11 December 2014
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Veterinary Medicines Division

## Committee for Medicinal Products for Veterinary Use (CVMP)

# CVMP assessment report for Suvaxyn CSF Marker (EMEA/V/C/002757/0000)

Common name: Classical swine fever vaccine (live)

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

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## *Introduction*

On 30 January 2013 the applicant Zoetis S.A. submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Suvaxyn CSF Marker in accordance with Regulation (EC) No 726/2004.

The product was considered eligible for the centralised procedure by the Committee on 14 June 2012 under Article 3(1) of Regulation (EC) No 726/2004 as it is a medicinal product developed by means of a biotechnological process. It also contains a new active substance which on the date of entry into force of the Regulation was not authorised in the European Union (EU). The product contains a genetically modified organism (GMO). The rapporteur appointed was M. Blixenkrone-Møller and co-rapporteur B. Urbain.

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

Classical swine fever control is strictly regulated within the EU through Council Directive 2001/89/EC and Commission Decision 2002/106/EC, the accompanying OIE (the World Organisation for Animal Health) Diagnostic Manual. Prophylactic vaccination is prohibited but emergency vaccination in pig holdings and of feral pigs is a legal option when following an EU approved vaccination plan. Therefore, the use of any vaccine is strongly dependent on different pre-conditions and the control policy applied.

This vaccine has been developed to discriminate marker vaccinated pigs from natural infected pigs with a classical swine fever virus (CSFV).

Suvaxyn CSF Marker contains live recombinant bovine virus diarrhoea virus containing the classical swine fever E2 marker (CP7-E2alf) and is presented in cardboard boxes containing 1 vial with 10 or 50 doses of lyophilisate and 1 vial with 10 or 50 ml solvent.

The proposed route of administration is intramuscular and the target species are pigs.

On 11 December 2014 the CVMP adopted an opinion and CVMP assessment report.

On 10 February 2015, the European Commission adopted a Commission Decision for this application.

# Part 1 - Administrative particulars

#### *Detailed description of the pharmacovigilance system*

The applicant has provided a detailed description of the pharmacovigilance system (dated 8th March 2011) which fulfils the requirements of Directive 2001/82/EC. 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 EU or in a third country.

#### *Manufacturing authorisations and inspection status*

A valid good manufacturing practice (GMP) certificate has been submitted.

The manufacturer Zoetis S.A., Belgium was inspected on 1 April 2011 and it is stated in the certificate that the manufacturer complies with the principles and guidelines of GMP. It is considered that no product specific inspections are necessary.

## *Overall conclusions on administrative particulars*

The detailed description of the pharmacovigilance system and GMP certification of the manufacturing sites was considered in line with legal requirements.

# Part 2 - Quality

## *Composition*

Suvaxyn CSF Marker is presented as a lyophilisate and solvent for suspension for injection in vials containing 10 or 50 doses of lyophilisate and 10 or 50 ml solvent.

The composition of the finished product is divided into the composition of the freeze-dried fraction and the liquid fraction. The freeze-dried fraction consists of the active substance, a live recombinant bovine viral diarrhoea virus containing the classical swine fever E2 marker (CP7_E2alf virus) in a quantity of $10^{4.8}$–$10^{6.5}$ $TCID_{50}$/dose, and the excipients L2 freeze-drying stabiliser and Dulbecco's modified Eagle culture medium (DMEM) which contain Dextran 40, casein hydrolysate, lactose monohydrate, sorbitol 70% (solution) and sodium hydroxide. The liquid fraction consists of sodium chloride and water for injections.

The vaccine contains no adjuvant or preservative.

The composition of the freeze-dried and liquid fractions is considered acceptable.

The pharmaceutical form is a suspension for injection.

## *Container*

The finished product is presented in multi dose vials of freeze-dried fraction and multi dose vials of liquid fraction.

Each fraction (freeze-dried and liquid) is filled into 15 ml (contains 10 x 1 ml doses) or 59 ml (contains 50 x 1 ml doses) type I hydrolytic glass vials. The vials containing the freeze-dried fraction are closed with bromobutyl rubber stoppers, and the vials containing the liquid fraction (solvent) are closed with chlorobutyl rubber stoppers. All vials are sealed with aluminium caps.

The rubber stoppers comply with the requirements of European Pharmacopoeia (Ph. Eur.) monograph 3.2.9. All the packaging components meet Ph. Eur. requirements.

## *Development pharmaceutics*

In the past, vaccination against CSFV was done by inactivated or live attenuated vaccines. However, the use of live attenuated vaccines can confuse the identification of pigs infected by field CSFV, as the immune response cannot be distinguished from those produced by the vaccine.

Suvaxyn CSF Marker vaccine has been developed with the help of grants from the European Commission under Framework 6 and 7 programmes and a number of research institutions and official medicines control laboratories (OMCL) have been involved.

The applicant has therefore chosen to manufacture a "marker" vaccine, where the immune response can be distinguished from that caused by the natural infection. The vaccine is produced using a live chimeric pestivirus including both CSF and bovine viral diarrhoea virus (BVDV). The active substance CP7_E2alf is based on BVDV where the E2 protein has been replaced by E2 of the CSFV strain Alfort 187. The vaccinated pigs will then be antibody positive for only the E2 protein of the CSF, and it will therefore be possible to distinguish the vaccinated pigs from those infected by the field CSFV.

The rationale for the development of the product is considered reasonable, as the marker vaccine will allow differentiation of infected from vaccinated animals (DIVA), and the choice of BVDV as the antigen carrier is also considered acceptable, as the pigs are weakly susceptible to this virus.

Potential related to efficacy of such a replication competent chimeric pestivirus, could be the ability to distinguish field infected herds (vaccinated or non-vaccinated) from only CP7_E2alf vaccinated herds combined with a potential for stimulation of different arms of the immune system following replication of the vaccine virus in the host.

For the final formulation the applicant has chosen to lyophilise the antigen fluid, containing a stabiliser, and the lyophilisate is then suspended in diluent before injection.

The choice of formulation for the finished product has been properly justified.

## *Method of manufacture*

The manufacture of the freeze-dried fraction consists of two stages:

1. Production of CP7_E2alf vaccine antigen(s)

2. Preparation of the finished product (freeze-dried fraction).

*Production of CP7_E2alf vaccine antigen(s)*

The vaccine virus is grown in swine kidney cells (SK cells).

The SK cells are inoculated in culture flasks, incubated and subcultures are carried out. The cell suspension obtained after the last passage is either used to plant roller bottles or bioreactors.

In roller bottles the culture medium is replaced with infection medium (virus in DMEM), incubated, and then the antigen is harvested aseptically and stored in sterile containers frozen at –40 °C or below. For final bulk antigen, the antigen fluids are stabilised with L2 stabiliser solution before freezing to obtain a final concentration of 75% antigen and 25% L2, and then the bulk is stored frozen at –40 °C or below, until used in final formulation.

In the bioreactors, microcarriers are added, and the cells then form monolayers on the microcarriers. The cell suspension is incubated, and the SK cell suspension is then sedimented and the culture medium is replaced with maintenance medium (virus diluted in DMEM). The infected cell monolayers are incubated, and then the antigen fluid is harvested and filtered through a ≤ 20 µm filter during transfer to pooling tank. L2 stabiliser is added to reach a final concentration of 75% antigen and 25% L2. The final bulk is then dispensed in sterile containers and stored frozen at –40 °C or below, until used in final formulation.

*Production of freeze-dried fraction*

The frozen bulk antigen is thawed, filled aseptically into glass vials, loosely stoppered and then loaded into a freeze-dryer. There a primary and a secondary drying step are performed. After secondary drying the stoppers are inserted in the vials. The vials are unloaded from the lyophiliser and sealed with aluminium caps.

*Manufacture of liquid fraction*

Sodium chloride is dissolved in water for injections. After complete dissolution and addition of water for injections up to final volume, the sodium chloride solution is aseptically filled into glass vials through a ≤ 0.22 µm filter, and the vials are then aseptically stoppered, and sealed with aluminium caps.

## *Control of starting materials*

## *Active substance*

The active substance, live recombinant E2 gene deleted BVDV containing CSFV protein E2 (CP7_E2alf), is

produced in SK cells.

Detailed descriptions of the master (MSC) and working seed cells (WSC) and characterisation of master (MSV) and working seed virus (WSV) were provided. The seed lots were controlled according to Ph. Eur. and current EU regulations (Annex I of Directive 2001/82/EC).

Information was provided on the chimeric pestivirus CP7_E2alf including its development, construction and control of the genetic stability. The characterisation of the chimeric virus structure, including characterisation of the E2 protein, is sufficiently described, including the genetic stability of the chimeric strain during the vaccine production. Genetic stability of the chimeric virus structure during development of the vaccine strain was initially not sufficiently addressed and this was raised during the assessment. With the responses provided it has become clear that passages used in some of the initial clinical trials and the MSV/WSV are not completely identical to each other neither in nucleotide sequence nor in the corresponding amino acid sequence. However, the amino acid substitutions have not occurred in either the known neutralising epitope or the T-cell epitope sequences.

Stability of the bulk antigen has been presented, both for bulk stored at minus 80 °C ± 10 °C and at –40 °C or below. Data has been presented for batches stored at minus 80 °C ± 10 °C, and these data show that there is very little change in the titre, which indicates that the antigen is stable when stored at this temperature. For bulk stored at –40 °C or below, data up to 18 months has now been presented. A shelf life of 18 months when stored at –40 °C or below can be approved.

Active substance and SK cells were tested for extraneous agents (EA) in accordance with the relevant Ph. Eur. monographs and guidelines.

## *Excipients*

Sufficient information on starting materials of both biological and non-biological origin has been provided. For those materials not listed in Ph. Eur. in-house specifications and certificates of analysis detailing control tests have been provided. Furthermore, solutions and media have been used in the manufacture of the vaccine which has been made in-house. The qualitative and quantitative compositions of the in-house media and solutions, the methods of preparation, controls and tests performed and the storage conditions have been properly described. All the in-house media and solutions have been sterilised in accordance with the requirements in Ph. Eur. monograph 5.1.1.

## *Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies*

The following starting materials of biological origin were tested:

Listed in Ph. Eur.: bovine serum, gentamicin sulphate and lactose monohydrate;

Not listed in Ph. Eur.: SK cells, CP7_E2alf, foetal bovine serum, trypsin and casein hydrolysate.

All the possible sources of transmissible spongiform encephalopathy (TSE) contamination via the above mentioned starting materials have been thoroughly evaluated.

In general, the starting materials of biological origin comply with the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 rev.3) and Commission Directive 1999/104/EEC.

The overall TSE risk associated with this vaccine is considered negligible.

## *Control tests during production*

The following in-process controls are performed on the final antigen:

- Sterility (Ph. Eur. monograph 2.6.1)

- Virus titration (in-house method).

The batch test results showed that the batches are well within the acceptance criteria, and show consistency of the product.

## *Control tests on the finished product*

Descriptions of the methods used for the control of the freeze-dried fraction (description, identity, sterility, absence of mycoplasma, titration, residual humidity and absence of extraneous agents) and the liquid fraction (description, volume, identity, acidity/alkalinity, heavy metals, arsenic, sterility, endotoxins and content of sodium chloride) and their specifications are provided.

The results of the analysis of five consecutive production runs of freeze-dried vaccine (three 10-dose presentations and two 50-dose presentations) and four consecutive production batches of solvent were presented which all comply with the required specification.

## *Stability*

*Stability of the freeze-dried fraction:*

Potency results of three experimental technical batches, produced in roller bottles at the Olot site and stored at 5 °C ± 3 °C for different periods of time and tested at different intervals, have been provided. Stability data comply with specification.

Additionally, a full stability program in accordance with relevant International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines has been initiated with the consistency batches (batch release batches) made at site Louvain-la-Neuve in Belgium (LLN) to support 24 months shelf life at 2 °C – 8 °C after a previous storage at –20 °C or below for up to 15 months when filled in 10 and 50 dose presentations.

Data have been submitted, one for batches stored at 2 °C – 8 °C and one for batches stored first at –20 °C or below. Stability data up to 21 months are provided and the results comply with the acceptance criteria. A finished product shelf life of 18 months when stored at 2 °C – 8 °C is granted.

*In-use stability*:

The finished product will be used immediately and no in-use stability data are therefore presented.

