21 April 2016
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for CLYNAV (EMEA/V/C/002390/0000)

Common name: salmon pancreatic disease vaccine (recombinant DNA plasmid)

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

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Introduction

On 30 August 2013, the applicant Novartis Animal Health UK Ltd submitted an application for a marketing authorisation to the European Medicines Agency (The Agency) for CLYNAV through the centralised procedure, falling within Article 3(1) of Regulation (EC) No 726/2004 (product developed by means of biotechnological process). The applicant was subsequently changed during the procedure to Elanco Europe Limited (formerly Novartis Animal Health).

The eligibility to the centralised procedure was agreed upon by the CVMP on 15 July 2010 as the product is developed by means of a biotechnological process. The rapporteur appointed was A-M. Brady and the co-rapporteur was D. Murphy.

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

CLYNAV contains supercoiled (sc) plasmid DNA containing as active substance 5.1 – 9.4 µg pUK-SPDV-poly2#1DNA plasmid per 0.05 ml dose. The pharmaceutical form of the product is solution for injection and the proposed route of administration is intramuscular use. The target species is Atlantic salmon (*Salmo salar*).

The product is intended for the active immunisation of Atlantic salmon to reduce impaired daily weight gain, and reduce mortality, and cardiac, pancreatic and skeletal muscle lesions caused by pancreas disease (PD) following infection with salmonid alphavirus subtype 3 (SAV3).

The vaccine is presented in one plastic bag of 250 ml.

On 21 April 2016 the CVMP adopted an opinion and CVMP assessment report.

On 27 June 2017 the European Commission adopted a Commission Decision granting the marketing authorisation for CLYNAV.

Scientific advice

The applicant received scientific advice from the CVMP on 15 September 2011. The scientific advice pertained to the quality and safety sections of the dossier and the applicable MUMS requirements relevant to the development of the product. The advice was noted and acted upon by the applicant appropriately.

MUMS/limited market status

The applicant requested MUMS/limited market status classification for this procedure by the CVMP, and the Committee confirmed at the 16 September 2010 meeting that, where appropriate, the data requirements in the appropriate CVMP guidelines on "minor use minor species (MUMS) data requirements" would be applied when assessing the application. MUMS status was granted for the following reasons:

Salmon pancreas disease (SPD) in salmonids is included in the list enclosed in the CVMP Guideline on data requirements for veterinary immunological products intended for minor use or minor species/limited market use.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system was provided (version 2.2, dated January 31 2012) which fulfils the requirements of Directive 2001/82/EC, as amended. 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 reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

CLYNAV is manufactured by Elanco Canada Ltd. at the Charlottetown site, Canada and shipped to Europe. Batch release for the EU will be carried out by Elanco Animal Health, Eli Lilly and Company Limited, Speke Operations, Liverpool, UK.

All of the manufacturing sites have been inspected by the UK Veterinary Medicines Directorate for Good Manufacturing Practice (GMP) compliance and the test site and batch release site have been inspected. All are considered satisfactory and GMP certificates are provided.

Overall conclusions on administrative particulars

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

Part 2 – Quality Composition

CLYNAV consists of a DNA plasmid (pUK-SPDV-poly2#1) dissolved in phosphate buffered saline. There is no adjuvant or preservative. The quantitative and qualitative composition has been adequately defined as 'pUK-SPDV-poly2#1 DNA plasmid coding for salmon pancreas disease virus (SPDV) proteins', 5.1 - 9.4 µg/0.05 ml dose (101.6-188.4 µg/ml).

Container

The final product is filled into 250 ml volume, single-chambered, sterile, intravenous-like, flexible, ethyl vinyl acetate (EVA) single port bags for parenteral use.

A single, multi-purpose port is used for filling, injection or transfer. A sterile and individually packaged transfer tube set, made of medical grade DEHP free polyvinyl chloride resin (PVC), will be included as part of the product package. The container complies with specifications in European Pharmacopoeia (Ph. Eur.) monograph 3.1.7, including closure integrity testing.

A detailed description of the design and function of the transfer tubing kit was provided, and compliance with the "Guideline on requirements for production and control of IVMPs" has been demonstrated. The aseptic closure system consists of a body, a puncture septum and a locking snap-down type cap that is sealed in place, post-filling and which does not make contact with the final product. The tip of this cap can be snapped off, which will expose the puncture septum and make ready the final product container for a transfer spike or injection needle.

Development pharmaceutics

SPD is an important disease of European, farmed, Atlantic salmon as it can result in significant losses due to morbidity, mortality and reduced production. PD was first recognised in Scotland in 1976 but not actually described in the literature until 1984. For many years there was some controversy over the aetiology of the disease, with various nutritional and infectious causes being suggested. The causative agent was isolated in 1995 and was shown to be an alphavirus, now known as SPDV. A total of six different subtypes of salmon alphavirus (SAV) have been identified and outbreaks of PD have been attributed to all six SAV subtypes. The salmonid alphavirus subtype 2 (SAV2) used for cloning the SAV gene sequence found in the pUK-SPDV-poly2#1 plasmid was isolated from Atlantic salmon tissue collected from fish in Scotland. The subtypes show a degree of geographical separation in Atlantic salmon, with SAV3 only detected thus far in Norway, SAV1, 4 and 6 found in Ireland and SAV 1, 2, 4 and 5 found in Scotland, and SAV-2 in parts of Norway. CLYNAV was developed in Canada based on the technology used to establish an existing product, Apex-IHN, licensed to the applicant in Canada since 2005. It comprises the same plasmid backbone and uses the same manufacturing process as Apex-IHN.

The plasmid contained in CLYNAV was created by insertion of the open reading frame for SAV2 into *E. coli* strain DH5α. The plasmid backbone includes regulatory gene sequences required for replication in *E. coli* and expression in the target species. Some of the regulatory sequences are derived from mammalian species, although the key sequences concerned with expression (human cytomegalovirus (CMV) promoter and Bovine Growth Hormone poly-A sequence) are derived from pcDNA3, a vector widely use in DNA vaccines in a wide range of species. Functioning of the vaccine construct is demonstrated empirically in the clinical studies in Atlantic salmon. Whilst the vaccine codes for subtype 2 expression, efficacy studies have only been carried out using a challenge virus belonging to subtype 3, which demonstrates some cross protection/homology between subtypes. A dose-titration efficacy study showed significant protection of fish vaccinated with plasmid DNA doses between 4.54 and 18.15 µg following challenge with a SAV3 isolate 35 days (388 degree days) post vaccination using a co-habitation model. A further dose-titration efficacy study showed significant protection of fish following challenge in saltwater approximately 400 degree days post vaccination. A plausible justification that a vaccine produced against one PD virus isolate may be efficacious against multiple serotypes of SAV. However, since only a SAV3 challenge was used in the pivotal saltwater challenge efficacy study the only proposed indication is for protection against SAV3.

The lower limit for the dose of the vaccine (5.1 µg supercoiled DNA (scDNA)/dose) was chosen on the basis of the batch of vaccine used in the definitive saltwater challenge study. An upper limit of 9.4 µg scDNA/dose was set on the basis of the calculated scDNA concentration of the batch used in a safety study.

No preservative is included and should not be needed because the vaccine bag has a single-use entry port.

Method of manufacture

Flow charts of the production processes and detailed description of production steps were provided.

The final product is aseptically filled into the final product containers with the required volume delivered by weight equivalence to each container through the filling port, which is then aseptically sealed.

A detailed description and purpose of the plasmid extraction method has been provided, as well as the validation of the purification process through the provision of data. The production process has also been investigated to determine the efficiency of removal of remnants of production Protein levels were also assessed at the finished product stage and a revised specification set. This is considered adequate to

confirm compliance with the CVMP note for guidance on DNA vaccines (CVMP/IWP/07/98) with respect to adequate detail and validation of the purification process.

The bioburden levels are controlled through the manufacturing process.

Control of starting materials

Starting materials listed in a Pharmacopoeia

The following starting materials in a pharmacopoeia are listed by the applicant:

Acetic acid, glacial, Ammonium molybdate tetrahydrate, Ammonia solution, Ammonium sulphate, Borax, Calcium chloride dehydrate, Cobalt (II) chloride hexahydrate, Copper sulphate pentahydrate, Dipotassium phosphate, Disodium phosphate heptahydrate, Ferrous sulphate heptahydrate, Glucose, Glycerol, Hydrochloric acid, Magnesium sulphate heptahydrate, Manganese sulphate monohydrate, Phosphoric acid, Potassium acetate, Potassium chloride, Potassium dihydrogen phosphate, Sodium laurel sulphate, Sodium chloride, Sodium hydroxide, Trometamol, Zinc sulphate heptahydrate. Certificates of analysis all conform to specifications in the Ph. Eur.

Starting materials not listed in a Pharmacopoeia

Starting materials of biological origin

The active substance of the vaccine is a scDNA plasmid, designated pUK-SPDV-poly2#1.

The salmonid alphavirus subtype 2 (SAV2) used for cloning the SAV gene sequence found in the pUK-SPDV-poly2#1 plasmid was isolated from Atlantic salmon tissue collected from fish in Scotland

The master and working seed lots have been characterised with respect to purity, identity and genetic stability. The identity of both the *E. coli* DH5a host cells and the pUK-SPDV-poly2#1 vaccine plasmid have been confirmed by DNA sequencing as well as more conventional methods. The fidelity of the DNA sequence of the finished product has been verified by DNA sequencing. It has been confirmed that the finished product specifications confirm 100% homology with the expected sequence, therefore the production process and finished product specifications are adequate to confirm that consistent product will be produced with respect to the fundamentally important DNA sequence of the product.

Master seed has been shown to be stable and an appropriate specification to ensure genetic stability during production has been set. The number of passages during production have been defined and justified.

Other materials of biological origin used for production of CLYNAV are: yeast extract, terrific broth powder and RNase A. Terrific broth powder is produced using materials of bovine origin and the origin of the material has been confirmed as milk fit for human consumption. RNase A is produced from bovine pancreas tissue. An assessment of the risk of contamination with extraneous agents from starting materials taking into account the nature of the raw materials, their source and origin, the controls and treatment and their use in production has been provided. The risk is considered to be negligible.

Starting materials of non-biological origin

Polypropylene glycol 2000 and kanamycin are listed.

Polypropylene glycol 2000 is used as an anti-foam reagent during fermentation; a satisfactory specification and certificate of analysis has been provided.

Kanamycin was only used to select cell populations carrying the plasmid encoding for the SAV genes and the kanamycin resistance cassette. No records of the sourcing of the kanamycin used for production of these seeds were kept. However, since this material is not used during routine vaccine production the absence of this information can be accepted.

Specific Measures Concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

An assessment of TSE risks has been provided, consistent with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3). This assessment takes into account the source, nature of tissue used and manufacturing process control measures.

