243/2006-Rev.3).

Interactions

No data about compatibility of Versiguard SARS-CoV-2 with any other vaccine were provided and an appropriate warning is included in the SPC.

Field trials

No dedicated field trial was provided. This is acceptable for a MUMS/limited market indication as the laboratory efficacy studies adequately established and validated the efficacy and it is justified that they are representative of efficacy under field conditions, according to the Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (MUMS)/limited market (EMA/CVMP/IWP/123243/2006-Rev.3).
Pharmacovigilance data of the 3,089,977 doses administered from June to September 2021 showed that the mortality rate remained in the normal range for this industry. That could suggest some protection where the virus circulated.
And the same reasoning applies to the zoo animals.

Overall conclusion on efficacy

Versiguard SARS CoV2 is intended to immunise mink against SARS CoV-2 infection. In an experimental challenge study at the onset of immunity (3 weeks), the vaccination of 9.5-month-old minks decreased the viral load in their respiratory tract and their central nervous system 4 days after the challenge and decreased nasal viral shedding in the late phase of the infection. However, the applicant should still justify that these results adequately support: 1) a biologically relevant

decrease of the shedding of virulent viruses which lowers the risk of transmission across minks and to other species and chiefly to humans and 2) a clinical benefit for the vaccinates when the viraemia and the viral load of the control minks was not associated with any noticeable disease. The applicant should also explain how the supportive results can be extrapolated to the lowest age proposed for the onset of immunity, which is 6-8 weeks.
The minimum age of vaccination has been set at 6 weeks based on pharmacovigilance data and this is questionable. The absence of investigation of the duration of immunity has been reported in the SPC and this is acceptable for a MUMS/limited market indication; however, some data on whether the vaccination provides immunity over the production lifetime of the minks should be collected. These results have been buttressed by serological evidence (neutralising antibodies) in minks and by pharmacovigilance data collected during emergency SARS CoV-2 vaccination programs in farmed minks, where most of the vaccinated minks were 6-weeks old. In addition, complementary data on efficacy in other non-target species (cats, dogs, zoo animals) have been presented.

The efficacy of vaccines containing spike based on Wuhan-1 SARS CoV2 strain has been shown less protective with the new SARS CoV2 variants infecting humans (Andrews, 2022). The applicant should justify that the protection afforded to minks by Versiguard SARS CoV2 against new variants of concern (B.1.1.529/BA.1; B.1.1.529/BA.2; B.1.1.529/BA.3) and predictable evolutionary variants remains biologically relevant. This is considered a major objection.

The results of efficacy studies in minks are not adequately reported in the SPC and this should be amended according to comments and suggestion made in the PI document.

Experimental challenges of vaccinated cats are debatable to be considered supportive because the vaccines used had a different composition (another adjuvant, formulation is unknown, BRP is not provided) than the one proposed for standard production.

Pharmacovigilance data collected during emergency use programs in farmed minks and a wide variety of zoo animals should be updated, but based on the studies available, justification to support a 6-week age to start vaccination still needs to be provided.

Part 5 – Benefit-risk assessment

Introduction

Versiguard SARS CoV2 is a new subunit vaccine for an emerging zoonotic coronavirus. It contains a modified spike protein of the Wuhan-1 SARS CoV2 strain.
The indication for use in mink has been classified as MUMS/limited market and therefore reduced data requirements apply that have been considered in the assessment.

Benefit assessment

Direct therapeutic benefit

The vaccination of 6-month-old minks is expected to decrease the viral load in their respiratory tract and their central nervous system as well as their nasal shedding.
The onset of immunity is 3 weeks after completion of the basic vaccination scheme.
The duration of protection has not been investigated and this is indicated in the SPC.

Additional benefits

The decrease of nasal shedding is expected to lower the spreading of the virus among congeners as well as susceptible in-contact animals and humans and the decrease of internal infection, and to lower the selection of escape variants which were a concern in the epidemiology of this bidirectional zoonosis.

Risk assessment

Risks for the target animal:

Quality:

The risk associated with the manufacture of this subunit vaccine has been overall mitigated by the control parameters set throughout the manufacturing process from the starting material to the release of the batch, however some outstanding points remain.

Safety:

The good safety profiles of the vaccine when it is administered in accordance with SPC recommendations have been demonstrated in mink as well as in other species (dogs and cats) and has been corroborated from pharmacovigilance data from vaccinated minks and zoo animals.

Efficacy:

The risk of lack of efficacy is considered low by the applicant but this point was questioned during the assessment.

Risk for the user:

A complete assessment has concluded that the vaccine posits an acceptable risk for the user when used according to the SPC recommendations.

Risk for the environment:

A full environmental risk assessment concluded that Versiguard SARS CoV2 contains no ingredients which are considered harmful to the environment.

Risk management or mitigation measures

An adequate text has been included in the product information to tackle the conventional risks associated with the use of any injectable product. Special precautions to be taken by the person administering the veterinary medicinal product to animals have been included in the SPC section 4.5 and in the package leaflet section 12.

Evaluation of the benefit-risk balance

In the presence of major and other concerns, no conclusions could be taken on the benefit-risk balance of the application.

Conclusion

Based on the data on quality, safety and efficacy presented by the applicant, the Committee for Veterinary Medicinal Products (CVMP) considered that the application for Versiguard SARS CoV2 was not approvable at day 120 since major objections and other concerns were identified which preclude a recommendation for marketing authorisation.

The details of the outstanding issues were provided in the list of questions.

On 15 September 2022, during the clock-stop, Zoetis Belgium communicated the withdrawal of the marketing authorisation application to the Agency.