*Stability of the diluent:*

The diluent was also tested for stability. The only parameter tested at all six time points was acidity/alkalinity. This is considered acceptable, as acidity/alkalinity is the most relevant parameter to control at the intermediate time points, as a change in this parameter can have an influence on the reconstituted finished product.

## *Overall conclusions on quality*

The manufacturing process (including development and validation) as well as control tests and specifications for both active substance and excipients were described in detail. The TSE risk associated with this vaccine is considered negligible. Batch release and stability data showed compliance of the product's quality

attributes with the specifications. A finished product shelf life of 18 months when stored at 2 °C – 8 °C has been supported by data.

# Part 3 – Safety

## *Introduction*

The vaccine is intended for administration in pigs from 7 weeks of age only for use in emergency vaccination in outbreak situations in pig holdings.

The vaccine was administered intramuscularly (IM) in the neck muscles at one site or both following the recommended schedule stated in the summary of product characteristics (SPC). The piglets in the majority of the safety studies were at minimum age and all were seronegative for antibodies against CSFV, BVDV and border disease virus (BDV). The sows used in the safety studies were in the different days of gestation.

It has been reported that BVD virus can infect pigs but most of the infections are asymptomatic; in some circumstances, reproductive events or weak CSF-like symptoms can occur (Terpstra, 1988; Deng, 2012). The vaccine virus safety has been addressed as below.

## *Laboratory tests*

## *Safety of the administration of one dose and repeated administration of one dose*

The study included piglets of the youngest recommended age and tested seronegative to pestiviruses. All piglets vaccinated via the intramuscular route seroconverted after two vaccinations given 2 weeks apart. The experimental vaccine batch with a titre of $10^{6.0}$ $TCID_{50}$/ml is close to the maximum titre $10^{6.5}$ $TCID_{50}$/ml stated in the SPC and MSV+3, stated to be the least attenuated passage level that will be present in a batch of vaccine.

The clearly described deviations of the experimental test batch used in the study compared to the final vaccine, and deviations related to excipients and formulation (frozen versus freeze-dried) are considered acceptable, as the deviations are not likely to influence the relevant safety issues.

Furthermore, reference is made to the safety of an overdose also in part 3.B.3 to cover the safety of administration of one dose, as outlined in the Ph. Eur. monograph 0065. This is acceptable.

Results show that there was little general indication for hyperthermia from data on average rectal temperatures per group. There were some pigs with a temperature above 40 °C which is generally considered as a critical point for pigs although piglet temperatures may be higher e.g. due to handling stress but these were sporadic findings. There were similar findings for occasional increases of temperature in the control group animals. Statistical evaluation of mean temperature data between vaccinated and control group did not show any statistically significant increase. The study is considered acceptable for evaluation of safety of administration of one dose, when the pivotal 10-fold overdose study defines the precise maximum titre of a single dose.

## *Safety of one administration of an overdose*

The study included piglets of 6–7 weeks of age (seronegative to pestivirus) overall compatible with the minimum age. The experimental vaccine test batch (MSV+3 which is the lowest passage that could be present in the vaccine) was given in 2 X 2 ml volumes resulting in a 10-fold overdose ($10^{7.5}$ $TCID_{50}$) compared to the single dose stated in the SPC. Owing to the experimental batch which was not freeze-dried

to reduce its volume, injection of a divided overdose at each side of the neck was given. These deviations are acceptable.

There was little general indication for temperature rise from data on average rectal temperatures per group. Data from one IM vaccinated piglet with a temperature rise for three days (up to 40.7 °C) related to hind leg inflammation was excluded from the temperature data. There were some other pigs with a temperature above 40 °C (generally considered as a critical point for pigs although piglet temperatures may be higher e.g. due to handling stress) but these were sporadic findings. There were similar findings for occasional increases of temperature in the control group animals. Statistical evaluation of mean temperature data between IM vaccinated and corresponding control group did not bring out significant differences.

Scientific literature supports that vaccination with CP7_E2alf does not exert significant impact on blood leukocyte counts. A proposed maximum titre for Suvaxyn CSF Marker of $10^{6.5}$ $TCID_{50}$/dose is acceptable.

### *Examination of reproductive performance*

Safety was also tested in a limited number of laboratory studies in pregnant sows. Reproductive parameters (abortions and observations of newborns) and seroconversion of newborns were investigated.

Study 1, a 10X overdose study included 15 pestivirus negative sows, 13 vaccinated and 2 control sows between 51 and 72 days of gestation, which covers the gestation phase between 55–80 days stipulated in the Ph. Eur. monograph 0065.

Study 2 included a max 1X dose in sows at different stages of pregnancy (6 control sows, i.e. 2 sows of phase 1, 2 and 3, respectively; and 10 sows in 1st phase, 12 sows in 2nd phase, and 13 sows in 3rd phase).

Note that in study 1 all piglets were delivered by caesarean section and caesareans were carried out for most deliveries in study 2.

The experimental vaccine batch (same batch used for studies 1 and 2) contained more than 10 times the maximum dose of $10^{6.5}$ $TCID_{50}$/ml stated in the SPC and MSV+3, which is stated to be the highest passage level that will be present in a batch of vaccine. The clearly described deviations of the experimental test batch used in the study compared to the final vaccine composition and deviations related to excipients and formulation (frozen versus freeze-dried) are considered acceptable, as the deviations are not likely to influence the relevant safety issues.

In conclusion, the two studies in sows did not reveal indications for major safety concerns for the sows as such, i.e. no signs of anaphylactic reaction or death of any sows during the studies. Hyperthermia above 41 °C was only detected in one sow 4 hours post-vaccination (temperature increase was 2.91 °C compared to its basal temperature). Temperatures above 40 °C were not observed in other sows. Minor local reactions which resolved within one day were observed in a few sows.

The lack of seroconversion in newborn piglets which had not received colostrum was found in both studies, which indirectly could indicate lack of transplacental transmission of vaccine virus, while the majority of the sows seroconverted after vaccination. Two mummified and one stillborn piglet of study 1 was tested directly for presence of vaccine virus by real-time polymerase chain reaction (RT-PCR), and with negative result. Healthy offspring was investigated for seroconversion, exclusively, so detection of potential CP7_E2alf persistently infected immunotolerant individuals was not addressed in the study.

However, these very limited studies did not reveal indications for potential transplacental transmission of vaccine virus.

In study 2, two vaccinated sows aborted of which one had been vaccinated in 2nd phase and one vaccinated in the 3rd phase of pregnancy. The abortions took place 16 days and 35 days post-vaccination. Further

investigations indicated that the abortions were not likely to be due to the vaccination and that mummified piglets were often of small size which probably meant that most of them died before vaccination.

It could be confirmed that the most sensitive target animals have been included in the laboratory studies investigating safety of the vaccine related to reproductive performance and transplacental vaccine virus transmission.

Reproduction anomalies were found in both studies. There was a lack of balance between control group of two animals and test groups between 10 and 13 animals in the studies, that makes comparison generally more difficult and much reliance is made on expected birth/defects normally observed in general rather than the outcome (e.g. 10X overdose: mummified piglets (2%) and stillbirth (0.7%)) reported of the experiments themselves.

Further safety data from field use cannot be gathered as sows are not supposed to be vaccinated in emergency situations. Therefore the safety cannot be assessed in a sufficient number of sows and does not allow the use of statistical analyses of results to investigate for potential reproductive safety issues.

In conclusion, vaccination of sows is contra-indicated due to the risk of birth of immunotolerant and persistently infected piglets, as the vaccine has shown lack of protection against transplacental transmission of challenge virus infection. Also additional beneficial safety consideration is related to the contraindication for sows is related to reduction of the potential risk of recombination events to occur during concurrent infections with the chimeric vaccine virus and wild type pestiviruses in foetuses and neonates including persistently infected immunotolerant individuals, being considered the most sensitive targets for pestiviruses.

## *Examination of immunological functions*

Adverse effects on immunological functions have not been addressed specifically by the applicant in the dossier. Scientific justification for the omission of evaluation of the parameter of leucopenia as indicated in the OIE recommended minimum requirements (OIE Terrestrial Manual, Chapter 2.8.3 on classical swine fever) has been provided. The significance of this parameter for a first rough indication of the safety of the Suvaxyn CSF Marker vaccine and immunological functions has been discussed and reference to data in support of findings, that the vaccine does not induce leucopenia has been provided.

In conclusion, there is no indication that the chimera has a negative effect on immunological function, as no detectable adverse effect on blood leukocytes numbers, B-cell activation, levels of pro-inflammatory cytokines or clinical health following vaccination were noted in additional supportive studies provided. A finished product shelf-life of 18 months when stored at 2 °C – 8 °C is granted. The potential impact of the chimeric virus on immunocompetence of foetuses has not been investigated. This is acceptable as the vaccine is contraindicated for use in sows.

## *Special requirements for live vaccines*

### Spread of the vaccine strain

A check for non-transmissibility was carried out in six week old piglets according to the Ph. Eur., except for the disease problems and medications in the animals during the trial period. There was no evidence provided that the vaccine virus could spread to in-contact piglets after IM or oral route. The trial results are primarily based on the lack of detectable antibody development. The appropriateness of the vaccine batch considering the notion of using the least attenuated passage level of this chimeric virus has not been substantiated, while piecing together results from safety studies of this dossier with studies in peer reviewed scientific

papers provides evidence that roughly no overt clinical disease development have been found in laboratory studies.

In conclusion, the study on spread of the vaccine is acceptable, as the documentation provided for this vaccine supports that it is only to be used in an outbreak situation in herds within restricted and controlled zones. Regulations related to emergency vaccination and a time period of quarantine after vaccination applies due to CSF being a notifiable disease.

**Dissemination in the vaccinated animal**

The results of the dissemination study provide evidence that the vaccine virus does disseminate after IM inoculation as virus was detectable post-mortem in tonsil tissue. Additional studies show that vaccine virus is detected in the draining lymph node and muscle tissue at the injection site on day 4 post-vaccination and dissemination to blood, other lymphoid tissues in the body and the spleen takes place. Wide dissemination of the chimera and isolation of infectious virus from tonsils and detection of viral genome by RT-PCR in tonsils for more than 1 month after vaccination could indicate potential for virus transmission, while this has not been reported yet in laboratory studies.

In a boar dissemination study performed in 2014, chimera-specific RT-PCR positive EDTA blood samples were reported in 3 of 7 pigs 4 days but not 7 days after vaccination. Additionally lymphatic tissues like lymph nodes, tonsil and spleen tested RT-PCR positive in animals 4 and 7 days after vaccination. Occasionally, pigs also tested RT-PCR positive in salivary gland, liver, bone marrow and lung. Samples that tested positive for viral RNA were subjected to virus isolation. Three samples were found positive by virus isolation: Tonsil and *Lymphonodus parotideus* of animal OM 2, and *L. parotideus* of animal OM 4. Both animals were sampled 4 days post-vaccination and only one duplicate well per organ was found positive.

A study to assess the dissemination risk of CP7_E2alf in semen, faeces and urine was conducted. The results of the study reported that reproductive organs and associated glands do not harbour detectable amounts of CP7_E2alf following vaccination and that therefore there is limited risk that the vaccine strain is transmitted by semen of breeding boars under field conditions. Vaccine virus was not detectable in faeces and urine. Presence of the vaccine virus in milk has not been investigated.

In conclusion, dissemination studies in piglets have been carried out. Due to the warning against use in sows (section 4.4 of the SPC: “Challenge studies have shown lack of protection against transplacental transmission of CSFV. Therefore sows should not be vaccinated, due to the risk of birth of immunotolerant persistently infected offspring. Persistently infected immunotolerant piglets represent a very high risk since they are shedding field virus and they cannot be identified serologically due to their seronegative status.” and the warning that vaccine is only to be used in emergency vaccination in outbreak situations in restricted control zones, the investigations and results are acceptable for the intended use.

**Increase in virulence**

In the study, tests for increase of virulence have been performed using less than worst-case scenarios compared to those stated in the Ph. Eur. monograph 0065 on swine fever vaccine (prepared in cell cultures), classical. Experimental documentation for the safety profile of the chimera should have been provided in accordance with a strict principle of worst case scenarios. This should as minimum in principal include the youngest age group indicated or animals even younger and passage of material from tonsils/draining lymph nodes, by use of the recommended IM administration, and carefully chosen time points which most likely coincide with expected maximum levels of virus in tonsils/lymph nodes.