It is concluded that the risk of transmitting TSE infectivity through the use of this vaccine is negligible.

Control tests during production

Control tests carried out at various stages during vaccine production include Gram stains, culture on lysogeny broth plates, microscopic observation and biochemical profile to confirm acceptable culture purity, bioburden test, optical density and wet cell weight to verify acceptable culture growth and total and super coiled DNA concentration to verify the presence in harvested cultures and to quantify at later stages. Data are provided to demonstrate the consistency of production of three batches. All in-process tests were performed during the manufacture of the consistency batches and the results were within specifications.

Control tests on the finished product

Description of the following methods used for quality control of the vaccine finished product and their respective specification were provided: plasmid homogeneity, plasmid identity, total DNA concentration, DNA Purity, residual RNA content, residual genomic DNA content, residual protein content, endotoxin content, sterility, scDNA concentration, appearance, pH, volume and batch potency. Tests for plasmid homogeneity, plasmid identity, total DNA, DNA purity, residual RNA content, residual genomic DNA, residual protein, supercoiled DNA concentration, endotoxins, appearance, pH and volume are performed. Detailed methodology and validation data have been provided for all tests and are acceptable.

The following tests are conducted: sterility and *in vitro* expression (potency). Methodology and validation reports have been provided and are acceptable.

For potency testing an *in vitro* test is used. This includes two different steps: a quantitative test to directly quantify (µg/ml) the active ingredient scDNA and a qualitative test for viral capsid protein expression in a cell line of fish origin.

Validation and detailed description of these *in vitro* tests have been provided and are considered satisfactory.

The proposal for the use of an *in vitro* test to measure the potency of the vaccine is considered a positive step and, in principle, the overall strategy and suitability of the tests proposed can be accepted.

The results of the analysis of three consecutive production runs of vaccine were presented. These complied with the indicated manufacturing specifications. It has been adequately demonstrated that these batches have been produced according to the method of manufacturing proposed for the finished product and are therefore adequate to demonstrate consistency of manufacturing.

Stability

The three consistency batches of CLYNAV were analysed at time points 0, 5, 12, 21 and 27 months after storage at 2-7ºC to demonstrate stability of the finished product.

Batches were prepared according to the proposed manufacturing process and are representative of standard and consistency batches.

At the time of testing the consistency batches in the stability program, an *in vivo* test was used as a measure of vaccine potency. This *in vivo* potency test has now been replaced by an *in vitro* test based on the quantitative amount of scDNA in the vaccine.

Analysis of the physical integrity of the stability batches as a percentage of the total DNA demonstrated that there was no considerable change in the percentage of scDNA content of the vaccine over a 27 month time period supporting the statement that the active ingredient of CLYNAV is stable over that period of time. Therefore, this is sufficient to support 24 months shelf life.

Potential contamination of the product over time was assessed via the endotoxin test, microbial sterility test, and DNA purity (260/280 nm ratio).

A further stability study is planned with the first three commercial batches of vaccine, in the bag presentation to be used for the commercial product i.e. 250ml EVA bags. The protocol and timelines for this stability programme have been provided. The results at the end of the study are recommended for submission to the competent authority

The proposed 24 months shelf life is supported, especially considering that it has been demonstrated that the content of sc plasmid (active substance) remains stable over time and that this application has been classified within the MUMS framework. It has also been confirmed that batches stored for 27 months are still efficacious.

There are no accelerated stability data and therefore the product will be subject to refrigerated transport. This is stated in the SPC.

In-use stability data have been generated over 10 hours using two batches of vaccine. Two studies were carried out, one to demonstrate the sterility of the product during that time and the second looking at the physiochemical properties, including potency. Both studies adequately support 10 hours in-use stability for the vaccine.

Overall conclusions on quality

CLYNAV is a DNA plasmid–based vaccine presented as a solution for injection in 250ml EVA bags. Each 0.05 ml dose will contain 5.1 - 9.4 µg of scDNA in phosphate buffered saline. There is no adjuvant or preservative.

The containers are of an acceptable standard. There is a transfer tubing kit included, which has been adequately described and included in the SPC.

No preservative is included and should not be needed because the vaccine bag has a single-use entry port.

A total of six different subtypes of SAV have been identified. Efficacy studies have been carried out using a challenge virus belonging to subtype 3, which demonstrate some cross protection/homology between subtypes. A plausible justification was provided that a vaccine produced against one PD virus isolate may be efficacious against multiple serotypes of SAV. However, because protection has only been demonstrated using a SAV3 challenge, the indication is for protection against SAV3.

The method of manufacture is well described and sufficient detail has been provided on the different steps of the production process.

Maximum holding times between each manufacturing step and the different types of container used for each manufacturing step have been defined.

The starting materials are well described and of adequate quality. Data have been provided to suggest that the complete ORF is relevant for protection against a range of SAV subtypes (although only SAV3 is claimed) and a complete history of the seed lots between construction of the vector and transformation into the host cells and the use of the construct to produce finished product has been provided.

The identity of both the *E. coli* DH5a host cells and the pUK-SPDV-poly2#1 vaccine plasmid have been confirmed by DNA sequencing as well as more conventional methods. The fidelity of the DNA sequence of the finished product has been verified by DNA sequencing. The specifications confirm 100% homology with the expected sequence therefore the production process and finished product specifications are adequate to confirm that consistent product will be produced with respect to the fundamentally important DNA sequence of the product.

The main risks concerning TSE have been addressed and are considered negligible.

Control tests carried out at various stages during vaccine production are adequate and include scDNA quantification and bioburden. Data are provided to demonstrate the consistency of production of three batches. Tests are carried out on the final product to confirm plasmid homogeneity, plasmid identity, total DNA concentration, DNA purity, residual RNA content, residual genomic DNA content, residual protein content, endotoxin content, sterility, scDNA concentration, appearance, pH, volume and batch potency.

An *in vitro* test is used to measure the vaccine potency. This includes two different steps: a quantitative test to directly quantify (µg/ml) the active substance scDNA and a qualitative test for viral protein expression in a cell line of fish origin. The tests will be done within the EEA and GMP certificates for each site are satisfactory. Validation and detailed description of these *in vitro* tests have been provided and are considered satisfactory.

The results of the analysis of three consecutive production runs of vaccine were presented. It has been adequately demonstrated that these batches have been produced according to the method of manufacturing proposed for the finished product and therefore are adequate to demonstrate consistency of manufacturing.

The 24 months shelf life proposed can be agreed, especially considering that it has been demonstrated that the content of scDNA plasmid (active substance) remains stable over time, and this application has been classified within the MUMS framework. It has also been confirmed that batches stored for 27 months are still efficacious.

There are no accelerated stability data and therefore the product will be subject to refrigerated transport. In-use stability data have been generated over 10 hours using two batches of vaccine.

In addition, the applicant is recommended to provide the following information post-authorisation:

The results of an on-going stability study for one development batch of CLYNAV packaged into the new EVA bags.

The results of a further stability study with the first three commercial batches of vaccine.

Part 3 – Safety

Safety documentation

CLYNAV is a DNA vaccine for use in Atlantic salmon from a minimum weight of 25 g for the active immunisation of Atlantic salmon to reduce impaired daily weight gain, and reduce mortality, and cardiac, pancreatic and skeletal muscle lesions caused by pancreas disease following infection with SAV3. The vaccine consists of a genetically constructed plasmid pUK-SPDVpoly2#1.

Salmon pancreas disease is listed in the appendix of the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/1234243/2006-Rev.2) (the MUMS Guideline) and therefore appropriate reduced data requirements can apply. Particular areas where the applicant has submitted reduced safety data requirements indicated in the MUMS Guideline are not to carrying out a single dose safety study, relying instead only on an overdose study and in not carrying out a field safety study.

Although it is not a live vaccine, the applicant has carried out additional studies to investigate the biodistribution and possible integration of the plasmid in vaccinated salmon and to investigate colonisation of gut microflora with the plasmid and the possibility of horizontal transfer.

Laboratory tests

Safety of the administration of one dose

A single dose safety study was not carried out, the applicant relying instead only on the overdose study in line with the provisions of the MUMS guideline.

Safety of one administration of an overdose

This study was conducted by administration of a dose of 115 µg DNA to salmon weighing between 12.4 and 13.3 g by intramuscular (i.m.) injection. The study was not conducted to Good Laboratory Practice (GLP) (which is permissible under MUMS), however adequate study monitoring and QA procedures were in place.

Due to animal welfare concerns it was not possible to administer 10 doses of the finished product to Atlantic salmon. Therefore the study was conducted with an approximately ten-fold concentrated formulation of three conformance batches of CLYNAV dose. The vaccine meets the requirements of the CVMP note for Guidance: DNA vaccines non-amplifiable in eukaryotic cells for veterinary use (CVMP/IWP/07-98-FINAL) regarding overdose studies and was in line with the MUMS provision to use standard doses of vaccine in safety studies.

No serious adverse events were observed during the conduct of the study and no mortalities attributable to administration of the vaccine or placebo. The main observations were:

- Visible needle damage was very common at sites of injection in fish that received either vaccine or placebo at sampling points up to and including 21 days post vaccination (DPV). Severity and prevalence was greater in fish that received vaccine and vaccination related effects could be most easily differentiated on 21 DPV.

- External visible needle damage was most severe at 4 DPV after which it gradually resolved and was observed in 77%, 57% and 20% of vaccinated fish at 4 DPV, 8 DPV and 21 DPV, respectively compared to 70%, 30% and 10% of fish that received placebo.
- Internal visible haemorrhage at site of injection was most severe at days 4 and 8 DPV and was observed in up to 97%, 95% and 45% of vaccinated fish sampled at 4 DPV, 8 DPV and 21 DPV, respectively compared to 100%, 95% and 0% of fish given placebo. From 46 DPV there was no internal evidence of haemorrhage for any of the fish.
- Whilst visible needle damage was not observed in any fish sampled at 46 DPV it was noted that at 90 DPV external visible needle damage was observed in 5% (4/83) of fish that received the vaccine and 2% (1/41) of fish that received placebo.
- Fin condition in both groups was comparable; at the beginning of the study most fish were rated fair, but most were rated poor by the end of the study.
- Histopathology evaluation of tissue at the injection site revealed that necrosis was most severe in fish given vaccine or placebo 4 DPV, after which it gradually resolved. The prevalence and/or severity of necrosis were substantially greater in vaccinated fish than controls 8 DPV (Tanks A1 and A2) and 21 DPV (Tank A1 only); moderate inflammation was also present 4 DPV. For both tanks, the severity of inflammation peaked in vaccinated fish 21 DPV, at which time both the prevalence and severity of inflammation decreased in fish given placebo. The peak in severity 21 DPV was primarily attributable to increased lymphocytic infiltrates in vaccinated fish. Myocyte regeneration was a prominent finding 8 DPV in vaccinated and control fish from both tanks, and the severity of this response peaked in vaccinated fish 21 DPV. The greatest prevalence and severity of fibrosis also occurred 21 DPV, this response was otherwise very low. Inflammation, myocyte regeneration and fibrosis all receded in both groups after 21 DPV and no significant differences were apparent in these responses between vaccinates and controls by 90 DPV.
- The histopathology scores were not significantly different between the groups vaccinated with any of the vaccine batches within either tank on any day post vaccination.
- The length of time for Atlantic salmon to return to normal colour, normal swimming behavior and feeding after vaccination were recorded. At 2 DPV 75% of fish showed dark colouration, which reduced over time until all fish had returned to normal coloration by 8 DPV. At 2 DPV 50% of fish showed normal feeding, which improved over time until all fish showed normal feeding by 9 DPV. All fish showed normal swimming behavior by 2 DPV. These observations were the same whether the fish had been vaccinated with CLYNAV or with control PBS, which suggests that the reactions are related to the i.m. vaccination process rather than specifically associated with the product. Nevertheless, the applicant has reflected these observations in the SPC.