Second passage of virus in vivo was not accomplished, notably using the chosen less than worst-case scenario. Furthermore, the Ph. Eur. monograph (and VICH GL41 on target animal safety: examination of live veterinary vaccines in target animals for absence of reversion to virulence (EMEA/CVMP/VICH/1052/2004))

actually specifies that if no vaccine virus is found after first passage, the test should be repeated again with a second series of passages; and also at this point compliance has not been demonstrated.

Therefore, the studies are non-compliant, and a less than worst-case scenario has been chosen. This is as such not acceptable. Taking into consideration, that the vaccine virus has not been attenuated during numerous series of passages e.g. in cell cultures with the possibility for accumulation of essential changes at several sites of the genome, passage studies in vivo could have provided some first line of evidence regarding in vivo genome variability, if they had been carried out appropriately. Also, the general text monograph (Ph. Eur. chapter 5, section 2.6.c) states special requirements for live vaccines including recombinant ones to be carried out, and these laboratory tests include increase in virulence tests. On the other hand, it is agreed that the test for monitoring of "Increase in virulence" designed for classically attenuated live vaccines (section 2-3-4 in Ph. Eur. monograph 0065 on classical swine fever vaccine (live, prepared in cell cultures)) is probably not very useful for obtaining new safety data for this chimera concerning first line of evidence of extent of genetic and phenotypic variability.

The applicant was asked to carry out the relevant studies adapted to this chimera to provide the necessary experimental first line of documentation concerning extent of genetic and phenotypic variability including potentials for recombination events with other pestiviruses [as stipulated in Ph. Eur. monograph 5.2.6 on special requirements for live vaccines. Laboratory tests. Recombination or genomic reassortment of strain]. The studies should reflect scenarios and probability of recombination events with the chimeric virus and wild-type viruses (such as CSFV, BVDV).

In conclusion, only very limited and solely in vitro studies were eventually presented. This has been accepted, taking into consideration the limitation of the use of the chimera solely in outbreak situations in domestic herds within restricted control zones and further limitations related to warnings in the SPC (see sections on Biological properties of the vaccine strain, Recombination of the vaccine strain below and recommendation for post-authorisation study).

**Biological properties of the vaccine strain**

Data were presented that the chimera is relatively stably adapted to cell culture and exhibits mutation rates in these cell cultures comparable to those of the other pestivirus strains examined in these cell-cultures. For genetically heterogenic RNA viruses belonging to the pestiviruses genus the mutational events occurring in their genomes have been found to include point mutations (quasi species), recombinations, insertions of cellular sequences, duplications, deletions and rearrangements.

It was highlighted in the dossier that type 1-IFN production is recognised as one of the major factors for control of early cp BVDV replication in the host. Overall, it has not been shown to which extent immuno-compromising factors with disturbance of innate antiviral host responses may result in increased replication of the chimera in vivo.

In conclusion, the significance of these in vitro studies for in vivo scenarios, where other cell types and complex virus-host interactions at different levels are relevant, are not evident.

Therefore a recommendation for post-authorisation surveillance is considered necessary.

**Recombination or genomic reassortment of vaccine and field strains**

The CP7_E2alf has been constructed by use of a novel approach commonly termed "synthetically provoked recombination event" between the two different pestiviruses belonging to separate virus entities, i.e. BVDV and CSFV. Notably, two or more genome deletions involving replication competence/efficiency have not been included in the construction of this chimera.

The chimeric nature of CP7_E2alf virus poses new questions, compared to knowledge already accumulated from naturally occurring pestiviruses, such as risks of development of viable mutant viruses including recombinations between the chimera and other pestivirus strains (CSFV, BVDV a.o.) with gain of new constellations of biological properties of importance for further spread (e.g. related to increased replication efficiency, ncp biotype, broadening tissue tropism, and host-range).

Worst-case scenarios of individuals harbouring CSFV and BVDV-CSFV chimera concurrently, is a scenario which should be taken into consideration regarding safety of the vaccine. It has been shown that the properties of the chimera also at the levels of tissue and target cell tropisms share essential overlap with those of other pestiviruses.

Recent scientific literature highlights that recombinations, insertion/deletion events in vivo allow another level of genome plasticity of pestiviruses (BVDV), in addition to the well-known polymerase-induced single nucleotide variations. Observed duplication events have been suggested to be related to in vivo properties such as virulence (Jenckel et al., J. Virol., June 2014, 88(12), 6983–6992).

Argumentation in the dossier provided that limited experimental studies in pigs are not a manageable experimental in vivo scenario for detection of recombinations. New essential properties developed with importance for further spread of chimera have not been addressed in in vivo experiments.

However, it can be stated that the risk of genetic recombination related to this vaccine is considered low based on the typical relatively limited replication of the vaccine virus in the host, and also the assumed limited circulation of wild-type viruses among the vaccinated animals. In addition, a strong interference phenomenon appears to hamper infection of hosts already infected with a pestivirus to become infected with another pestivirus concurrently. Importantly, given (i) the indication for use of this vaccine in healthy CSFV naïve domestic herds (i.e. excluding back-yard holdings or wild boar populations), and (ii) vaccination is implemented solely for emergency vaccination in controlled zones related to outbreaks; the risk appears very low for generation of viable mutant viruses including recombinations between the chimera and wild-type CSFVs and other pestiviruses.

In conclusion, the line of argument against carrying out laboratory in vivo studies addressing the extent of genome variability by use of worst-case scenarios has been met by precautionary approaches in the form of additional warnings and contraindications in the SPC.

The agreed post-authorisation recommendation to investigate the genetic stability of the vaccine in the control zones during use of the vaccine in an outbreak situation could provide further information. The importance of full-genome deep sequencing in combination with manual in-depth data analysis for proper post-marketing investigations is recommended to be included in the applicants post-marketing surveillance and diagnostics. Also, the methods applied are expected to be adapted and updated in a timely manner to detect all levels of genome plasticity related to use of the chimera.

## GMO

Aspects related to the extent of genetic variability of the chimeric construct in vivo, are of critical importance for assessment of the environmental risk. The mutational events occurring in pestivirus genomes have been found to include point mutations (quasi species), recombinations, insertions of cellular sequences, duplications, deletions and rearrangements. Data and scientific publications have been gathered to address the issue whether this vaccine represents increased risk compared to those of already circulating pestiviruses, under field conditions. Safety aspects that make persistence in the population highly unlikely are:

- The backbone CP7 is highly attenuated in pigs (Reimann et al., 2004).

- The chimera has not shown replication in other species than swine (tested in calves, young goats, lambs and rabbits) König et al., 2011. After oral inoculation of a dose of $2x10^7$ $TCID_{50}$ of the chimera, ruminants do not seroconvert (König et al., 2011).
- There was no evidence provided that the vaccine virus could spread to in contact piglets after IM vaccination.
- The cytopathogenic biotype is proposed to lead to self-limitating infections (Peterhans et al., 2011), which everything equal is suggested to counteract development of persistent vaccine virus replication in vaccinated animals.
- The use of the vaccine is restricted to outbreak situation in production herds within restricted control zones. The SPC includes a warning against the use of the vaccine in sows.

In conclusion, important safety aspects related to the use of this chimera make the risk related to persistent circulation of the vaccine virus itself in animal host populations is highly unlikely.

### *Study of residues*

Residue studies are not required. The active ingredient being a substance of biological origin intended to produce active immunity does not fall within the scope of Regulation (EC) No. 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin. In addition the other components of the vaccine are either listed in table 1 of the annex of Commission Regulation No. 37/2010 or considered as not falling within the scope of Regulation (EC) No. 470/2009 when used as in this product.

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The excipients listed are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this veterinary medicinal product.

The withdrawal period is set at zero days.

### *Interactions*

No studies have been performed using other vaccines at the same time as Suvaxyn CSF Marker. The appropriate wording is included in the SPC.

### *Field-like studies*

A small scale field study was carried out under controlled quarantine conditions. In a total of 30 pigs administered either CP7_E2alf (20 piglets) or saline (10 piglets). No injections site reactions, clinical signs of disease or adverse events related to administration were observed (potency of the used batch was $10^{6.0}$ $TCID_{50}$/IM dose). The vaccine dose used was close to the maximum potency of one dose. Transient increases in rectal temperature were observed in both treatment groups, with increased temperatures lasting for up to two days in the vaccinated group.

By only 14 days post-vaccination 19 out of 20 animals developed serum neutralising antibodies against CSFV and also E2 specific antibodies (commercial enzyme-linked immunosorbent assay (ELISA) test kit). Vaccination did not induce any CSFV $E^{rns}$ antibodies (commercial ELISA test kit). Both serological tests were qualitative (positive/negative) measures of antibodies to CSFV. The presence of CSFV-antibodies as such is not sufficient to prove efficacy, as long as antibody levels correlating to a certain degree of protection has not been a focus of solid attention for this chimera.

In conclusion, as CSF is a notifiable disease the regulations prevent conventional field studies to be undertaken in the EU. A small scale trial was performed under quarantined conditions in 8 week old piglets, relatively close to the youngest age group indicated for vaccination. The results demonstrated that the vaccine did not provoke adverse reactions in this age group, which support the safety profile found in the other laboratory studies carried out.

## *User safety*

BVDV and CSFV are not considered zoonotic. There is a negligible risk that the virus can infect humans. Excipients do not present a risk to the user.

The user safety for this product is acceptable when used as recommended in the SPC.

## *Environmental risk assessment*

The chimeric CP7_E2alf is a single stranded positive sense RNA virus. It appears to be the first live replication competent chimeric RNA virus which could eventually be used for domestic animals in EU in restricted control zones as emergency vaccination in an outbreak situation.

Only, a single focus of genome switch (E2) between BVDV and CSFV has been made to construct the chimeric CP7_E2alf. Introduction of deletions in different parts of the genome has not been made for further limitation of the risk that reversion and transmissibility occur.

The extent of genetic variability of the chimeric construct in vivo is of critical importance for the environmental risk assessment.

Properties of the carrier virus, the strain CP7 of BVDV giving rise to the live vaccine, Suvaxyn CSF Marker, derives from a backbone cytopathic biotype of BVDV. In general RNA viruses have greater genetic variability, since RNA viruses do not possess proof-reading mechanisms to repair errors occurring during genome replication. Moreover, like a number of genetically heterogenic RNA viruses, BVDV is best considered, not as a single entity, but rather as a heterogeneous group of related viruses that differ in their antigenicity, cytopathogenicity, and virulence.

The mutational events occurring in pestivirus genomes have been found to include recombinations, insertions of cellular sequences, duplications, deletions and rearrangements. The studies provided indicate that the GMO resides very little potential to spread from healthy vaccinated piglets under laboratory conditions.

The different pestivirus species (CSFV, BVDV, and BDV) infect cells of ruminant or porcine origin, and the highest susceptibility and the most efficient replication are observed in cells derived from the homologous animal species.

Provided that the vaccine is only used in emergency situations in restricted controlled zones in domesticated healthy pigs in outbreak scenarios the risk e.g. of recombination and increase in virulence or gain of new altered virulence traits could be regarded as minimal.

One scenario envisioned to be likely encountered during emergency vaccination situation is that dissemination taking place in individuals under field conditions is different from that found in laboratory studies.

In some individuals, e.g. immunocompromised by other viral infections, dissemination in the body and shedding could be of other dimensions that those found in laboratory studies in healthy piglets. In vaccinated pigs at the same time incubating a systemic infection with a CSFV field strain known to exert immunosuppressive effects in the host and immunotolerance in foetal infections, the probability of

recombination events between field strain and vaccine strain poses an increased risk (Ph. Eur. monograph 5.2.6) for generation of viruses with new biological properties.

Investigations of the vaccine in animals that might be immunosuppressed is a requirement in the CSF chapter 2.8.3 guidance for modified live vaccines (MLV) vaccines section ii) safety (OIE Terrestrial Manual 2008, Chapter. 2.8.3 on classical swine fever).

DIVA principals related to this chimera represents an essential means of control with regard to elimination of herds where wild type viruses are introduced in vaccinated herds. The marker potential of the vaccine constitute a principal positive point allowing for the use of discriminatory tests although serological DIVA results presented do not allow a clear differentiation of infected from vaccinated animals in some cases. Also delayed or inexistent detection of some of the vaccinated and subsequently infected animals appear to be a problem. It also remains to be documented how genetic DIVA principles would work in the field.

In general, the argumentation is reasonable to support the view that the release of the GMO would not present a hazard to humans, other non-target species or the environment, when the use of the chimera are restricted to emergency vaccination campaigns in domestic pig herds in restricted controlled zone, and with contraindication for sows and other warnings included in the SPC.

However, a principal issue will be to assess whether the use of this vaccine presents increased risk compared to e.g. the risks from already circulating pestivirus under field conditions. Data has not been presented from in vivo studies to assess the level of risk for generation of viable mutants including recombinations of the chimera during mass emergency vaccination campaigns.

Indications for the vaccine are narrowed down to essentially domesticated pigs for slaughter and living in captivity.

Vaccination of sows is contraindicated, since challenge studies have shown lack of protection against transplacental transmission of CSFV and a risk of birth of immunotolerant and persistently infected offspring. Persistently infected immunotolerant piglets represent a very high risk since they are shedding the virulent virus and in principle they cannot be identified serologically due to their seronegative status.

Further risks related to the chimera due to lack of foetal protection. The risk from use of the chimera in field scenarios involving reproductive sows should be avoided as it is associated with increased risk concerning the generation of viable mutant viruses including recombinations between the chimera and wild-type strains.

In conclusion, the use of the chimera should be narrowed down to emergency vaccination purposes, and regarding emergency vaccination the use of the chimera should be reduced to circumstances where these risks related to the chimeric status of the vaccine are minimal.

### *Assessment of the overall risk to the environment*

As the vaccine strain CP7_E2alf is a genetically modified organism (GMO) a full environmental assessment has been carried out to address this issue and data provided to confirm the extent of genetic and phenotypic stability. Recent scientific literature highlights that recombinations, insertion/deletion events in vivo allow another level of genome plasticity of pestiviruses (BVDV), in addition to the well-known polymerase-induced single nucleotide variations (Jenckel et al., J. Virol., June 2014, 88(12), 6983–6992); observed duplication events have been suggested to be related to in vivo properties such as virulence.

The extent of genetic instability, including properties related to recombination, is an essential safety issue of relevance for the use of this live chimeric RNA virus vaccine. The applicant has addressed this issue.

The indications are that the vaccine virus infections do not exhibit virulence in healthy animals, and the

vaccine virus infection disseminates in the body and can be detected in cells of the lymphoid system including pharyngeal region (tonsil) from where spread is a possibility. Shedding via semen, urine, or faeces, transmission to sentinels, or transplacentally to foetuses or effects on the immune system have not been detected in the laboratory studies carried out. In addition the vaccine strain was considered harmless for humans.

The virus can stay infectious for considerable time in the environment but needs porcine cells for its replication.

Importantly, the applicant argues from use of the vaccine in outbreak situations and in case of emerging mutants that the control that is associated with CSFV in EU would lead to detection and enforcement of legal controls.

Overall the risk to the environment is indicated to be minimal given the vaccine is only used for emergency vaccination of farmed pig herds in controlled zones in outbreak scenarios.

## *Overall conclusions on the safety documentation*

In a safety study in piglets the vaccine did not cause any clinical signs or injection site reactions, and there was little indication for hyperthermia from data on average rectal temperatures per group. There were some pigs with a temperature above 40 °C which is generally considered as a critical point for pigs (although piglet temperatures may be higher e.g. due to handling stress) but these generally appeared as sporadic findings. Histology of the injection site revealed no macroscopic or microscopic lesions at 14 or 28 days after vaccination with a single dose.

The overdose study does not comply with all the requirements set out in the OIE manual for CSF live vaccines as no monitoring of peripheral blood leukocyte counts has been made. Supportive studies show that vaccination did not have significant detectable impact on peripheral blood leukocyte counts.

Safety was tested in sows in two laboratory studies. Sows were seronegative against both CSFV and BVDV. Tenfold overdose given by the intramuscular route did not provoke adverse reactions. In trial 2 one sow showed transient hyperthermia 4 hours post-vaccination (+ 2.91 °C for one day) which was probably related to the vaccination, and a few sows had small and transient palpable injection site reactions. Trial 1 (10x overdose) with 13 sows at 51–72 days of pregnancy, which covers the gestation phase stated in the Ph. Eur. monograph 0065, appeared without identification of overt safety concerns. It is noted that offspring were delivered by caesarean section due to unspecified delivery problems which occurred in the preceding trial on reproductive performance.

For investigation of potential impact of the chimera on reproductive parameters large scale field studies would be needed.

Further safety data from field use cannot be gathered as sows are not supposed to be vaccinated, therefore the safety cannot be assessed in sufficient amount of sows to allow for use of statistical analyses of results to investigate for potential reproductive safety issues.

A warning against use in sows is present in the SPC. Challenge studies have shown lack of protection against transplacental transmission of CSFV leading to the risk for birth of immunotolerant persistently infected offspring. Persistently infected immunotolerant piglets represent a very high risk for lack of success in eradication of CSFV, since they are shedding the virulent virus and they cannot be identified serologically due to their seronegative status. In addition, potential risk of concurrent chimera and wild-type pestivirus infections in foetuses and newborns in turn leading to increased risk of recombinations is further minimized by avoiding the use of the vaccine in sows.

Adverse effects on immunological functions have been addressed by the applicant in supportive studies.

Adverse effects with impact on blood leukocyte counts and relative amounts of lymphocyte subsets in peripheral blood have not been observed after use of the vaccine. The possible impact of the chimeric virus on immunocompetence of foetuses has not been presented in the safety trials. This is acceptable as the vaccine is only to be used in emergency vaccination in outbreak situations within restricted control zones, and vaccination of sows is contraindicated.

Specific studies required for live vaccines were conducted. Check for non-transmissibility was carried out in six week old piglets according to the Ph. Eur., except for the disease problems and medications in the animals during the trial period. There was no evidence provided in highly limited studies that the vaccine virus could spread to in contact piglets after intramuscular or oral administration. Notably, the latter trial results are primarily based on the lack of detectable antibody development. Transmission of vaccine virus to target and non-target species is possible, and cannot be ruled out.

Evidence has been provided that the vaccine virus disseminates after IM inoculation and was detectable post-mortem in tonsil tissue. Additional studies show that vaccine virus is detected in the draining lymph node and muscle tissue at the injection site on day 4 post-vaccination and dissemination to blood, other lymphoid tissues in the body and the spleen takes place. It is noted that despite wide dissemination of the chimera and virus isolation from tonsil for more than 1 month after vaccination, which could indicate potential for virus transmission, this has not been reported yet in limited laboratory studies. In summary, transmission of vaccine virus to target and non-target species is possible, and cannot be ruled out.

Increase in virulence studies was not carried out following a worst-case scenario. In the preliminary studies carried out consecutive passage of virus was not accomplished.

As CSF is a notifiable disease the regulations prevent conventional field studies to be undertaken. A small scale trial was performed under quarantined conditions in 8 week old piglets, which demonstrated that the vaccine did not provoke adverse reactions in this age group, which support the safety profile found in laboratory studies.

**Concerning extent of genetic instability and recombination**

The chimeric CP7_E2alf is a single stranded positive sense RNA virus. It appears to be the first live replication competent chimeric RNA virus which could eventually be used for domestic animals in the EU in restricted control zones as emergency vaccination in an outbreak situation.

Only, a single focus of genome switch (E2) between BVDV and CSFV has been made to construct the chimeric CP7_E2alf. Specifically, introduction of deletions in different parts of the genome have not been made for further limitation of the risk that reversion and transmissibility could occur.

The extent of genetic instability of the chimeric construct in vivo is of critical importance for the full environmental risk assessment and the interrelated GMO risk assessment, which have been carried out for this vaccine.

Recent scientific literature highlights that recombinations, insertion/deletion events in vivo allow another level of genome plasticity of pestiviruses (BVDV), in addition to the well-known polymerase-induced single nucleotide variations (Jenckel et al., J. Virol., June 2014, 88(12), 6983–6992); observed duplication events have been suggested to be related to in vivo properties such as virulence.

The applicant argues that limited experimental studies in pigs are not a manageable experimental in vivo scenario for detection of recombination events.

The likelihood that new essential properties develop with importance for further spread of chimera has not been addressed in vivo experiments.

Overall, the risk of genetic recombination is considered as low based on the typical relatively limited replication of the vaccine virus in the host, also the assumed limited circulation of wild type viruses among the vaccinated animals in the restricted controlled zones where vaccination takes place. In addition, a strong interference phenomenon appears to hamper concurrent infections of hosts already infected with one type of pestivirus.

Importantly, given (i) the indication for use of this vaccine in healthy CSFV naïve domestic herds (i.e. excluding back-yard holdings or wild boar populations), and (ii) vaccination is implemented solely for emergency vaccination in controlled zones related to outbreaks; the risk appears very low for generation of viable mutant viruses including recombinations between the chimera and wild type CSFV.

In conclusion, additional warnings in the SPC text have been included to meet the applicant's theoretical line of arguments against carrying out laboratory in vivo studies addressing genome instability including recombination events.

In general, the understanding is that the vaccine virus infection does not exhibit virulence in healthy animals, and the vaccine virus infection disseminates in the body and can be detected in cells of the lymphoid system including pharyngeal region (tonsil) from where spread is a possibility. Horizontal transmission has however not been detected in the studies carried out. Indications of transplacental transmission of vaccine virus infection to foetuses, shedding of vaccine virus via semen, urine, or faeces, or detectable effects on the immune system were not substantiated in the studies carried out. In addition, the vaccine strain is considered harmless for humans. The virus can stay infectious for considerable time in the environment but essentially needs porcine cells for its replication.

In conclusion, the risk to the environment is indicated to be minimal when used as recommended exclusively in outbreak situations in farmed pig herds and in restricted control zones.

Furthermore, the recommendation on implementation of surveillance plans in the control zones during use of the vaccine in relation to outbreak situations adds to risk mitigation.

The methods applied to the agreed surveillance post-marketing study to be conducted should be adapted and updated in a timely manner to detect all levels of genome plasticity related to use of the chimera. Data from use of the vaccine in outbreak situations remains to be presented from this study once conducted. Concerning serologic and genetic DIVA (marker properties) this has been shown to work in principle and adds to the positive side of the safety evaluation of the chimera, while tools fit for purpose in outbreak situations for differentiation of exposure to this GMO from wild type CSFV of BVDV infections remain to be investigated.

# Part 4 – Efficacy

## *Introduction*

Classical swine fever (CSF) is a highly contagious viral disease of both wild and domestic pigs. Due to its worldwide relevance for animal health and the pig industry, it is notifiable to the World Organisation for Animal Health (Office International des Epizooties, OIE).

Although considerable progress has occurred in the eradication and prevention of the disease, the threat for an epidemic still exists in EU. Some European wild boar populations are endemically infected.

CSF is caused by systemic infection with classical swine fever virus (CSFV), an enveloped, positive-strand RNA pestivirus, belonging to the Flaviviridae family. CSFV is structurally, molecular biologically and antigenically closely related to the ruminant BVDV and border disease virus (BDV). Three genotypes each

comprising three to four subgenotypes and antigenic differences have been indicated in preliminary results from cross neutralisation tests presented by the applicant.

The current control strategy in EU is a strict stamping out strategy without vaccination, while Community legislation also foresees the possible use of emergency vaccinations, and especially use of vaccines that allow differentiation of infected from solely vaccinated animals (DIVA) (Council Directive 2001/89/EC).

Two types of vaccines have previously been available for emergency vaccination of CSF: MLV and E2 subunit vaccine (E2subV). While MLV type vaccine is highly efficacious, E2subV is somewhat less efficacious but has the advantage of DIVA properties, i.e. properties to differentiate infected from vaccinated animals. The risk from meat of vaccinated and infected animals depends on the type of vaccine used, the field virus strain and the time between vaccination and field infection. Early infections bear a higher risk of viraemia, especially for E2subV vaccinated pigs (Scientific Opinion / Statement / Guidance of the EFSA Panel on Animal Health and Welfare on a request from Commission on "Control and eradication of Classic Swine Fever in wild boar", The EFSA Journal (2009) 932, 1-18).

At the individual animal level the DIVA principle by use of discriminatory serology related to classical swine fever (CSF) vaccines and field infections have not yet been documented to be fully robust (OIE Terrestrial Manual 2008, Chapter. 2.8.3 and references therein).