The minimum weight for vaccination, 25 g, that has been cited on the SPC (and double the weight of fish used in the 10 fold overdose study) is based on the results of an efficacy study which demonstrated a reduced the risk of adverse reactions associated with immunisation of very small fish.

- The applicant has also discussed the practical implications of volume inaccuracies observed in multi-dose equipment in usage under field conditions, it is noted that these inaccuracies are not vaccine-related per se, and it is expected that users will be appropriately trained and will use correctly calibrated equipment in accordance with manufacturer's specifications.
- The applicant has adequately justified that there would be no significant impact on grading of fish at slaughter. This is based on observations for fish vaccinated with the applicant's related DNA vaccine Apex IHN using the same dose route, dose volume and type of device and is acceptable.

- Growth data have been investigated up to 27 months in studies, both of which showed that weight gain was either significantly greater or not significantly different between fish vaccinated with CLYNAV and fish injected with saline. In addition, the applicant investigated possible interactions with other products which showed that CLYNAV did not have an adverse effect when used in conjunction with routinely used oil emulsion vaccines. Taken together, the data provided give assurance that CLYNAV does not have an adverse effect on weight gain.

The applicant has justified the use of 115 ug DNA per dose in the 10x dose in one study this is in excess of a 10x overdose of the revised specification (5.1 – 9.4 µg per dose) of plasmid DNA.

In conclusion, the applicant has adequately supported safety following the administration of 10x the recommended dose of CLYNAV for the target species.

Safety of the repeated administration of one dose

The vaccine is only intended for administration once and therefore no studies on the safety of repeated administration were carried out.

Examination of reproductive performance

The applicant has not provided data to examine the effect of the vaccine on reproductive performance and has included a contraindication for the vaccine against use in broodstock. The question whether the DNA plasmid could become integrated into the Atlantic salmon genome is considered in the section on 'Dissemination in the vaccinated animal' below. The applicant conducted an integration study that investigated gonads and muscle tissue. Gonad samples were collected 1 day after vaccination with a 10x dose of CLYNAV at a time when the levels of vaccine plasmid are highest according to the applicant's bio-distribution study. The applicant developed a method to extract and separate plasmid DNA from genomic DNA, then enriches for DNA containing plasmid sequence and analyse by sequencing. No evidence of integration has been found. The applicant used the worst case Limit of Detection (LOD) as the basis for a Qualitative Risk Assessment (QRA). The QRA assessed the risk of transmission of integrated plasmid DNA into the genome of wild salmon and found the risk to be negligible. The applicant evaluated different scenarios using a range of parameters which are relevant to a range of ecosystems and interaction scenarios relevant to production in Europe. In addition, the applicant conducted a consequence analysis. The assessment of the consequences of risk to the wild salmon population should an integration event occur has been conducted thoroughly and the likelihood of an integration event conferring a selective advantage is low. Should integration occur in coding and/or regulatory DNA sequences, a malignant event is possible, however it is acknowledged that the likelihood of integration into such sequences is low. Should integration occur in non-coding DNA sequences, this would not lead to any phenotypic change and the consequences are therefore low. The potential consequence that fragments of plasmid sequence may be detectable in the genome of CLYNAV vaccinated fish or their progeny in future years was only possible when modelled within the context of extreme worst case scenarios. The consequence of integration at muscle tissue is limited to the individual fish affected and there are no issues in terms of environmental safety.

Overall, it can be accepted that the consequences of integration, in terms of environmental effects on future populations of wild Atlantic salmon, are negligible.

It is accepted that the consequences of integration to individual fish are impossible to extend to the population level unless the change is heritable. It is also accepted that the likelihood of integration occurring at the gonads is negligible. This is because the data submitted show that plasmid is only detectable in gonad tissue at a low level for a brief period (1 day) after administration, and is

undetectable 7 days after vaccination. If integration had occurred, it would be expected that plasmid would be detectable after 1 day post vaccination. In addition, integration at the gonads would require a sequence of significant events to occur which are difficult to imagine occurring in practice. An acceptable reasoned argumentation supported by the current state of knowledge was provided on DNA vaccines that conclude that the risk of integration is a theoretical rather than a real risk. In addition, published data do not show any evidence of integration of plasmid sequences into eukaryotic genomes over evolutionary time. Fish, like other species, have been continually exposed throughout evolution to plasmid sequences (from the constant exposure to bacterial DNA both internally and externally via cuts and abrasions), yet there is no evidence of integration of such plasmid sequences into eukaryotic genomes. In addition, the plasmid is engineered to reduce the potential for integration. Accordingly there is no potential additional risk associated with the low plasmid exposure from this vaccination strategy. In the absence of a mechanism for stably existing in the genome, it is not possible to envisage how such a transfer to the gonad of wild salmon could occur.

Nevertheless, a QRA was conducted modified in response to questions and comments which concluded that the risk to the environment is effectively zero. Appropriate scenarios and parameters were used in the model, which included uncertainty about certain parameters allowing for a worst case scenario analysis for 40 generations (roughly equivalent to approximately 200 years), assuming a worst case at every generation. The QRA was conducted using a model which generates an output that is robust to extreme scenarios.

The data provided allow the conclusion that the risk of integration of CLYNAV is negligible and that the risk to the environment following the use of CLYNAV as recommended is acceptable.

Examination of immunological functions

No data was provided to examine the effect of the vaccine on immunological functions which is acceptable as stated in the CVMP MUMS guidelines and CVMP scientific advice.

A detailed explanation of the mechanism of action of DNA vaccines in fish was provided which also includes a justification for the relevance of the mammalian gene regulatory sequence for expression in the target species, with extensive cross-reference to published literature. The possibility of adverse effects on the immune system, such as auto-immune responses, is also addressed by reference to published literature. Whilst there is a small possibility of induction of autoimmunity by the use of DNA plasmid vaccines, it is accepted that this is very low. One study demonstrated the safety of use of CLYNAV at the same time as two other inactivated multivalent, oil-adjuvanted vaccines, and also demonstrated that concurrent vaccination with CLYNAV did not negatively affect the protection against infectious pancreatic necrosis (IPN) challenge, and therefore does not have an immunosuppressive effect.

Special requirements for live vaccines

Although CLYNAV is not a live vaccine, some of the same considerations are considered relevant for this vaccine.

Dissemination of plasmid in the vaccinated animal

A study was provided to investigate the bio-distribution of the vaccine plasmid and its potential for integration into the Atlantic salmon genome.

The bio-distribution, clearance and potential risk for genomic integration of CLYNAV following vaccination of Atlantic salmon with either a 2x or 10x dose of plasmid was investigated. The fish used weighed 9 g± 1.3 g at one day post-vaccination. The plasmid used for formulation of the vaccine batches was a small

scale developmental batch which was prepared by a process reflective of that used to prepare product for market. The key specification for the product (scDNA content) has been confirmed which is above the proposed minimum set by the applicant, therefore the use of this batch has been adequately justified.

Vaccinated fish were followed for up to 822 days after vaccination. For bio-distribution, samples were taken from a range of tissues up to 60 days post vaccination, which included epaxial muscle at the injection site, gut, spleen, gonad, head kidney and heart. These were analysed by qPCR to detect the presence of vaccine plasmid. In addition, further samples of the epaxial muscle from the site of injection were taken up to 822 days after vaccination and also analysed by qPCR for the detection of vaccine plasmid. An investigation for integration was also conducted on epaxial muscle from the site of injection at 436 days post vaccination.

Bio-distribution studies showed that plasmid was detectable at all sites 1 day post vaccination, in gut samples 21 days after vaccination and at the injection site at the last time point studied (822 days post vaccination).

Testing for bio-distribution involved testing for the presence of plasmid by qPCR without establishing whether this was integrated or not. The study design also included investigation of integration as part of this bio-distribution study, however this investigation had some limitations and the applicant provided further data from a new integration study.

This study investigated the gonads and muscle tissue from fish vaccinated with a 10x overdose of CLYNAV. Injection site (muscle) samples were taken at 14 days post vaccination. Gonad samples were collected 1 day after vaccination, at a time when the levels of vaccine plasmid were highest according to the applicant's bio-distribution study. A state of the art method was developed to extract and separate plasmid DNA from genomic DNA. High molecular weight genomic DNA samples were then tested for presence of plasmid sequence, using three different qPCR assays. No plasmid sequence was detected in any of the gonad tissue samples. As plasmid sequence was detected in all muscle samples, these samples were further tested for chimeric sequences (plasmid sequence connected to DNA from the *Salmo salar* genome). There was no evidence of integration. A QRA of the risk of transmission of integrated plasmid DNA into the genome of wild salmon found the risk to be effectively zero.

Scientific advice recommended that the choice of tissue sampled during any investigation of the potential for integration should be based on tissues which have the greatest potential for the detection of an integration event. The choice of muscle cells at the injection site satisfies this from the point of view of the site with the highest concentration of vaccine plasmid. In line with the scientific advice samples from the reproductive organs were also investigated for evidence of integration at a timepoint (1 DPV) when plasmid levels at the gonads are highest. The timing of tissue analysis was considered appropriate, but with respect to the gonads, an additional time point would have been ideal for a more robust assessment. However, this time data gap can be overcome by the availability and use of the bio-distribution data which show that plasmid was below the limit of detection at the next time-point (7 days post vaccination) which is reassuring, because if no plasmid DNA could be detected in gonads it means that no plasmid DNA was integrated (given the LOD).