Suvaxyn CSF Marker is a genetically constructed attenuated live vaccine candidate for intramuscular administration to pigs to induce immunity against classical swine fever virus (CSFV).

The vaccine strain, CP7_E2alf is a chimeric pestivirus which has been constructed using CP7, an infectious cDNA clone of BVDV. After deletion of the envelope protein E2-encoding region, the respective sequence of CSFV strain Alfort 187 (genotype 1) was inserted (Fig. 1 below).

In principle, as only the E2 envelope gene section is from a CSFV strain, pigs vaccinated with Suvaxyn CSF Marker will be antibody positive for only the E2 protein of CSF. If they subsequently are exposed to infection, then their immune system is confronted with other CSFV antigens, including the CSFV $E^{rns}$ protein. Assays (e.g. ELISAs) to detect antibodies against CSFV $E^{rns}$ and E2 therefore candidate to be used to distinguish pigs that have been exposed to CSFV infection (unvaccinated or previously vaccinated with Suvaxyn CSF) from those, that have only raised an immune response following vaccination with Suvaxyn CSF marker vaccine.

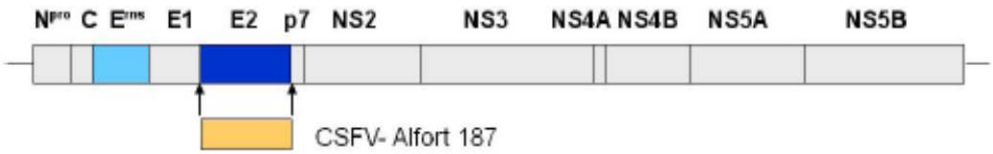


Figure 1. Schematic presentation of CP7_E2alf construct

Potential benefits of such chimeric pestivirus construct should be the opportunity to distinguish field infected herds (vaccinated or non-vaccinated) from only Suvaxyn CSF Marker vaccinated herds combined with a potential efficacy profile following replication in cells of the vaccine with broad range of immune stimulation of the host.

Notably, the antigenic relationships and especially differences between CSFV and BVDV play a role in the field for the possibilities of distinction between the immune responses raised against CSFV and BVDV epitopes (of the major envelope glycoprotein (E2) and the minor envelope protein with RNAse activity ($E^{rns}$).

Efficacy requirements are stipulated in Directive 2001/82/EC and the Ph. Eur. monograph 5.2.7. There is a specific Ph. Eur. monograph 0065 for attenuated live CSF vaccines, and also specific minimum OIE demands have been formulated in OIE Terrestrial Manual 2008, Chapter 2.8.3 on classical swine fever.

## *Laboratory trials*

### Establishment of a challenge model

The natural main entry of CSFV occurs via the oronasal pharyngeal route and primary replication starts in lymphocytes and macrophages in pharyngeal lymphoid tissues with the induction of fever. In fully susceptible swine the virus infection spreads via the blood throughout the body to all tissues, with predilection for replication in the lymphoid system, vascular endothelia and the epithelia of the gastrointestinal tract. Also spread to the central nervous system and passage across the placenta to foetuses are typical pathogenetic events as well as the typical pathological and clinical manifestations accompanying a virulent infection. Briefly, the CSF disease can manifest as acute haemorrhagic fever with respiratory, gastrointestinal and neurological symptoms resulting in high mortality rates, as persistent infections with subacute to chronic disease symptoms, or even remain mostly unapparent. Factors like the age and general health status of the animals, and virulence factors of the virus isolate determines the virulence of the infection and disease severity. Shedding occurs via excretions and secretions of saliva, urine and faeces, notably including excretions from congenitally persistently infected immunotolerant offspring.

Laboratory challenge trials were carried out to establish the efficacy of the vaccines using an oronasal challenge model. The applicant has stated that methods for serological and virological tests used in the clinical studies and described in the study reports were either based on commercial test kits, the OIE Terrestrial Manual 2008 or the Technical Annex accompanying the EU Diagnostic Manual (Commission Decision 2002/106/EC). Also all laboratories which had performed these tests were accredited according to ISO/IEC 17025, and appropriate and representative validation reports have been provided.

### Challenge strain

The challenge strain Koslov was used in all pivotal vaccination-challenge studies. The strain appeared highly virulent in the performed efficacy challenge studies. The strain is closely related to the Alfort strain behind the CSFV E2 component, and heterologous to the chimeric vaccine strain.

It induced severe clinical disease in piglets with central nervous signs and animals with severe clinical signs were preferably euthanised within seven to ten days after infection.

One issue to note is the choice of performing challenge studies only with the highly virulent strain Koslov in sows. Here the strain Koslov also led to acutely virulent infections, which did not appear suitable for carrying out tests for persisting infections of sows for a 5–6 week period after challenge and the birth of infected offspring as laid out in section 2-3-5-2 Ph. Eur. monograph 0065 on classical swine fever vaccine (live, prepared in cell cultures).

This is a dilemma also in respect to recommendations according to the OIE Terrestrial Manual 2008, Chapter 2.8.3 on classical swine fever, and also in respect to strain relevance to the CSFV situation in EU for the last decades.

In conclusion, a well-chosen oronasal challenge route reflecting the natural transmission pathways has been used. Concerning acute virulence the oronasal challenge dose was proven efficient in young piglets in compliance with the Ph. Eur. monograph 0065 section 2-3-5-1.

## Determination of the vaccine dose

### Preliminary immunogenicity studies

The preliminary controlled “proof of concept” trial (Study 1) supported that a single dose of CP7_E2alf vaccine, administered by the intramuscular route, can induce protection against clinical disease and prevent mortality after a virulent CSFV strain Koslov challenge.

The study (Preliminary Immunogenicity Studies, Study 2) was performed in piglets to evaluate the dose response of efficacy of a pilot batch containing vaccine strain CP7_E2alf against a challenge with CSFV strain Koslov. The pilot batch of the vaccine was used undiluted ($10^{5.0}$ $TCID_{50}$/dose), and in two dilutions 1:40 ($10^{3.5}$ $TCID_{50}$/dose) and 1:160 ($10^{2.6}$ $TCID_{50}$/dose). In brief, vaccination IM in piglets 6–8 weeks old indicated efficacy against an oronasal virulent CSFV challenge even at the lowest dose tested (titre of $10^{2.6}$ $TCID_{50}$).

In conclusion, a titration of the vaccine dose was carried out to determine $PD_{50}$ (the dose of antiserum or vaccine that protects 50% of the animals challenged) and define the minimum dose. A minimum dose of not less than 100 $PD_{50}$ was established, which is in overall compliance with section 2-3-5-1, Ph. Eur. monograph 0065 on classical swine fever vaccine (live, prepared in cell cultures).

### Onset of immunity – piglets

The study carried out at the National Food Chain Safety Office in Budapest, was controlled, while not specifically documented in compliance with good laboratory practice (GLP) and included at total of 40 piglets, 7–10 weeks old, which all tested negative for antibodies against BVDV and BDV. Animals were tested in commercial CSFV specific E2 antibody ELISA and in serum neutralisation test (NT) against CSFV. The study was negatively controlled, randomised by body weight and pen, and blinded from the time of challenge when co-mingling was done. Three groups of pigs were included (10 unvaccinated controls; 15 piglets administered 1 ml intramuscularly; 15 piglets orally administered 1.6 ml). A pilot batch (MSV+5 passages, manufactured according to the dossier and to GMP) was used with the potency $10^{4.5}$ $TCID_{50}$/IM dose and $10^{5.6}$ $TCID_{50}$/PO dose. Only IM vaccination is relevant for the applied indication, and only results from IM use will be dealt with below. Fourteen days post-vaccination all pigs were challenged oronasally (0.5 ml in each nostril and 1 ml in the mouth) with 2 ml CSFV strain Koslov (containing $10^{6.1}$ $TCID_{50}$/ml). Follow-up included clinical observations, measurement of rectal temperatures, virus isolation from blood before vaccination until 3 weeks post-challenge (in most animals on days 4, 7, 10 14 and 21 days post-challenge, post-mortem investigations of organ samples (histopathological and immunohistochemical), and serology. Results from nasal swaps collected on days 4, 7, 10, 14, and 21 have not been presented.

Vaccination did not provoke adverse reactions with the exception of an increase in temperature 4 days post-vaccination (40.5 °C) in vaccinated animals, while individual measurements from control animals are missing in this period.

Results showed that IM vaccination with a single dose of CP7_E2alf in pigs conferred protection against challenge in compliance with the requirements of the Ph. Eur. monograph 0065 on classical swine fever vaccine (live, prepared in cell cultures). The challenge with CSFV induced infection, clinical signs and mortality in 10 out of 10 non-vaccinated control pigs within 21 days after challenge. Challenge appeared severe. Virus was isolated in cell culture from whole blood samples of 8 (80%) out of 10 controls and 1 out of 13 animals (missing tonsils from two vac animals) tested (8%) of 13 IM vaccinated animals (only on day 4 post-challenge) tested positive post-challenge. By antigen ELISA, all serum samples of control animals tested positive from 4 days after challenge, while four vaccinated animals (27%) tested positive 4 days post-challenge. Taken together, in 5 out of 15 animals (38%) viraemia was detected in intramuscularly vaccinated animals.

Typical post-mortem histological manifestations of virulent CSFV infection were reported in control animals involving multiple organs and tissues including kidney, skin, lymphoid system (tonsil, lymph nodes and spleen), respiratory, gastrointestinal, and central nervous system. By immunohistochemistry on organ samples, CSFV antigen was found widespread in control animals, exclusively. Piglets vaccinated by the IM route developed antibodies against CSFV E2 (almost all by 14 days post-vaccination), had few clinical signs and were protected against mortality. Virus isolation or real-time quantitative polymerase chain reaction (RT-qPCR) on post-mortem samples were not done.

In conclusion, the piglets were representative for the most sensitive class of piglets indicated for vaccination. The IM vaccine dose was $10^{4.5}$ $TCID_{50}$, which is close to the minimum potency for this vaccine stated in the SPC (potency $10^{4.8}$ to $10^{6.5}$ $TCID_{50}$). This study is pivotal for documentation of onset of immunity 14 days after a single vaccination with a 3 weeks observation period (prevention of mortality and reduction in infection and disease severity) in piglets. The minimum age supported in the presented data is 7 weeks.

Limited studies in young pigs support protection against CSFV of genotype 2 (strains CSF1045, and CSF1047 from 2009) originating from Germany and Israel, respectively (Blome et al., Vet. Microbiol., 169, pages 8-17, 2014).

Serology and virus antigen detection were made using commercial kits (ELISA by HerdChek CSFV Ab test kit (IDEXX) and antigen ELISA by HerdChek CSFV Ag/Serum test kit (IDEXX). Both these tests are qualitative rather than quantitative. Serum samples were not tested further to investigate the antibody responses post-vaccination and challenge with regard to the marker properties of the vaccine and the DIVA principle.

### The influence of maternal antibody on the efficacy of the vaccine – piglets

A study was conducted while not documented in compliance with GLP (except for the GMP produced vaccine) to include a total of 40 piglets, 6–7 weeks old. The study was negatively controlled, randomised by body weight and pen, and blinded from the time of challenge. Six sows were vaccinated 4 weeks before farrowing with CSFV strain “Thiveral” (Lot number 1612S1D1, Ceva-Phylaxia Rt.). Piglets from these sows were included in the study on maternally derived antibodies (MDA). Antibody titres in two weeks old piglets has not been presented (the randomisation was done according to these results). Three groups of pigs were included (10 unvaccinated controls; 15 piglets administered 1 ml intramuscularly; 15 piglets orally administered 1.6 ml). The vaccine was not efficacious when given by the oral route, and as this route is not indicated these results are not dealt with further. The pilot batch used (VMRD-12-005 MSV+5 passages; potency: $10^{4.8}$ $TCID_{50}$/IM dose, was close to the minimum titre and did not provoke detectable unwanted side-effects in the animals.

Fourteen days post-vaccination all pigs were challenged oronasally (0.5 ml in each nostril and 1 ml in the mouth) with 2 ml CSFV strain Koslov (containing $10^{5.7}$ $TCID_{50}$/ml). Follow-up included clinical observations, measurement of rectal temperatures, RT-qPCR testings, serology and post-mortem investigations of organ samples. Nasal swaps were collected, but not investigated.

The challenge with the CSFV strain Koslov was severe, causing clinical signs and death of 70% of the control animals. There is no requirement for the challenge to meet the Ph. Eur. monograph 0065 specifications in seropositive animals. It is possible that MDA did result in some protection against the challenge as the three animals with the highest MDA titres were mostly protected from the challenge, and these animals also developed E2 antibodies following challenge.

Piglets vaccinated by the intramuscular route developed VN antibodies (almost all by 14 days after vaccination) and E2 antibodies by 7 days after challenge. Challenge appeared to retain liveliness and eating of animals and few symptoms are observed until they suddenly succumbed. The vaccinated animals had a reduction in clinical signs compared to unvaccinated controls, and mortality was not observed. Notably, an unusual finding was that the organ samples were for the majority tested positive by immunohistochemical analysis to an overall similar extent in both controls and vaccinated animals. Also the blood samples from vaccinated animals (day 4 post-challenge) tested positive (12 out 13 tested) in RT-qPCR. Significant differences between controls and vaccinated animals with regard to the RT-qPCR results have not been documented. Reduction of antigen detection (commercial ELISA) +/– in blood was statistical significant (significance at P 0.05 level) when comparing control group to the group vaccinated intramuscularly on days 4, 7, 10, 14, and 21. Serum samples were not tested further to investigate the antibody responses post-vaccination and challenge with regard to the DIVA principles of the vaccine.

Whether the MDA interfered with vaccination cannot be directly evaluated as a comparison of the results in a design including groups with and without MDA+/– has not been designed.

The results on reduction of infection strongly suggests that MDAs do interfere significantly with vaccination when comparing indirectly to the presented results in onset of immunity study in MDA negative piglets, where CSFV antigen was found to be widespread in organs by immunohistochemistry in control animals, exclusively. In contrast, considerable systemic dissemination of virus antigen was found throughout the body in control animals as well as in vaccinated piglets with MDA. The results indicate widespread replication in the body (including tonsils, spleen, mesenterial lymph node, ileum, kidney and lung and brain as judged by the comparable and large amounts of virus antigen observed by immunohistochemistry in tissue samples collected 3 weeks after challenge. Notably the issue on reduction of infection after vaccination is not apparent from the post-mortem investigations. The results could indicate that low level MDA+ vaccinated pigs risk to develop subclinical subacute/chronic infections.

Therefore the SPC includes information that the vaccine has shown reduced protection in studies of piglets with maternally derived antibodies compared to other studies of vaccinated piglets without maternally derived antibodies. Decision to vaccinate piglets with maternally derived antibodies should be taken based on the outbreak situation and the associated control zones.

### Duration of immunity – piglets

The study was conducted according to GLP standards and included a total of 40 piglets, 7–8 weeks old and without antibodies to pestivirus. The study was negatively controlled, randomised by body weight and pen, and blinded. Three groups of pigs were included (10 unvaccinated controls; 15 piglets administered 1 ml intramuscularly; 15 piglets orally administered 1.6 ml). Information from the orally vaccinated piglets is not dealt with further as they are not relevant to the indications. A pilot batch (191010 MSV+5 passages; potency: $10^{4.5}$ $TCID_{50}$/IM dose) of minimum titre was used. Prior to vaccination the piglets were tested CSFV seronegative and free from any CSFV infection (blood were subjected to virus isolation attempts, RT-qPCR, and antigen ELISA) and were thus representative for the most sensitive piglets indicated. The vaccine did not induce temperature rises which appeared correlated to vaccination. Following vaccination blood was tested for CSFV antibodies by ELISA and virus neutralisation test (VNT) at monthly intervals.

Six months post-vaccination all pigs were challenged oronasally with 2 ml CSFV strain Koslov (containing $10^{5.5}$ $TCID_{50}$/ml). Follow-up included clinical observations, measurement of rectal temperatures, testing of blood samples for CSFV RT-qPCR, antigen ELISA, serology and post-mortem investigations of organ samples (RT-qPCR and antigen ELISA). It was necessary to give various antibiotic treatments during the trial to piglets in all groups to treat different conditions (respiratory infections, external ear infection, lameness and abscess) and it was concluded that the treatments did not interfere with the trial.

The challenge with the CSFV strain Koslov was very severe, causing clinical signs in all of the control animals and nine of ten animals had to be euthanised. CSFV was isolated in cell culture from most of the control animals and the presence of CSF antigen was detected in the sera of all controls. Typical manifestations of CSFV infection were seen at post-mortem and live virus was detected in most organ samples 3 weeks post-challenge.

Piglets vaccinated by the intramuscular route developed antibodies against CSFV (both neutralising antibodies and E2 antibodies (Herdcheck CSFV Ab ELISA (Idexx Laboratories) by one month after vaccination, had few clinical signs and were fully protected against mortality. Samples from 14 out of 15 animals were RT-qPCR positive from day four post-challenge. Positive RT-PCR results were obtained for nearly all the post-mortem organ samples from all piglets.

EDTA blood samples were positive in the CSFV specific RT-qPCR showing increasing genome levels (lowest cq values <17 corresponding to $3.5x10^7$ copies per microliter blood) until slaughtering of the animals.

Significantly higher viral genome loads were found in the blood of the unvaccinated animals compared to the IM vaccinated group. Tonsil samples of all vaccinated animals were positive with cq values of between 28 and 36 corresponding to $7.7x10^3$ and $2.5x10^1$ genome copies per microliter, respectively. The large majority of spleen and salivary gland samples were positive in CSFV specific RT-qPCR, while no virus isolation in vitro were successful from blood or tissue samples of IM vaccinated piglets and they stayed negative in the antigen ELISA.

Nine of the 10 control animals did not raise detectable levels of CSFV-specific antibodies in any of the employed serological tests upon challenge infection. In contrast, neutralising antibody titres rose quickly in all intramuscularly vaccinated animals at 10 days post-challenge, and from day 7 to day 22 post-challenge (end of study) CSFV specific E2 ELISA-antibodies (Herdcheck CSFV Ab ELISA (Idexx Laboratories) were detected. Vaccinated animals (14 out of 15 animals tested) were positive for $E^{rns}$ antibodies in the commercial kit PrioCHECK $E^{RNS}$ ELISA (Prionics Lelystad).

Thus the vaccine prevented mortality and reduced severity and duration of clinical symptoms. A reduction of infection in the 22 days observation period expressed as reduction in viral genome copy numbers compared to control animals was demonstrated for days 4 to 22 post-challenge infection.

Attempts to isolate cell-culture infectious virus from blood and from organs post-mortem of vaccinated animals have been carried out all with negative results. This is not the same as non-shedding, and nasal and tonsil shedding was indeed possible, but not solidly investigated during the 21 days post-challenge.

Furthermore, virus isolation is not as sensitive as shedding tested in transmission studies in vaccinated animals after natural infection as outlined in OIE Terrestrial Manual 2008, chapter 2.8.3 on classical swine fever. The OIE formulation on minimum demands for CSF marker vaccines also includes: “The vaccine should provide protection against any natural-contact challenge, i.e. it should prevent clinical signs and re-excretion of the virus. The efficacy of vaccination should be shown experimentally by studies in which transmission of wild-type virus in vaccinated groups of pigs is studied.” Such investigations on shedding and transmission have not been presented solidly.

Serum samples were also tested by the $E^{RNS}$ commercial kit, PrioCHECK $E^{RNS}$ ELISA, and all vaccinated animals were seronegative for $E^{RNS}$ antibodies until after challenge. Nearly all of the vaccinated animals became $E^{RNS}$ positive after challenge. This demonstrates the principle that solely vaccinated animals could at least for the majority of pigs be distinguished from “naturally” infected animals and thus the serological Marker/DIVA principle was supported in all vaccinated animals before challenge.

Whether the DIVA principle works at herd level in the field can only be investigated by emerging assays concerning specificity and sensitivity of the tests on large panels of samples from emergency field situations.

In conclusion, a single dose of the vaccine given intramuscularly prevented mortality and reduced clinical symptoms and virus infection when challenge was given 6 months after vaccination and with an observation period 3 weeks post-challenge as indicated in the Ph. Eur. monograph 0065 (CSFV) section 2-3-5-1. The studies are pivotal for demonstration of duration of immunity by virulent challenge to last at minimum six months after vaccination. Significant higher genome loads, loads of infectious virus by isolation in cell culture, and viral antigen load were determined in blood of unvaccinated animals in comparison to the intramuscularly vaccinated group of animals substantiating reduction in infection after vaccination. It appears that CSFV persists in vaccinated animals well beyond the 3 weeks observation period post-challenge.

### Prevention of transplacental transmission

The objective of study was to evaluate the efficacy of Suvaxyn CSF Marker vaccine against a virulent challenge in sows to evaluate protection against transplacental transmission with infection of foetuses.

Antibodies against CSFV was measured in HerdCheck CSFV Ab ELISA, Idexx Laboratories) and neutralisation test against CSFV.

The study carried out by the Food Chain Safety Office was controlled while not clearly stated as compliant with GLP standards (except for the GMP-produced vaccine batches). Ten sows were included of which two served as unvaccinated controls, six sows were administered 1 ml Suvaxyn CSF Marker vaccine intramuscularly, and two sows were orally administered 1.6 ml vaccine. Two batches were used, Batch 12-005 MSV+5 passages with the potency: $10^{4.8}$ $TCID_{50}$/IM dose (minimum titre) and pilot batch 191211 MSV+5 passages with the potency $10^{5.6}$ $TCID_{50}$/PO dose. Fourteen days post-vaccination (at day 60 of gestation) all sows were challenged oronasally (0.5 ml in each nostril and 1 ml in the mouth) with 2 ml CSFV strain Koslov (containing $10^{5.5}$ $TCID_{50}$/ml).

Follow-up included clinical observations for 60 minutes after administration to observe any systemic reaction (anorexia, anaphylactic reaction, vomiting etc.). Blood (with and without anticoagulant) was sampled at D0, D14 (before challenge); D21, D23 (7 and 9 days post-challenge), and D56 (at euthanasia). Blood samples were analysed by ELISA for CSFV antibodies and CSFV antigen and by neutralisation test for antibodies, and by virus isolation and RT-qPCR for CSFV. Necropsy at D56 (6 weeks after challenge) and collection of tissue samples from tonsils, lymph nodes (submandibular and mesenterial), lung, spleen, liver and ileum as well as from all the foetuses (spleen, kidney, lymph nodes).

Intramuscularly vaccinated sows carried between 2 to 13 foetuses. Control sows carried 9 and 10 foetuses, respectively. Tissue samples which was (formalin fixed) from sows have not yet been investigated. From foetuses pools of the three organ samples (stored at minus 75 °C were analysed by virus isolation and by a serum antigen ELISA, both at Food Chain Safety Office and some were also tested in RT-qPCR at Friedrich Loeffler Institute, from foetuses of one of two control sows, and from foetuses of 2 of 6 vaccinated sows. Serum samples were not tested further to investigate the antibody responses post-vaccination and challenge with regard to the DIVA principle.

The challenge with CSFV strain Koslov was severe, causing clinical signs and death of one of the control animals. The other control sow survived without clinical signs. Live CSF virus was isolated from the foetuses of the apparently unaffected sow so clearly transplacental transfer of the infection did take place. Two sows vaccinated by the intramuscular route seroconverted in HerdCheck CSFV Ab ELISA (Idexx) 14 days post-vaccination, while 2 additional animals showed results in the grey zone between positive and negative samples. There were no clinical signs in the vaccinated sows after CSFV challenge.

The highly virulent infections with the strain Koslov were not suitable with respect to reflecting a strain of moderate virulence to allow for survival of the control sows up till farrowing (for 5–6 weeks after challenge). Viral transmission to foetuses was only investigated in one of two control sows, one sow succumbing at day 6 post-challenge, and tests for transmission to foetuses was not carried out.

The challenge resulted in viraemia including transplacental infection, clinical signs and mortality in non-vaccinated and PO vaccinated sows.

The Ph. Eur. monograph 0065 states: "the vaccine complies with the test if no virus is found in the blood of vaccinated sows and in foetuses from the vaccinated sows, and no antibodies against classical swine fever virus are found in the serum of the foetuses from vaccinated sows". The results presented do not comply with the monograph.

Only offspring of two vaccinated sows were tested, and their offspring was found to be infected by commercial antigen ELISA testing on blood samples and by RT-qPCR. In addition in the majority of foetuses from the two sows investigated transplacental transmission of infection was detected.