Study of residues

CLYNAV consists of DNA and phosphate buffered saline; it does not contain any adjuvants or preservatives. The active substance, being a principle of biological origin intended to produce active immunity, does not fall within the scope of Regulation (EC) No 470/2009. The excipients listed in section 6.1 of the SPC 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 are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product. Therefore a withdrawal period of zero days

was proposed. Scientific advice recommended that if plasmid is still detectable at the end of the observation period that further investigations would be necessary to establish the actual duration of this persistence. It was shown that low levels of plasmid are present at the injection site 331 days after vaccination (0.23 % of Day 1 level), and that this level continues to fall until the last timepoint tested (822 days) when levels have fallen to 0.001% of the levels recorded 1 day post vaccination. The final timepoint is relevant to commercial production of salmon, therefore the applicant has adequately investigated the persistence of vaccine plasmid. Whilst this DNA vaccine is not considered a GMO the EFSA (European Food Safety Authority) opinion (EFSA statement on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed 19 July 2007) concerning the survival of GMO plant DNA in the gastrointestinal tract of humans and animals has some contextual relevance. This view was that after digestion any biologically active genes and proteins are rapidly degraded into short DNA or peptide fragments.

Consequently, it is considered that the risk from consumption of residual vaccine is not a major safety concern as the vaccine DNA will be digested as would any DNA found within the normal diet. A withdrawal period of zero days is therefore acceptable.

Interactions

No data were provided to investigate interactions of the vaccine with other veterinary immunological products and therefore proposes to include a statement in Section 4.8 of the SPC that: '*No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis*'.

In view of the fact that farmed Atlantic salmon are commonly vaccinated against several diseases during production, some of which may be compulsory, vaccination with CLYNAV alone is not likely to occur. A study was conducted to investigate the use of the vaccine in combination with other commercially available vaccines. The study did not raise any additional safety concerns, therefore it is concluded that the use of CLYNAV with other vaccines in routine use has been adequately considered. However, given that the efficacy of CLYNAV was not evaluated in the concurrent use study, the applicant has not proposed a claim for compatible use with any other vaccines. This is considered appropriate.

Field studies

The applicant has not provided data from field studies which is acceptable in accordance with the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (EMA/CVMP/IWP/123243-rev.2) and consistent with CVMP scientific advice to the applicant. The omission of field studies was dependent on the caveat that laboratory studies demonstrated the absence of a safety risk and that it was sufficiently justified that data from the laboratory studies are representative for safety under field conditions. This was accepted because no specific concerns were raised by the overdose safety study However, according to the CVMP Guideline on the design of studies to evaluate the safety and efficacy of fish vaccines (EMA/CVMP/IWP/314550/2010), safety studies should allow for a prediction of the safety profile over the average life span.

The applicant addressed this issue by presentation of data from their bio-distribution laboratory study which monitored fish for 822 days post vaccination, which is relevant for commercial production of farmed salmon. These data did not raise any significant safety concerns. In addition, the applicant investigated possible interactions with other products which showed that CLYNAV did not have an adverse effect when used in conjunction with routinely used oil emulsion vaccines. The applicant has also provided pharmacovigilance data for the related DNA vaccine APEX-IHN (3 adverse event reports from >83 million

Atlantic salmon vaccinated since 2005), the relevance of which has been adequately justified. Therefore, data were provided from a number of sources to adequately support the safety profile of CLYNAV over the average commercial life span.

User safety

A Final User Safety Risk Assessment for the vaccine has been prepared in accordance with the CVMP Guideline on user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006). As CLYNAV is a DNA vaccine, the CVMP Note for Guidance: DNA vaccines non-amplifiable in eukaryotic cells for veterinary use (CVMP/IWP/07/98-FINAL) was taken into account in this assessment.

The following scenarios were identified in which exposure to the vaccine could occur:
1. vaccine storage and preparation of the vaccination equipment; *spillage*
2. administration of the vaccine to fish (trained individuals); *accidental self-injection*
3. handling vaccinated fish; *environmental contamination*
4. cleaning the vaccination equipment, and disposal of empty vaccine bags; *spillage*

Spillage of the product onto the user or handling vaccinated fish is not considered to pose any hazard. Parenteral exposure following accidental self-injection poses the most risk to the user in terms of frequency of occurrence and potential consequences following exposure. This includes any risk from active ingredients and as injection needles are used for multiple fish vaccinations there is a risk of infecting the injection site with naturally occurring bacteria present on the surface of the fish which the applicant notes could result (in rare cases) in the onset of cellulitis. The vaccine is indicated for the vaccination of smolts and would therefore be administered in hatcheries to fish held in tanks by trained individuals. CLYNAV was developed on the basis of a product authorised in Canada, APEX-IHN, which has the same plasmid backbone and manufacturing process. Pharmacovigilance data covering the period 01 January 2005 to 06 February 2013, during which more than 65 million fish were vaccinated with APEX-IHN, report a single accidental injection of a human. No signs resulting from injection could be seen one day after exposure, and no long term adverse reactions were reported. The minimum weight indicated in the SPC has been increased to 25 g (based on study data), which reduces the potential risk of self-injection associated with vaccination of large numbers of small fish. In response to concerns raised, the potential risks to the user following accidental self-injection were considered in greater detail. The concern that accidental self-injection may give rise to formation of autoantibodies against DNA was addressed and the argumentation is accepted that this was not a cause for concern, and this is also adequately supported by literature in the public domain including the use of DNA vaccines for human use. The potential for the development of hypersensitivity following repeated exposure through accidental self-injection was considered to be acceptable. The SPC recommends that medical advice is sought following accidental self-injection.

Environmental risk assessment

CLYNAV contains a genetically constructed plasmid, pUK-SPDVpoly2#1, which contains a pUK21-A2 backbone and genes of SAV. It was concluded that the DNA vaccine is not itself a genetically modified organism (GMO) since the plasmid is not a living organism and can only be replicated in bacterial cells. Data presented and a QRA concluded that the risk of integration was negligible.

Scientific advice from CVMP at the time contained a recommendation regarding ERA to be carried out in line with the requirements of EC Directive 2001/18 as well as the normal ERA for any immunological veterinary medicinal product in accordance with the requirements of EC Directive 2001/82. In line with this advice an ERA was submitted which is based on a six step approach (as recommended by 2002/623/EC) and is intended to take into account any potential hazard that may be linked directly or

indirectly with the DNA vaccine itself, including an evaluation of all the genes present in the plasmid construct. Within the ERA experience with APEX-IHN vaccine was taken into account, which uses the same plasmid backbone and is authorised in Canada since 2005. A QRA was concluded as part of the overall ERA. This assessment drew together the applicant's data, published data and expert opinion in complex modelling of different scenarios and included a consequence analysis of any potential risks.

The construction of the vaccine plasmid and the genetic elements were described and presented. The potential for genetic information to mobilise from the plasmid by a number of routes was considered. The absence of the ability of the plasmid to replicate outside of bacterial cells is also highlighted and the potential for integration of the plasmid into fish cells or the transfer to environmental bacteria acknowledged.

The mode of use of the DNA vaccine was described, and the potential risks to humans, animals and the environment were identified as: spillage of the vaccine, accidental inoculation of the user, leakage of the vaccine from the injection site of the fish, fish may escape from the containment tanks or nets in seawater and come into contact with wild fish, and indirectly the DNA of the plasmid could become integrated into the chromosomal DNA of the fish, humans or animals eating the fish could be exposed to the DNA of the plasmid or parts of it, potential plasmid release via effluent water from vaccinated fish, the DNA of the plasmid may become integrated into bacteria present in the fish. Each of these potential risks was discussed.

The greatest risk identified from spillage of the vaccine or leakage from the injection site of vaccinated fish would be potential uptake by environmental bacteria. A specific study was conducted which investigated the potential for the spread of kanamycin resistance associated with the vaccine, to environmental bacteria. Some concerns were raised regarding this study and a new study was conducted. Based on the conclusions of the study, it is accepted that the risk of spread of kanamycin resistance associated with the vaccine is negligible.

The risk of accidental self-inoculation by the user was considered using evidence from use of the related DNA vaccine, APEX-IHN, to assess the severity of the local reactions, which are low. The potential for the development of hypersensitivity associated with repeated accidental self-injection has been examined as part of the QRA and found to be no different to existing conventional vaccines and is acceptable.

The escape of vaccinated fish was considered a very low risk due to the farm management measures in place to avoid such an event. Escape rate was provided in a QRA to assess the risk of transfer of integrated vaccine plasmid DNA to the environment. As part of the QRA the current situation was reviewed with respect to escapes and it was noted that there are a number of active measures in place to minimise escape in Norway, Scotland, and Ireland. These are set down in either legislation or industry guidance. There is a trend toward reducing the number of escapes in recent years. Appropriate parameters and scenarios have been examined in the QRA, which has assessed the risk of integrated plasmid DNA passing to wild salmon in extreme worst case scenarios and found that the risk to the environment was acceptable when the vaccine is used as recommended and therefore the risk associated with escaped vaccinated fish has been adequately addressed.

The theoretical potential for integration of the vaccine plasmid into the fish germ-line DNA was acknowledged, and the potential for integration of vaccine plasmid DNA into vaccinated salmon was investigated and the potential fate of the integrated plasmid DNA assessed. There are two key elements to the approach: Laboratory integration study; and QRA to assess the risk of integration in the germ-line of wild salmon. The laboratory study found no evidence of integration.

In the QRA, a modification of the model described by Hindar et al (2006) was used and the outcome using worst case estimates of the frequency of integration was assessed. The worst-case was based on the limit of detection (LOD) of the method used to detect integration at the injection site in the

applicant's integration study. The worst case integration rate was used in the model to generate outputs modelling the fate and persistence of integrated plasmid DNA in the wild salmon population. In extreme worst case scenarios the model predicts that integrated plasmid DNA would be transferred to wild salmon, which is not unexpected. The model also predicts that if vaccination is ceased then the integrated plasmid DNA would effectively die out as a consequence of stochastic fade-out, i.e. self-propagation in the absence of the influx of vaccinated fish is unlikely. However, it is considered that the risk should not be evaluated assuming that vaccination will cease, and the risk has also been assessed when vaccination was continued for 40 generations (approximately 200 years), and found that persistence was still only possible in extreme worst case scenarios. The output of this model predicts that under a set of extreme worst case scenarios there is a probability of persistence of the plasmid in wild salmon at generation 40 of approximately 4 %. The study also investigated the presence of vaccine plasmid at the gonads. It has been shown that only low levels of plasmid could be detected at the gonads shortly (1 day) after vaccination and levels were below the LOD of the qPCR assay at the next time point (7 days post vaccination). Therefore it has been shown that no plasmid DNA could be detected in gonads after 7 days, which is reassuring, because if no plasmid DNA could be detected in gonads it means that no plasmid DNA was integrated. It is reasonable to consider this analysis as one element of the investigation into the potential for integration at the gonads using data from gonads rather than muscle. Using this limit of detection as an estimate of the frequency of integration showed that any potential integrated vaccine plasmid DNA has a zero probability of persistence even in extreme worst case scenarios.

As discussed in the previous section 'Examination of reproductive performance', the potential consequences of risk to the wild salmon population have been considered and it is accepted that the likelihood of integration occurring at the gonads is negligible. Therefore, it can be accepted that the consequences of integration, in terms of environmental effects on future populations of wild Atlantic salmon, are negligible.

Overall, it was considered that the QRA was conducted using an appropriate model and that the model generated an output that was robust to extreme scenarios. It is accepted that the data support the conclusion that the risk of integration of CLYNAV is negligible and that the risk to the environment following the use of CLYNAV as recommended, is acceptable.

The applicant has drawn on EFSA comments related to the risk from consuming meat from animals vaccinated with GMOs, which concluded that genes and proteins are rapidly degraded in the normal way as any biological material would be digested. A worse case for consumption of an injection site was considered. The potential frequency of transformation of enteric bacteria was also considered. The view that the potential for an adverse event from these factors is low is supported.

With regard to risk management strategies, it was proposed that it will only be necessary to apply the normal management strategies for water handling that are currently used in fish farms. The QRA supports the conclusion that the risk from water contaminated with vaccine is negligible, therefore normal management strategies for water handling are considered adequate.

Conclusions on the environmental risk assessment

The risks to the environment from spillage of vaccine and subsequent contamination have been adequately considered and found to be negligible. The most significant risk to the environment is if plasmid DNA were to become integrated into genomic DNA of vaccinated fish that escape from the containment tanks or nets in seawater and come into contact with wild fish. While measures are generally in place to prevent this happening some fish on occasions do escape and have been known to breed with wild salmon. There would be a risk of propagating the plasmid if it became integrated into the salmon genome. A study to investigate this possibility has been carried out using state of the art methods and

found no evidence of integration. Current scientific knowledge is that integration and transfer of this integrated DNA to future generations of fish is a theoretical rather than a real risk. A QRA of the risk of transfer of integrated vaccine plasmid into the wild salmon population has been conducted using extreme worst case scenarios and concluded that this risk to the environment is effectively zero.

It is strongly emphasised that the QRA was conducted on the assumption that integration in gonad tissue occurs. There is no available evidence to indicate that this will occur. The conclusions of the QRA, assuming that integration does occur, and in an extreme set of worst case scenarios, still demonstrate that there is a negligible risk of plasmid sequence persisting in future generations.

Consequently it is concluded that the risks to the environment have been adequately investigated and support a negligible risk, which is acceptable.

Overall conclusions on the safety documentation

Four studies were provided to investigate: a) the safety of a 10-fold overdose; b) the potential spread of the vaccine construct to non-vaccinated cohabiting fish and to environmental bacteria; and c) the bio-distribution of vaccine plasmid in the vaccinated animal; and d) the potential for integration of plasmid DNA into salmon genomic DNA.

No data were provided on safety of administration of one dose of vaccine. This was in accordance with the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/123243) and CVMP scientific advice to the applicant which states that a one dose administration study need not be carried out as this aspect can be covered by the overdose test.

The overdose study was supportive of a minimum weight of 25 grams. Fish below the minimum weight were used and the majority of adverse reactions seen were associated with needle injury.

CLYNAV is recommended for use as a single administration, therefore no information has been provided in support of safety of the repeated administration of one dose. Furthermore, no data were provided to examine the effect of the vaccine on reproductive performance. This is in line with the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/123243-Rev.2) and CVMP scientific advice to the applicant and is in principle acceptable. The vaccine is contra indicated for use in broodstock.

An integration study was concluded that investigated gonads and muscle tissue. Gonad samples were collected 1 day after vaccination with a 10x dose of CLYNAV at a time when the levels of vaccine plasmid are highest according to the bio-distribution study provided. A method had been developed to extract and separate plasmid DNA from genomic DNA, then enrich for DNA containing plasmid sequence and analyse by sequencing. No evidence of integration has been found. The worst case LOD was used as the basis for the QRA. The QRA assessed the risk of transmission of integrated plasmid DNA into the genome of wild salmon and found the risk to be negligible. Different scenarios had been evaluated using a range of parameters which are relevant to a range of ecosystems and interaction scenarios relevant to production in Europe. In addition, a satisfactory consequence analysis has been conducted. The consequence of integration at muscle tissue is limited to the individual fish affected and there are no issues in terms of environmental safety.

Overall, it can be accepted that the consequences of integration, in terms of environmental effects on future populations of wild Atlantic salmon, are negligible.

It is accepted that the consequences of integration to individual fish are impossible to extend to the population level unless the change is heritable. It is also accepted that the likelihood of integration

occurring at the gonads is negligible; this is because the applicant's data show that plasmid is only detectable at a low level in gonads for a brief period (1 day) after administration, and is undetectable 7 days after vaccination. If integration had occurred, it would be expected that plasmid would be detectable after 1 day post vaccination. In addition, integration at the gonads would require a sequence of significant events to occur which are difficult to imagine occurring in practice. A reasoned argumentation was submitted by the current state of knowledge on DNA vaccines that concludes that the risk of integration is a theoretical rather than a real risk. In the absence of a mechanism for stably existing in the genome, it is not possible to envisage how such a transfer to the gonad of wild salmon could occur.

Nevertheless, an ERA was conducted which included a QRA, modified in response to questions and comments which concluded that the risk to the environment is effectively zero. Appropriate scenarios and parameters was used in the model, which included uncertainty about certain parameters allowing for a worst case scenario analysis for 40 generations (roughly equivalent to approximately 200 years), assuming a worst case at every generation. The QRA was conducted using a model which generates an output that is robust to extreme scenarios. The data provided support the conclusion that the risk of integration of CLYNAV is negligible and that the risk to the environment following the use of CLYNAV as recommended is acceptable.

Although CLYNAV is not a live vaccine, some of the same considerations are considered relevant for this vaccine. Specific studies to investigate the spread of the vaccine construct and dissemination in the vaccinated animal were submitted. Vaccine plasmid was shown to persist at the site of injection in muscle tissue until at least 822 days post-vaccination, however at very low levels.

User safety is considered and draws on information from the field use of the applicants' related product APEX-IHN, which is a DNA vaccine based on the same plasmid backbone and which has been used in Canada since 2005. Based on the assessment of the nature and likelihood of the hazards identified, the use of CLYNAV presents a negligible risk to the user. The risk of adverse reactions in the user has been adequately considered by the applicant, and has been supported by literature references concerning the use of candidate DNA vaccines in humans. The vaccine is not oil adjuvanted and therefore there are no risks associated with oil adjuvants. It is accepted that the risk from self-injection of a fish DNA vaccine is acceptable, the applicant has mitigated risks to the end user associated with vaccinating large numbers of small fish by increasing the originally proposed weight to 25g indicated on the SPC, in reality whilst this is the minimum authorised it will probably be used in larger fish.

CLYNAV consists of DNA and phosphate buffered saline only; it does not contain any adjuvants or preservatives. It is considered that the risk from consumption of residual vaccine is not a major safety concern as the vaccine DNA will be digested as would any DNA found within the normal diet. Given that there are no components of the vaccine for which an MRL would be required, a withdrawal period of zero days was acceptable.

Data were provided on interactions of the vaccine with two other veterinary immunological products, however although the safety of concurrent use of CLYNAV with two other vaccines has been demonstrated, in the absence of efficacy data regarding concurrent use, no claim for compatibility with any other veterinary medicinal product is made and the standard statement reflecting this is included in Section 4.8 of the SPC.

Data from field studies have not been provided. This can be accepted as no safety risk was observed in any of the studies conducted with CLYNAV, including the overdose study conducted with up to 10x the proposed dose. Taking into account that the product does not contain mineral oil adjuvant and is not injected ip, overall therefore there is no reason to consider that there is a need to follow longer term reactions in specific field studies.

Data from a bio-distribution study which followed fish for 822 days post vaccination, which is relevant for commercial production of farmed salmon, were provided. These data did not raise any significant safety concerns. In addition, the applicant investigated possible interactions with other products which showed that CLYNAV did not have an adverse effect when used in conjunction with routinely used oil emulsion vaccines. Pharmacovigilance data for the related DNA vaccine APEX-IHN (3 adverse event reports from >83 million Atlantic salmon vaccinated since 2005) were also provided and the relevance of which was adequately justified. Therefore, the applicant has provided data from a number of sources to adequately support the safety profile of CLYNAV over the average commercial life span.

An ERA was at the time submitted in line with the recommendations of the scientific advice from CVMP. This ERA was prepared in line with the requirements of EC Directive 2001/18 as well as the normal ERA for any immunological veterinary medicinal product in accordance with the requirements of EC Directive 2001/82, considering the risks to the target species, the environment and end user and consumer. The risk associated with the use of CLYNAV is negligible. This conclusion was based on a combination of factors where data have been generated or has been drawn from published literature, some of which includes comment from EFSA regarding the risk to the food chain and the environment. The proposed estimates of risk of genome integration and transmission to environmental bacteria rely to large extent on specific studies.

The data and risk assessment provided confirming support for an absence of risk of transmission of the selective marker from plasmid vaccine to environmental bacteria is satisfactory.

Part 4 – Efficacy

SPD is caused by several different subtypes of SAV. SAV1 was described as the causative agent of PD in farmed Atlantic salmon in Ireland and Scotland. The closely related sleeping disease virus (SDV or SAV2) has been reported to occur in Atlantic salmon in Norway and in fresh water rainbow trout in France, England, Scotland, Spain, Italy and Germany. The SAV3 has been described to cause PD in farmed Atlantic salmon and sea reared rainbow trout in Norway.

Based on partial sequence data, SAV strains can be assigned to six different groups, termed subtypes. While all outbreaks of sleeping disease examined to date have been as a result of infection with SAV2, outbreaks of SPD have been attributed to all six SAV subtypes. The subtypes show a degree of geographical separation in Atlantic salmon, with SAV3 only detected thus far in Norway, SAV 1, 4 and 6 found in Ireland and SAV 1, 2, 4 and 5 found in Scotland.

PD was first recognised in Scotland in 1976 but not actually described in the literature until 1984. For many years there was some controversy over the aetiology of the disease, with various nutritional and infectious causes being suggested. The causative agent was only isolated in 1995 and was shown to be an alphavirus, now known as SPDV.

CLYNAV is a vaccine against SPD which contains a DNA plasmid (pUK-SPDV-poly2#1) which encodes the major structural proteins of salmon alphaviruses. The plasmid was constructed based on RNA first isolated from partially purified SAV2 isolated from infected salmon tissues grown in tissue culture. Each 0.05 ml dose of vaccine contains 5.1 – 9.4 µg of DNA plasmid in a phosphate buffered saline solution. It is indicated for administration by i.m. injection to salmon at least 25 g in size. The claim is for "the active immunisation of Atlantic salmon to reduce impaired daily weight gain, and reduce mortality, and cardiac, pancreatic and skeletal muscle lesions caused by pancreas disease following infection with salmonid alphavirus subtype 3 (SAV3)".