Thus, due to lack of testing of foetuses (only foetuses of two of the 6 IM vaccine sows was tested), it can only be concluded that in 5 of 6 vaccinated animals viraemia was detected. As foetal tissue is most likely the most sensitive predilection site for virus replication, the trial indicate that in from 84% to 100% of sows the vaccine afforded protection did not meet the criteria of the Ph. Eur. monograph 0065 and the recommendation of the OIE (OIE Terrestrial Manual 2008, Chapter 2.8.3 on classical swine fever) with regard to solid protection against transmission of virus to offspring.

The results do not show compliance with the protection stipulated in the Ph. Eur. monograph 0065, where the criteria for compliance include that no virus is found in the blood of vaccinated sows and no transmission to foetuses should occur. In the presented study, virus was found in the blood of 4 of 6 IM vaccinated sows on days of testing (days 21 and 23 post-challenge), while in two out of 8 vaccinated sows virus was transmitted to foetuses without detection of virus in the blood of the sows.

No argumentation has been provided to show that transplacental transmission is taking place to a higher degree in highly virulently infected animals compared to challenge with more moderately virulent strains that could induce subacute and chronic infections in the sow which again could lead to the birth of infected litters.

The vaccine was at the minimum titre and prepared using the virus strain at the most attenuated passage. The challenge study does not meet the ideal and stringent requirements of the monograph. However the applicant considers that this monograph is only for conventionally attenuated live vaccines and not completely applicable to this chimeric vaccine construct.

Emphasis is placed on the fact that high level of protection against transplacental infection documented in laboratory studies is finally considered a mandatory demand for CSF vaccines in view of the success and benefit in case of emergency and control and eradication of this devastating animal disease. The birth of persistently infected, immunotolerant piglets that are healthy and survivable but continuously shedding virus should be avoided as these are a main reservoir of CSFV infection.

In conclusion, challenge studies have shown lack of protection against transplacental transmission of CSFV. Persistently viremic, in utero infected pigs seldom produce specific antibodies. These piglets can constantly shed large amounts of virus and are a dangerous virus reservoir, spreading the disease and maintaining the infection within the pig population (Van Oirschot and Terpstra, Vet. Microbiol., 1977, 2, 121-142; Reviews: Moennig, Vet. Microbiol., 2000, 73(2-3), 93-102; Moennig et al. Vet. J., 2003, 165, 11-20). Therefore sows should not be vaccinated, due to the lack of protection against transplacental transmission of field CSFV and the risk of birth of immunotolerant persistently infected offspring. Persistently infected immunotolerant piglets represent a very high risk since they are shedding field virus and they cannot be identified serologically due to their seronegative status.

The applicant notes that in the event of an outbreak situation it is unlikely that pregnant sows would be vaccinated. The applicant further notes, that it may be of value to carry out future experimental tests to aid in the decision of whether to vaccinate or not. It should be kept in mind that infection of foetuses can lead to immunotolerant and persistently infected (PI) animals that shed high amounts of virus lifelong and are essentially considered as virus reservoir.

In addition, the use of the chimera in field scenarios involving such persistently infected individuals should be avoided as it is associated with increased risk concerning the generation of viable mutant viruses including recombinations between the chimera and wild-type strains.

### *Field trials*

Please refer to Part 3.

## *Overall conclusion on efficacy*

The efficacy of a single intramuscular dose of the vaccine has been demonstrated against virulent CSFV challenge in pigs from 7 weeks of age. It was shown that the vaccine induced antibodies against CSFV in most pigs and by only 14 days after vaccination the pigs were fully protected against mortality after intramuscular administration. In addition there were reductions in the level of clinical signs in the challenged animals and in the infection, so the vaccine also reduced infection and disease caused by CSFV.

Use in MDA positive piglets is associated with a warning in the SPC as the vaccine has shown reduced protection in studies of piglets with maternally derived antibodies compared to studies of piglets without maternally derived antibodies.

Studies in vaccinated breeding boars addressing potential shedding of virulent challenge virus in semen have not been conducted. Use of the vaccine in experimental studies in breeding boars has not revealed safety concerns. Therefore the decision to vaccinate breeding boars and piglets with maternally derived antibodies should be taken based on the actual outbreak case and associated control zones.

It was also demonstrated that efficacy of the vaccine lasted for at least six months. No studies on revaccination were provided.

The vaccine was tested by both intramuscular and oral routes and the data shows that efficacy reach higher levels when the intramuscular route is used. The applicant only included indications for the intramuscular route in the SPC. Challenge studies have shown lack of protection against transplacental transmission of CSFV.

Emphasis is placed on the fact that high level of protection against transplacental infection documented in laboratory studies is finally considered a mandatory demand for CSF vaccines in view of the success and benefit in case of emergency and control and eradication of this specific animal disease. The birth of persistently infected, immunotolerant piglets that are healthy and survivable but continuously shedding virus should be avoided as these are a main reservoir of CSFV infection.

In addition, the use of the chimera in field scenarios involving such persistently infected individuals should be avoided as it is associated with increased risk concerning the generation of viable mutant viruses including recombinations between the chimera and wild-type strains. The SPC section 4.4 has been amended with warnings accordingly. Wording of the SPC to convey that the indications have been shown for genotype 1 and only apply partly to recent wild types (genotype 2) has been added to the SPC.

The presence of CSFV-antibodies was shown especially in all intramuscularly administered pigs but as such it is not sufficient to prove efficacy as long as antibody levels correlating to a certain degree of protection has not been a focus of solid attention for this chimera.

The marker properties of the vaccine have also been investigated in some of the studies. The vaccine induces antibodies against the E2 envelope protein which can be detected in a commercially available test kit (PrioCheck CSFV Ab). However, the vaccine does not induce antibodies against the $E^{rns}$ structural protein of CSFV which can be detected in infected animals in a commercially available test kit (PrioCheck CSFV $E^{rns}$). While the serological DIVA principle has been supported in the presented studies, the field use of the DIVA principle for the vaccine has not been fully shown at this stage.

The genetic DIVA concept has been outlined. The benefit of its systematic use in emergency situations in domestic pigs remains to be revealed.

Regarding detection of $E^{rns}$, the PrioCHECK CSFV Erns ELISA (Prionics) was the only one tested for this purpose even though some improvements were needed with regard to its sensitivity, selectivity and especially robustness. The improved version of the test was evaluated in a ring test. It was shown that the

ELISA CSFV infections in vaccinated pigs with a sensitivity ranging from 82-94% (95% CI, sera taken from domestic pigs vaccinated once with CP7_E2alf ≥21 days post-infection). The range implies that the test must be applied on a herd level to reliably detect infection. The given sensitivity is not solely based on test capacity, but also based on a lack of anti-$E^{rns}$ responses in some vaccinated pigs. These animals are not considered to present a problem in terms of CSFV transmission since the lack of $E^{rns}$ antibodies could indicate a lack of field virus replication in these animals. Specificity was 89-96% (95% CI, sera from domestic pigs vaccinated once with CP7_E2alf).

The recombinant vaccine virus has potential marker properties for use in DIVA (differentiation between field virus infected and solely vaccinated animals). Diagnostic tools targeted to detection of antibody responses could enable DIVA strategies. Serological DIVA tools should be able to differentiate between antibody responses after solely herd vaccination with CP7_E2alf from responses after natural field CSFV infection.

DIVA efficiency depends on the performance of tests related to fitness for purpose in outbreak situations. Serological DIVA concept has been shown in principle, while actual DIVA tools remain to be tested on large panels of samples from emergency vaccination in outbreak situations.

Discrimination of vaccine from field virus could enable monitoring of the spread of an outbreak in the face of a concurrent vaccination campaign and supports the decision on whether to continue vaccination (and/or other control methods) or whether to cease control methods because the outbreak is contained. Furthermore, discrimination of serological responses to field or vaccine virus could be used to support lifting trade restrictions on meat and movement restrictions on finished fatteners for slaughter during and following an outbreak.

A post-authorisation recommendation to investigate the systematic use of DIVA concept following a CSFV outbreak with restriction zoning implementation for control and eradication of the infection has been proposed (see below).

Notably, as the chimera has not been used in an outbreak situation and the utility of the vaccine can only be proven if it were used.

## Recommendation for post-authorisation

In addition the applicant is recommended to follow the following proposal for data to be collected in the event of use in an outbreak situation:

***Genetic stability during a vaccination campaign:***

If possible, tonsil material and blood samples will be collected from dead vaccinated pigs (that died within 14 days after vaccination) from different herds and assessed for the presence of chimera by chimera specific RT-PCR and for the presence of field strains by CSVF RT-PCR. Positive samples are used for virus isolation. Isolated viruses are sequenced and the sequences compared with that of the vaccine chimera to investigate the rate of mutation of the chimera and of genetic exchange with field strains.

***Data from field use:***

Blood and organ samples are collected from all pigs with clinical signs suspicious of CSF and belonging to different vaccinated herds within the vaccination zone. Samples are analysed for presence of field and vaccine virus and antibodies against field and vaccine virus. Information on vaccination details, abnormal clinical signs (incl. temperature), mortality including aborted material) due to CSF and welfare destruction rates are gathered for each herd. Sampling time points are prescribed in the EU Diagnostic Manual, Chapter IV, Checking and sampling procedures. Results are used to provide information on spread of the infection within a herd, spread of the outbreak, duration of measures, costs, etc. and can be used to feed future simulation modelling.

***Effectiveness of the DIVA strategy:***

If possible, blood sample are collected from pigs of a number of herds a) with diseased pigs, b) suspected to have been exposed and then vaccinated and c) not suspected to have been exposed, but prophylactically vaccinated early during an outbreak, and analysed by chimera specific RT-PCR and CSFV RT_PCR to demonstrate genetic DIVA concept. Sampling time points are prescribed in the EU Diagnostic Manual, Chapter IV, Checking and sampling procedures.

A prescribed proportion of pigs are included in the samplings, including all pigs with fever or signs suspected to be related to CSFV infection in different herds a) with clinically affected pigs, b) with clinically affected pigs and vaccination regime and c) non-affected pigs and vaccination regime at least 30 days after last affected case or last outbreak in the area (final screening). Samples are by $E^{rns}$ ELISA (e.g. Priocheck) and E2 ELISA (e.g. Priocheck or Herdchek) or the Luminex system (Final Report CSF-Go-DIVA) to demonstrate serological DIVA concept. Sampling time points are prescribed in the EU Diagnostic Manual, Chapter IV, Checking and sampling procedures.

# Part 5 – Benefit-risk assessment

## *Introduction*

Suvaxyn CSF is a live vaccine against CSF based on the genetically engineered pestivirus chimera CP7_E2alf. Its backbone is the cytopathic bovine viral diarrhoea virus strain “CP7” in which the region encoding the main immunogen of pestivirus, the surface glycoprotein E2, has been replaced by the corresponding part of CSF virus (CSFV) strain “Alfort”. The chimeric principle for pestivirus is new, and the first live replication competent chimeric RNA virus which could eventually be used in domestic animals in EU in restricted control zones for emergency vaccination in an outbreak situation.

Classical swine fever is a serious and often fatal highly contagious viral disease of both wild and domestic pigs. Due to its worldwide relevance for animal health and the pig industry, it is notifiable to the World Organisation for Animal Health (Office International des Epizooties, OIE). Classical swine fever is a serious and often fatal disease of pigs. Upon infection, animals suffer from a multi-systemic disease that can present manifestations of disease in almost all organ systems and even go with signs of a viral haemorrhagic fever. In young animals, mortality often reaches 100% and the contagion can be high.

Although considerable progress has been made in the eradication and prevention of the disease, the threat for an epidemic still exists in EU. Some European wild boar populations are endemically infected.

In fully susceptible animals typically generalised acute virulent infections lead to multi-systemic disease manifestations involving most organ systems and signs of viral haemorrhagic fever can occur. In young animals, mortality often approaches 100%.

The vaccine is intended for the active immunisation of pigs from 7 weeks of age, to prevent mortality and reduce infection and disease caused by CSFV. The onset of immunity is 14 days after a single intramuscular administration and the duration of immunity is 6 months after completion of the basic vaccination. No information on revaccination is provided.