Sufficient information was submitted to justify the choice of subtype used to construct the vaccine plasmid. The parental sequence for the SAV genes in CLYNAV was SAV2, however it has not been

investigated if the vaccine will protect against subtype 2. This vaccine is not directly akin to an attenuated form of live virus which would be expected to confer protection to the wild type parent strain. However, a sequence alignment also confirms high homology with published SAV sequences and that the differences are within strain variation. Challenge studies were only conducted using SAV3, which is documented to be exclusive to Norway. However, the applicant has provided a plausible justification that a vaccine produced against one PD virus isolate may be efficacious against multiple serotypes of SAV, thereby supporting the epidemiological relevance of the vaccine. However, since only SAV3 challenge was used in the pivotal seawater challenge efficacy study the applicant revised the proposed indication to protection against SAV3 only.

SPD is included in the list attached to the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/123243/2006-Rev.2). Some reduction in requirements compared to those mentioned in Annex I of Directive 2001/82/EC as amended may therefore be considered as outlined in the guideline.

Laboratory trials

The applicant has submitted reports of six laboratory trials designed to demonstrate the efficacy of the vaccine.

Severe cardiac and skeletal myopathies are key features of this disease and therefore it is appropriate to use these parameters in a laboratory model of the disease. Efficacy data from studies in which vaccinated fish were challenged in fresh water has been supplemented by data from saltwater challenge studies. These demonstrated similar heart histopathology to that observed in freshwater an efficacy laboratory study e.g. poor weight gain and mortality. The explanation of the clinical signs and lesions observed in naturally occurring PD is satisfactory. It is accepted that the parameters investigated in these studies are relevant to naturally occurring PD. The relevance of heart lesions as a parameter to measure disease following challenge has been satisfactorily addressed. A clinical benefit of vaccination on the reduction of additional histological lesions of PD is supported by the pivotal efficacy study

The virulent challenge strain used in the earlier efficacy studies carried out entirely in fresh water was isolated from tissue homogenates prepared from the heart of clinically symptomatic fish collected from an outbreak in Norway. The SAV challenge model did not result in mortality under laboratory conditions in fresh water, therefore heart and pancreas histopathology and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) were selected as appropriate measures to investigate the efficacy of the vaccine during freshwater challenge studies. However, mortality did occur following challenge of unvaccinated fish in a saltwater model.

Note that the challenge points within laboratory efficacy studies is expressed either in degree days (mean water temperature in °C multiplied by number of holding days) or calendar days (duration of immunity)

Proof of concept

A study was carried out to demonstrate the safety and efficacy of a plasmid DNA vaccine against SPD. Five groups of 440 salmon were used with an average weight of 44.9 g at the beginning of the study. Two groups received an experimental batch of CLYNAV at doses of 10 and 20 µg DNA/dose respectively. A third group received a commercial conventional vaccine against PD. The other two groups acted as controls and received either a commercial oil-adjuvanted vaccine that did not contain pancreas disease virus (an inactivated, oil adjuvanted multivalent vaccine against furunculosis, classical vibriosis, coldwater vibriosis, wound disease and infectious pancreatic necrosis) or PBS respectively. All fish except the PBS control group received a second vaccination with a commercial PD vaccine at 213 degree days after the

first vaccination. The fish were challenged by co-habitation with infected salmon either 518 degree days or 153 days at 12 ±1°C (5 months) after vaccination. Samples were collected from heart and pancreas tissue for histopathology and virus detection by RT-PCR on days 18, 22 and 26 post-challenge. Significantly reduced histopathology scores were recorded for both pancreas and heart tissues from fish vaccinated at both time points compared to the control groups and the group vaccinated with another commercial PD vaccine. The concentration of pancreas disease virus in heart tissue was also less in the groups vaccinated with the DNA plasmid than the other groups. There was no significant difference between the two different doses of DNA plasmid.

The vaccine batch used in this study was a development batch. The quantity of sc plasmid DNA was estimated using gel analysis to be 78.6% sc (compared to the open circle form), which is below the minimum specification of ≥ 90% scDNA. In addition, the fact that fish were vaccinated with another PD vaccine during the pre-challenge phase could have influenced the immune response against CLYNAV to some degree. The statistical analysis used for both the 518 degree day and 153 calendar day time points in this study were based on categorical variables (pancreatic necrosis, pancreatic inflammation, heart necrosis and heart inflammation) with data being ranked, transformed and used in an analysis of variance (ANOVA). No claims on the RT-PCR values, i.e. a reduction in viral load, are made. There was a clear positive effect of vaccination in both of the CLYNAV vaccine groups, thereby supporting proof of concept. However, the study was considered as only supportive of efficacy because the weight of the fish at the time of vaccination far exceeded the minimum weight recommended for vaccination.

To investigate efficacy following challenge in saltwater, a saltwater challenge model was developed. This study is described. Briefly, three tanks were used in this study; Tanks 1 & 2 were SAV 3-challenged, while Tank 3 was a negative control. Cohabitation challenged (i.e. SAV 3-challenged) fish from Tank 1 were used for serial sampling of blood and tissue for histological analysis throughout the in life phase of the study. RT-PCR was used to detect SAV3 in heart tissue samples. Fish in Tanks 2 (SAV 3-challenged) & 3 (saline mock-challenged / negative controls) were used to monitor clinical/behavioural signs and progression of PD in a relatively undisturbed environment, i.e. void of a sampling stressor; fish in these tanks were undisturbed after challenge until termination of the in life phase at 83 DPC. Heart, pancreas, and skeletal muscle samples were collected from ten fish from each of these tanks. All mortalities collected during the in-life phase were identified as either a naïve fish (i.e. SAV 3-challenged or negative control), or a trojan fish. Each of these fish were weighed, measured for fork length, and examined for the presence of gross abnormalities. Samples were also collected for blood and histological analysis. A weighted histological score index was calculated from the histological score data obtained from every fish within every tank.

Analysis of heart tissue from Tank 1 cohabitation challenged fish indicates that SAV3-positive samples begin to appear by 14 days post-challenge (with a 100% prevalence based on 5 samples). SAV3-positive RT-PCR prevalence remained ≥80% through to study day 41, after which it dropped, although prevalence remained in the 40 – 60% range throughout the remainder of the study. One of ten cohabitation challenged fish from Tank 2 (83 DPC) was SAV3-positive by RT-PCR, suggesting that this tank was also properly challenged. None of the ten samples tested from Tank 3 (83 DPC) were SAV3-positive (and all were β-actin positive), suggesting that this tank was not accidentally challenged with SAV3.

Throughout the length of the study, two SAV3-challenged fish and one trojan fish died in both Tanks 1 & 2, representing 2% and 4% of the SAV3-challenged population per tank, respectively. The kidney of each of these mortalities was cultured on BHI + 2% NaCl. Kidney cultures from one SAV3-challenged fish and one trojan fish from Tank 1 had mixed growth. The other SAV3-challenged fish kidney culture from Tank 1 produced no growth. None of the kidney cultures from Tank 2 produced any growth. These results suggest that none of these fish died from a systemic bacterial infection. No mortalities were observed in Tank 3.

Mean body weight change was significantly higher in the negative controls from Tank 3, with a mean weight change of 136.84 g (mean percent weight change of 131.8%) relative to SAV3-challenged fish from Tank 2 with a mean weight change of -8.55 g (mean percent weight change of 7.5%). Mean fork length was also statistically different between these populations, with mean length changes of 5.362 cm and 0.998 cm (mean percent length changes of 25.12% and 4.58%), for Tanks 3 & 2, respectively. After 83 days post challenge, notable differences in the overall size of negative control and SAV3-challenged fish were highly apparent.

The most notable clinical/behavioural differences that were observed between SAV3-challenged and negative control fish (from Tanks 2 & 3, respectively), were that the SAV3- challenged fish displayed inappetence (i.e. reduced feed intake and decreased interest in feed), faecal cast production, and lethargy, and were observed resting at the bottom of their tank for extended periods of time. SAV3-challenged fish in Tank 2 began to show signs of inappetence around the end of the second week post-challenge. By the end of the fourth week post-challenge, very few fish in this tank (approximately only 5-10%) showed an active interest in feed. This was in contrast to the appetite of negative control fish in Tank 3, which was normal except for 4 days during the three month study, three of which immediately followed a change in tank volume which was done to maintain stocking densities after continued growth in this tank.

Throughout the course of the challenge model development study, disease progression in red skeletal muscle started after pathologic changes had been seen in pancreas and heart but before such changes were seen in white skeletal muscle. Necrosis and inflammation in red skeletal muscle were apparent by 21 DPC, with both peaking in severity (mild to moderate) by 28 DPC, while necrosis and inflammation of white muscle was observed to peak at 41 DPC. Heart Index scores were lowest pre-challenge and increased through Day 28, and then generally decreased throughout the remainder of the study. Pancreas Index scores were lowest pre-challenge and increased through Day 14, and then decreased throughout the remainder of the study.

The results of challenge model development study demonstrated that the SAV 3 saltwater cohabitation challenge model is an effective means of inducing PD in naïve Atlantic salmon which could be used to investigate vaccine efficacy during challenge in salt water. The main features of PD were reproduced following saltwater challenge, although significant mortality did not occur during the timescale of this investigation.

Determination of the vaccine dose

A dose titration study was carried out in Atlantic salmon with an average weight of 11.6 grams. Triplicate groups of 39 to 41 fish per treatment, were vaccinated via i.m. injection with a single dose per fish of 0.46, 0.84, 1.97, 4.54, 8.47 or 18.16 µg pUK-SPDV-poly2#1 plasmid DNA diluted in 50 µl PBS or 50µl PBS as a negative control. Challenge was by co-habitation with infected fish on day 35 (388 degree days) post-immunisation. Efficacy was measured by determining the viral load in heart using RT-qPCR and tissue pathology by microscopic evaluation of heart tissue samples taken 19, 26 and 35 days post-challenge. The results showed significantly lower virus load and histopathological score in heart tissue for treatment groups receiving 4.54, 8.47 and 18.16 µg pUK-SPDV-poly2#1 plasmid as compared to the negative control and 0.46 µg pUK-SPDVpoly2#1 plasmid treatment groups at all points of time. Moreover, there were no significant differences between doses 4.54, 8.47 and 18.16 µg pUK-SPDV-poly2#1 plasmid by either SAV 3 RT-PCR or heart-pathology histopathology indexes at any point of time. The most efficacious dose was in the range of 4.54 to 18.16 µg plasmid DNA vaccine. It was concluded that there were significant differences between the negative control group and dose groups of 4.54 µg and above for the two efficacy parameters measured. The production of the vaccine batches used in this study was on an experimental scale and had some minor differences to the proposed production method, therefore the studies are only considered supportive.

A further dose titration study was carried out using saltwater challenge to demonstrate that the active component of the vaccine is sc pUK-SPDV-poly 2#1 plasmid and to quantify the minimum active concentration of sc pUK-SPDVpoly 2#1 plasmid required to elicit effective clinical protection. The intention was also to demonstrate the ability to detect, *in vivo*, a sub-potent batch of CLYNAV for future correlation with an *in vitro* quantification assay. An additional group was used to investigate the impact of residual genomic DNA on efficacy of the final product by comparing a commercial serial dose against a dose containing an equivalent concentration of sc pUK-SPDV-poly 2#1 plasmid with minimal residual genomic DNA.

Fish in eight groups of 30 fish were administered a single dose (0.05 mL) via i.m. injection of one of the following: a titrated dose of a commercial serial of CLYNAV, a laboratory preparation of CLYNAV with restriction enzyme digested active ingredient (pUK-SPDV-poly 2#1 plasmid), a laboratory preparation of the pUK-SPDVpoly 2#1 plasmid (from the serial master seed bank) with minimal residual gDNA, or PBS as the control veterinary product. The remaining 166 fish were held in a third tank and used as non-vaccinated, non-challenged (NVNC) controls (20 fish), Trojan shedders during challenge (120 fish), naïve fish to assess smolting status (20 fish) and fish to allow for incidental losses such as jumpers (6 fish). Following smoltification and an immunisation period of approximately 400 degree days (d.d.), the two tanks of vaccinated or mock vaccinated fish were challenged by introducing Trojan shedders experimentally infected with SAV 3. Efficacy was evaluated at 21 days post challenge (d.p.c.) (tank 1) and 49 d.p.c. (tank 2) by means of heart histology and RT-PCR to detect virus. A histological score index was calculated from data obtained from every fish within every group. The index was analysed using ANOVA to determine if differences exist among treatments. If death occurred, in addition to tests on heart tissue the kidneys were cultured to rule out a possible bacterial cause. At each assessment point, 10 NVNC fish were also sampled from tank 3 for comparative analyses. RT-PCR analysis of heart tissue in control and vaccinated fish demonstrated a valid SAV 3 challenge and confirmed that SAV was the primary pathogen in this challenge model.

The cumulative mortality results for 21 d.p.c. showed strong protection for the 100%, 50% and 25% doses with increasing mortality occurring as the dose decreased (10% and 5%). Digested CLYNAV had mortalities similar to the PBS control group (23% and 20% respectively). The laboratory (lab) preparation showed strong protection with a slight increase in mortality from the 100% batch (7% and 0% respectively). Statistics were not performed on the 21 d.p.c. results.

The cumulative mortality results for 49 d.p.c. were analysed and showed statistically significant protection for the 100%, 50%, 25% doses and lab preparation relative to the PBS control group. The 25%, 50% doses and lab preparation plasmid were not statistically different relative to 100% CLYNAV. The digested CLYNAV, 5% and 10% doses had mortalities not statistically different from the PBS control group.

Fish weight was measured for fish at vaccination and survivors, 21 (tank 1) and 49 d.p.c. (tank 2) to monitor weight change from vaccination through to challenge. Only the 49 d.p.c. body weight data were analysed. There was an overall statistical difference in post body weight between treatment groups and PBS after controlling for pre-weight. The mean body weight was significantly higher for 25%, 50%, 100% CLYNAV and lab preparation plasmid relative to the PBS control group. There were no statistically significant differences in body weights between 25%, 50% CLYNAV and lab preparation plasmid relative to 100% CLYNAV. There were no statistical differences in the body weights of 5% and 10% CLYNAV or digested CLYNAV relative to the PBS control group.

Histology was conducted on heart samples collected 21 d.p.c. Treatment with 100% CLYNAV resulted in near complete protection against the cardiac effects of alphavirus infection, whereas such protection was generally diminished in a dose-responsive fashion among fish that received lesser concentrations of CLYNAV. Digested CLYNAV provided no protection, but protection in fish that received the lab preparation

plasmid was roughly equivalent to that experienced by salmon of the 50% CLYNAV group. There was an overall statistical difference among treatment groups. There were no statistically significant differences for 5% and 10% CLYNAV or digested CLYNAV relative to the PBS control group. The mean heart index score was significantly lower for 25%, 50%, 100% CLYNAV, lab preparation plasmid and NVNC relative to PBS. The 25% and 50% CLYNAV heart index scores were significantly higher than the 100% CLYNAV group. There were no statistically significant differences for the lab preparation plasmid and NVNC relative to 100% CLYNAV.

It was concluded therefore that the minimum effective dose was 100% CLYNAV (5.6 µg scDNA/ 0.05 mL dose). The genomic DNA concentration of this batch was higher than the maximum specification. To address the possibility that genomic DNA could contribute to vaccine efficacy, adequate argumentation was provided based on analysis of the data and proposed revised gDNA specifications to satisfy this concern. The applicant is recommended to recalculate the acceptable gDNA range once at least 20 batches have been produced and to provide the information post-authorisation.

Onset and duration of immunity

A study was carried out to test the efficacy of the three batches of vaccine produced to demonstrate consistency of production. Salmon weighing between 10-20 g were divided into four groups, three of which were vaccinated with the consistency batches and the fourth received saline as controls. Efficacy was monitored by heart pathology of fish 24 days after challenge. Scores for all of the vaccinated groups were significantly lower than those for the controls. Two of the batches were slightly above the original minimum concentration (10.5 µg DNA per dose instead of 10.0 µg) and the other was at the original maximum titre (12.5 µg DNA per dose). The batch of vaccine used was produced from master seed lot # 22-Feb-2007 and this will be the same for all future commercial batches. However, the scDNA concentrations of the batches used were slightly higher (92.4 to 92.8 %) than the minimum now specified for the vaccine (90%) and this study was considered to be supportive only.

In the proof of concept study, fish were challenged at 153 calendar days (5 months) post-vaccination, although this was considered as a supportive study only. However, this study provides some useful information regarding immunity at 5 months post-vaccination, albeit in fish vaccinated at a larger weight than recommended. This study was conducted in freshwater. The smoltification process involves considerable physiological changes in the salmon and therefore the applicant was requested to provide data to confirm efficacy of the vaccine after the fish are transferred into salt water. Additional data were provided from a study conducted in a saltwater laboratory model that reproduced the typical clinical presentation of PD in Atlantic salmon observed in the field. The study employed the same laboratory model described in the challenge model development study and was designed to investigate both onset of immunity (OOI) and duration of immunity (DOI). The randomised, analytically blinded, controlled, single centre efficacy and DOI study used a single CLYNAV 0.05 mL dose administered intramuscularly in the target species Atlantic salmon parr, challenged using a SAV 3 cohabitation challenge model in saltwater.

In the OOI study groups of vaccinated fish (IVP) or control (CVP) fish injected with saline, with an average weight of 20.5 g, were challenged by co-habitation after smoltification (water temperature 12 ±2°C), at either 399 degree days to establish OOI or 92 days (approximately) 3 months post vaccination to establish DOI. Samples were collected on days 21, 52 and 84 post challenge. Relative weight gain was determined for each fish by subtracting the vaccination weight from the weight collected at sampling. Statistical analysis of survival was evaluated between IVP and CVP groups for both 399 d.d. and 92 days challenge tanks (Tanks 1 and 2, respectively). Mortality data were collected over the course of the study with the final sampling of the study considered as 'end the study' for mortality analysis purposes and all surviving fish were considered as censored. Kaplan-Meier product limit survival curves were presented for all groups. The kidneys of all mortalities were cultured to rule out a presumptive bacterial cause for mortality, and hearts of all mortalities were collected. At each of the sample times, tissue samples were

collected from up to 30 fish in the IVP and CVP groups and 10 fish from the NVNC group. Weights and any gross abnormalities were recorded. Following euthanasia, samples of somatic muscle, heart and pancreas were collected for histological examination. A histological score index was calculated from data obtained from every fish within every group. RT-PCR was used to confirm SAV 3 infection.

At the end of the challenge period (approximately 84 d.p.c) for Tank 1 (challenged 399 degree days post vaccination), the average bodyweight change was 102.3 g in the IVP group and 18.5 g in the CVP group, which was significantly different. Significant differences between average index scores for IVP and CVP in the heart existed and were significantly higher i.e. worse, within the CVP group, compared to the IVP group regardless of the sample time, post challenge. Average index scores for exocrine pancreas were also significantly higher in the CVP group compared to fish within the IVP group at all 3 time points, 21, 52 and 84 d.p.c. Furthermore, the average index score for red muscle was significantly higher in the CVP group compared to fish within the IVP group at all 3 time points, 21, 52 and 84 d.p.c. and the average index score for white muscle was significantly higher in the CVP group compared to fish within the IVP group at 52 and 84 d.p.c. The Kaplan-Meir survival estimate post SAV3 challenge was significantly higher in the IVP group than in the CVP group.

At the end of the challenge period (approximately 84 d.p.c.) for Tank 2 (challenged 92 days post vaccination), the average bodyweight change was 196.9 g in the IVP group and 54.4 g in the CVP group, which was significantly different. Significant differences between average index scores for IVP and CVP in the heart and exocrine pancreas existed and were significantly higher i.e. worse, within the CVP group, compared to the IVP group at 21 d.p.c. The average index score for red muscle was significantly higher in the CVP group compared to fish within the IVP group at 21 d.p.c. time points, whereas there was no significant difference between the average index for white muscle between IVP and CVP at the same time point. The Kaplan-Meir survival estimate post SAV3 challenge was significantly higher in the IVP group than in the CVP group.

This study demonstrated the efficacy of CLYNAV following challenge in saltwater. The vaccinated groups showed improved weight gain, reduced heart, pancreas and skeletal muscle lesions and reduction of death following challenge at 399 degree days and 92 days post vaccination. The genomic DNA concentration of the batch of CLYNAV used for this study was 69.9 µg/ml, which is outside the specification of ≤59.8 µg/ml maximum. The applicant acknowledged this but considered that this would not have affected the efficacy of the vaccine. In view of the absence of clear evidence that a higher gDNA concentration than standard would enhance efficacy, and in the interests of animal welfare, the suitability of this batch to demonstrated efficacy was accepted.