## *Benefit assessment*

## Direct therapeutic benefit

Suvaxyn CSF market has the following indication: For active immunisation of pigs from 7 weeks of age onwards to prevent mortality and reduce infection and disease caused by classical swine fever virus (CSFV).

In laboratory studies Suvaxyn CSF Marker was shown to prevent mortality and reduce infection and clinical signs in vaccinates upon infection with CSFV strains. Thus, a direct beneficial effect is seen on the basis of the individual animal that is protected from a devastating disease. In essence it is the early onset "additional benefit" of reduction of infection with virulent CSFV and possibly even before the documented onset of immunity at 14 days post a single vaccination, which is the potential asset of using the chimera in emergency vaccination in face of an outbreak.

## Additional benefits

The benefit of reduction of infection with virulent CSFV is an essential rationale for use in emergency vaccination campaigns.

- Suvaxyn CSF Marker provides protection documented from 14 days post-administration and only a single dose is needed.
- The vaccine is designed with marker potential to differentiate infected from solely vaccinated animals (DIVA). Emergency vaccination programs are usually accompanied by extensive surveillance programs, which could benefit from the marker nature of Suvaxyn CSF. The current control strategy in EU is a strict stamping out strategy without vaccination, while Community legislation also foresees the possible use of emergency vaccinations, and especially use of vaccines that allow differentiation of infected from solely vaccinated animals (DIVA)(Council Directive 2001/89/EC).
- Horizontal transmission of vaccine virus has not been revealed in limited laboratory studies.
- No significant local or systemic adverse reactions after vaccination of piglets (with the possible exception of transient mild fever).
- Replication of vaccine virus in pharynx makes oronasal excretion of the vaccine virus from vaccinated piglets possible, while this has not been detected in the laboratory studies.
- Use of its potential marker properties for use in (DIVA) and its added benefit compared to existing eradication strategies remains to be documented in actual outbreak situations.

Experience with the implementation of emergency control measures during CSF outbreaks in the domestic pig population is accumulating, while the excessive killing of pigs and destruction of animal products occurring during such outbreaks remains a critical and ethical concern. Emergency vaccination to control CSF ('vaccination-to-live' strategy) could significantly improve the situation by reducing mass-destructions, however requires an in-depth evaluation before being considered to be a valid alternative.

## *Risk assessment*

*Main potential risks:*

### *Transplacental transmission*

Challenge studies have shown lack of protection against transplacental transmission of CSFV. Therefore sows should not be vaccinated, due to the risk of generation of immunotolerant persistently infected foetuses. Persistently infected immunotolerant piglets represent a very high risk since they are continuously shedding the virulent virus and constitute a reservoir of CSFV infection, and they cannot be identified serologically due to their seronegative status.

The use of the chimera in sows is also contraindicated as immunotolerant persistently infected foetuses (foetuses are considered the most susceptible target for pestiviruses) and newborn piglets of vaccinated sows should be avoided due to increased risk of generation of viable mutant viruses by recombination events between the chimera and wild-type genomes during concurrent infections.

***Generation of viable mutants including recombinations of the chimera during mass emergency vaccination campaigns***

Taking into consideration the lack of prior knowledge in literature and the fact that no in vivo data has been presented to assess the level of risk for generation of viable mutants including recombinations of the chimera during mass emergency vaccination campaigns, this vaccine is only to be used in an outbreak situation in seronegative herds within restricted control zones, and should not be used in sows.

Safety aspects related to the use of this chimera make the risk related to persistent circulation of the vaccine virus itself in animal host populations highly unlikely. Notably, in vivo experiments designed for the purpose to obtain some documentation and insight on the risk/likelihood, that new essential properties related to the chimera develop by the range of mechanisms known in pestiviruses, have not been presented.

As the vaccine has shown lack of protection against transplacental transmission of challenge virus infection, warning against vaccination of sows (section 4.4 of SPC) is important, due to the risk of birth of immunotolerant and persistently infected piglets. Also safety consideration related to contraindication for sows is of importance for reduction of the potential risk of recombination events to occur during concurrent infections with the chimeric vaccine virus and wild type pestiviruses in foetuses and neonates (including persistently infected immunotolerant individuals) being considered the most sensitive targets for pestiviruses.

Documentation provided for this vaccine supports that it is only to be used in an outbreak situation in herds within restricted control zones.

***For the target animal and non-target animal***

There is a risk of a slight increase in body temperature following vaccination. The vaccine did not cause any clinical signs or injection site reactions.

The risk of modification of the immune system caused by the vaccine has not been confirmed in laboratory studies in piglets.

It is essential that despite wide dissemination of the chimera and virus isolation in cell culture from tonsils for more than a month after vaccination, which could indicate potential for virus transmission, this has not been reported in laboratory studies. In orally vaccinated animals the chimera was detected up to 63 days post-vaccination in one of two individuals tested (Tignon et al. 2010). Viraemia raises the risk of transplacental crossing of the vaccine. Nonetheless, offspring did not seroconvert in the study included in the dossier which is an indirect indication for lack of transplacental transmission of the vaccine virus. Generally, direct tests by use of RT-PCR techniques were not applied to test for transplacental transmission. The cytopathic vaccine alone is not expected to give birth to immunotolerant persistently infected piglets.

Studies on safety in animals are insufficient with respect to assessment on impact on reproduction parameters. Notably, a warning against use in sows is presented in the SPC.

Concerning non-target animals the chimera has not shown replication in species other than swine (tested in calves, young goats, lambs and rabbits as reported in König et al., 2011). After oral inoculation of a dose of $2x10^7$ $TCID_{50}$ of the chimera, seroconversion was not found in ruminants (König et al., 2011). There was no evidence provided that the vaccine virus could spread to in contact piglets after IM vaccination, while the spread of vaccine virus to tonsils provide a possibility spread to other pigs and also non-target species. The potential for spread to target as well as non-target animals is possible and cannot be ruled out when the vaccine is used in the field.

***DIVA principle***

The recombinant vaccine virus has potential marker properties for use in DIVA (differentiation between field virus infected and solely vaccinated animals).

In pigs the vaccine has been shown to induce antibodies against E2 of CSFV.

Diagnostic tools targeted to detection of antibody responses could enable DIVA strategies.

Serological DIVA tools based on detection of CSFV antibodies other than those raised against E2, such as $E^{rns}$ antibody detection, should be able to differentiate between antibody responses against herd vaccination with CP7_E2alf from responses against $E^{rns}$-CSFV after natural field CSFV infection.

DIVA efficiency also depends on the performance of tests related to fitness for purpose in outbreak situations.

Serological DIVA concept has been shown in principle, while actual DIVA tools remain to be tested to work for purpose on large panels of samples from emergency vaccination in outbreak situations.

Some relevant pestivirus RT-PCR strategies could be used in outbreak situations to differentiate between the vaccine virus genome and those of fields strains based on sequences unique to the CP7_E2alf (Leifer et al. J. Virol. Methods, 2009, 158(1-2), 114-122).

Genetic DIVA strategies could turn out to be an advantage from a diagnostic point of view and needs further testing in outbreak situations.

***For the user***

BVDV and CSFV are not considered zoonotic. There is a negligible risk that the virus can infect humans. Excipients do not present a risk to the user. Warnings in the SPC are appropriate.

***For the environment***

Critical basic knowledge on biological properties regarding frequency of recombinations between closely related pestivirus are apparently not known and this is critical for evaluation of the level of risk for the use of the chimera in the relevant emergency vaccination situations.

Data has not been presented to assess the level of risk for generation of viable mutants including recombinations of the chimera during mass emergency vaccination campaigns or in vivo experiments.

Experimentally, the pestivirus has an overlapping host spectrum: classical swine fever virus can be transmitted to cattle; bovine viral diarrhoea virus can infect swine, sheep, goats, some camelids, and a variety of other wild and domestic ungulates, including deer, antelope, and buffalo. In addition to cattle, BVDV infects a variety of other species. With regard to Suvaxyn CSF Marker, the mentioned findings that no serious adverse reactions have been identified in the investigated non-target species are accompanied by a reference to a scientific paper with further information on the involved species (calves, young goats, lambs, and rabbits).

***Unintended spread of vaccine strain***

Dissemination and the possibility of shedding oronasally from vaccinated individuals has been elucidated in laboratory studies in healthy piglets and is limited. No horizontal transmission has been confirmed in limited laboratory studies, while the possibility exists of spread to target and non-target animals, but this was not demonstrated in the limited studies carried out. This should be taken into consideration in an outbreak situation in case farmed ruminants are kept on the premises. Also the spread of vaccine virus transplacentally in sows, if vaccinated despite warnings on the SPC, is possible.

***Increase in virulence***

The biodistribution of the vaccine virus in pigs studied in tissues and organs have been provided. Crossing of the blood-brain barrier might be an indication of potential neurovirulence, which is known for CSFV. For further details see above in the section *Generation of viable mutants including recombinations of the chimera during mass emergency vaccination campaigns.*

***For the consumer***

Residue studies are not required. The withdrawal period is set at zero days.

## *Risk management or mitigation measures*

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user, environment and consumer and to provide advice on how to prevent or reduce these risks.

## *Evaluation of the benefit-risk balance*

The product has been shown to have a positive benefit-risk balance overall.

The vaccine induces an active immunisation with induction of early onset protection with reduction of infection of infection, and against mortality and severe disease as documented in challenge studies with CSFV in piglets. This live vaccine contains a genetically modified organism (GMO).

The fact that no in vivo data has been presented to assess the level of risk for generation of viable mutants including recombinations of the chimera during mass emergency vaccination campaigns, this vaccine is only to be used in an outbreak situation in seronegative farmed herds within restricted control zones.

Addition of a warning against use in sows and a warning “Documentation provided for this vaccine supports that it is only to be used in an outbreak situation in herds within restricted control zones.” renders the benefit risk balance favourable.

The indications are that viruses do not exhibit virulence in healthy animals, and the vaccine virus infection disseminates in the body and can be detected in cells of the lymphoid system including pharyngeal region (tonsil) from where spread is a possibility. Horizontal transmission has however not been detected in the limited studies carried out. Study on transmission of vaccine virus via placenta to foetuses has not revealed transmission in indirect tests for serum antibody response in foetuses prenatally. Shedding of the chimera via semen, urine, and faeces has been investigated and has not been observed. General effect on the immune system (leucopenia) has not been detected in the reports from scientific literature. In addition the vaccine strain is considered of negligible risk for humans. The virus can stay infectious for a considerable time in the environment but needs animal cells for its replication. Overall the risk to the environment is indicated to be minimal, and warnings in the SPC mitigate risks, and recommendations on post-marketing studies has been agreed upon, should the vaccine be used in the field for emergency vaccination in field outbreaks. The vaccine is only to be used under emergency mass vaccination situations in CSFV naïve domestic herds in restricted control zones related to outbreak situations and by the intramuscular route.

Studies have shown lack of protection against transplacental transmission of CSFV. The vaccine is contra-indicated in sows.

As no worst case in vivo studies addressing genome stability have been conducted, a post-marketing investigation in case of use of vaccine, has been asked. While it is necessary to obtain field evidence of genetic stability in animals with this chimeric virus related the use of the vaccine in emergency situations it is clear that this will not be possible in the current legislative environment where field studies are not allowed.

Data from field use of the vaccine should be used to investigate the efficacy of the vaccine with regards to efficacy to dampen infection spreading during CSF outbreaks in the EU and the effectiveness of the DIVA strategy in such circumstances. The applicant is recommended to characterise the genetic make-up of virus circulating in the field related to the CSF outbreak, and focus on mutations including recombination events between the vaccine virus and the wild types. In conclusion, the methods applied to the study should be adapted and updated to meet the three concerns raised above (extent of in vivo genome variability of the vaccine, field efficacy of the vaccine, practicability of the DIVA principles). Therefore a post-authorisation recommendation has been agreed as the studies will be controlled and defined by the veterinary authorities.

## *Conclusion on benefit-risk balance*

The overall benefit-risk evaluation for the product is deemed positive with a sufficiently clear and complete product information. A warning regarding use of vaccine in sows is present in the SPC.

## *Conclusion*

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP concluded that the quality, safety and efficacy of Suvaxyn CSF marker vaccine were considered to be in accordance with the requirements of Directive 2001/82/EC.

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP recommends the granting of the marketing authorisation for Suvaxyn CSF marker vaccine to be used only for emergency vaccination in an outbreak situation in herds within restricted control zones.