Although there was clear evidence of a successful SAV challenge based on the histopathological parameters, there was also evidence of bacterial infection. A recalculation of the survival results were submitted, removing the mortalities associated with bacterial infections. These results confirmed that mortalities due to SAV 3 infection were significantly reduced in the CLYNAV-vaccinated groups compared to the saline controls. A reduction of mortality claim was therefore supported

This study demonstrated that following an immunisation period of either 399 degree days (d.d.) or 92 days (approx. 3 months), survival among CLYNAV vaccinated fish was significantly higher than saline-vaccinated control fish over 12 subsequent weeks exposure to SAV 3. The average bodyweight change of salmon vaccinated with CLYNAV in this study was also significantly higher than equivalent saline-vaccinated control fish at 12 weeks post SAV 3 challenge. On average, CLYNAV-vaccinated salmon gained 102.3 g or 197.0 g compared to saline-vaccinated control fish that gained only 18.5 g or 54.4 g for the 399 d.d. and 3 month immunisation periods respectively. Expert opinion confirms that although impaired weight gain can be considered a secondary effect of infection, vaccination with CLYNAV has a direct effect in reducing the adverse effect on weight gain caused by SAV infection and, while this has only been

demonstrated in a laboratory situation, it is indicative of what is likely in the field. It is considered that an indication for reduction of the adverse effects on weight gain is supported by the data presented.

This study provides adequate support for the revised claim proposed by the applicant. The batch of vaccine used contained 5.1 µg scDNA (plasmid), which is the minimum specified in the SPC and the average weight of the fish (20.5 g) accords to the minimum specified (25 g).

Field trials

No field trials have been presented in the dossier. With reference to the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species (EMA/CVMP/IWP/123243/2006-Rev.2) which states that if sufficient laboratory studies are performed, field studies are not required, although efficacy data from the field may be required as a follow-up measure. The CVMP had previously provided scientific advice that the omission of field studies can be considered acceptable as long as all relevant laboratory studies are performed. The design of the studies must be suitable to support the claims proposed for the vaccine under field conditions and it is also expected that data regarding DOI will be generated for the vaccine. The CVMP advice further pointed out that for fish it is particularly difficult to generate these data under true laboratory conditions and therefore the applicant was recommended to generate some field data. However, it was accepted that it was not practically possible to carry out suitable trials in the field and therefore demonstration of a meaningful DOI in a controlled laboratory environment was accepted since the design of the study was adequately representative of field use.

The data presented provide support for time of OOI (399 degree days) and DOI (3 months at12 ±2 °C) following challenge in saltwater. The limitations that the applicant faced with respect to conducting field trials given the ongoing discussions regarding the potential GMO status of vaccinated fish at the time of vaccine development led the applicant to develop what would represent the most high risk production setting and challenge in conditions that mimicked marine conditions in the new saltwater challenge model.

The suitability of a DOI of 3 months post vaccination at 12 ±2°C was questioned with respect to the likely timing of infection in the field and clinical relevance noting, in particular, that fish may be infected with SPDV and develop disease during the entire period at sea. CVMP accepted that the DOI demonstrated can be considered clinically relevant in that young fish, which are most susceptible to SAV3, will be protected for at least the period immediately after sea transfer. There are scientific reasons, based on knowledge of the immune response in general, why it would not be expected for immunity to suddenly drop off after 3 months. In addition, the plasmid does not disappear from the injection site after 3 months and remains for a prolonged period in the vaccinated fish. In addition, there is supportive evidence that the response is likely to last for longer than the 3 months shown in the pivotal study, provided by freshwater study (approx. 5 months.

It was therefore concluded by the CVMP that the duration of immunity demonstrated is acceptable.

Overall conclusion on efficacy

Reports of three detailed and well-designed efficacy studies were provided to establish proof of principle, efficacious dose, OOI and to confirm the efficacy of the three batches of finished product manufactured to demonstrate consistency of production. In these studies vaccinated fish were challenged in fresh water and were therefore considered only of supportive value. The first two studies were conducted using experimental batches of vaccine that did not correspond to the method of manufacture described in part 2 of the dossier and can therefore be considered only of value in establishing the 'proof of principle' and

determining a suitable quantity of DNA plasmid for the vaccine. The batches used for the third study were those manufactured to demonstrate consistency of production. The efficacy parameters investigated by the applicant were more restricted in the later studies. In the first study, both pancreas and heart histology and presence of SAV3 virus in heart tissues were studied. In the second study only heart histopathology and virus load was studied, while in third only heart histopathology was reported. Accordingly, the only efficacy supported by the results of the latter study was the reduction of heart pathology caused by SAV3 virus infection.

As SPD is predominantly a disease of salmon in the sea studies using a seawater challenge model were submitted. Development of this model is described in a fourth study The seawater challenge study, demonstrates that following an immunisation period of either 399 degree days (d.d.) or 92 days (approx. 3 months), survival among CLYNAV vaccinated fish was significantly higher than saline-vaccinated control fish over 12 subsequent weeks exposure to SAV3. The average bodyweight change of salmon vaccinated with CLYNAV in this study was also significantly higher than equivalent saline-vaccinated control fish at 12 weeks post SAV 3 challenge. On average, CLYNAV-vaccinated salmon gained 102.3 g or 197.0 g compared to saline-vaccinated control fish that gained only 18.5 g or 54.4 g for the 399 d.d. and 3 month immunisation periods respectively. This study provides adequate support for the claim proposed. The batch of vaccine used contained 5.1 µg sc (plasmid) DNA, which is the minimum specified in the SPC and the average weight of the fish (20.5 g) accords to the minimum specified (25 g).

Some data based on homology between SAV subtypes to support that the vaccine is epidemiologically relevant to the overall EU situation were provided, however the claim proposed is limited to SAV3 on the basis that this is the only subtype for which protection from challenge has been demonstrated.

The consistency of histopathological observations in salmon hearts in one study, the field and subsequent studies plus the correlation between microscopic lesions in the pancreas, heart and skeletal muscle and reduced clinical signs (weight loss) or mortality post challenge indicate that the laboratory assessments of CLYNAV efficacy are relevant indicators of expected field performance. The additional data presented in later studies are consistent with histopathological observations from the earlier efficacy study. In particular, lesions observed in cardiac tissue 24 days post SAV3 exposure in a freshwater laboratory study, were consistent to those made in naturally occurring pancreas disease in the field and observations at 21 d.p.c. in subsequent saltwater laboratory studies.

However, it is noted that the seawater challenge study, reflects the most high risk production setting for SAV3 exposure, because the study conditions represented intensive farming conditions with early induction of smoltification and a short interval of calendar days between initial vaccination at minimum weight and transfer to seawater. For less intensive farming systems, vaccinated fish may not be transferred to sea water within 3 months of vaccination. Challenge was conducted at two time points in sea water conditions, both of which were shortly after sea water transfer (8 calendar days and two months, respectively). The demonstrated DOI at 3 months post vaccination at approximately 12 ±2°C °C is noted and the applicant has provided a justification for the general clinical relevance of this period. There is supportive evidence that the response is likely to last for longer than the 3 months shown in the pivotal study, provided by freshwater study (approx. 5 months). In addition, it would not be expected for immunity to suddenly drop off after 3 months, particularly since the plasmid does not disappear from the injection site after 3 months and remains for a prolonged period. It is therefore concluded that the DOI demonstrated so far is sufficient for a marketing authorisation to be granted.

In addition, the applicant is recommended to recalculate the acceptable gDNA range once at least 20 batches have been produced and provide the information post-authorisation.

Part 5 – Benefit-risk assessment

Introduction

CLYNAV is a vaccine against SPD which contains a DNA plasmid (pUK-SPDV-poly2#1) which encodes the major structural proteins of salmon alphaviruses. There are no DNA vaccines currently authorised for use in animals in the EU. The vaccine is presented as a solution for injection. The dose is 0.05 ml containing between 5.1 to 9.4 µg of scDNA, for administration by the i.m. route to small fish, 25 grams and upwards.

SPD is listed in the appendix of the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMA/CVMP/IWP/123243/2006-Rev.2) and therefore appropriate reduced data requirements have been taken into account in the assessment.

The dossier has been submitted in accordance with Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

The proposed indication for the vaccine is for active immunisation of Atlantic salmon to reduce impaired daily weight gain, and reduce, mortality and cardiac, pancreatic and skeletal muscle lesions caused by PD following infection with SAV3. OOI occurs within 399 degree days (mean water temperature in °C multiplied by number of holding days) following vaccination.

DOI has been established at 3 months post vaccination at approximately 12 ±2°C.

Challenge studies have been conducted in both freshwater and sea water models. The demonstrated DOI in the sea water challenge model is 3 months at 12 ±2°C. This is not sufficient to demonstrate that the vaccine would be efficacious throughout the period that salmon are at risk but is clinically relevant, especially for young fish immediately after sea transfer.

Additional benefits

The vaccine is not adjuvanted and is formulated in buffered saline and is administered by the i.m. route. There are no risks of longer term lesion development in fish and down grading at slaughter, which has welfare benefits.

The vaccine does not contain any adjuvant and therefore has an improved user safety profile because the risk frequently associated with accidental self-injection with adjuvants are absent with the use of this vaccine.

CLYNAV can be administered to young fish from a weight of 25 g and therefore provides early protection.

Efficacy has been demonstrated against challenge with SAV3 and only protection against SAV3 is claimed. This is a scientific rationale to indicate that the vaccine should be efficacious against all subtypes of the virus, however this has not been confirmed in this application.

It is reasonable to infer that immunity will persist for longer than the minimum established given supporting challenge data in freshwater at 5 months post vaccination, the general fact that immunity does not suddenly wane and that the vaccine plasmid persists at the vaccination site.

The product is a non-adjuvanted, well purified and consistently produced and controlled active ingredient. An *in vitro* potency test is used for batch control of potency which means no use of experimental fish on a routine basis for batch release typically associated with fish vaccines. This confers a positive 3Rs aspect to this vaccine. This *in vitro* method of measuring potency is fully representative of the efficacy of the product in the target animal and eliminates biological variation in the batch potency test, which enables consistent production and efficient batch control.

Risk assessment

Main potential risks have been identified as follows:

Potential risks to quality:

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Potential risks to the vaccinated fish are:

The vaccine can cause local reactions at the injection site. No specific reactions to the vaccine were observed in the overdose safety study but macroscopic needle damage was common at the site of injection following administration. This was considered to be a result of use of the i.m. route of injection and judged to have negligible consequences for the fish and was therefore considered acceptable.

The vaccine can cause systemic reactions. Transient systemic reactions such as changes in the swimming behaviour, pigment changes and inappetence are very commonly observed in vaccinated fish. Similar reactions were also seen in control fish.

A study to investigate whether the DNA of the plasmid could become integrated into the chromosomal DNA of the fish has been carried out using state of the art methods and found no evidence of integration. Current scientific knowledge is that integration and transfer of this integrated DNA to future generations of fish is a theoretical risk rather than a real risk.

A QRA of the risk of transfer of integrated vaccine plasmid into the wild salmon population has been conducted using extreme worst case scenarios and concluded that this risk to the environment is effectively zero.

Potential risks to the user include:

The person(s) administering the vaccine could inoculate themselves with the vaccine. Pharmacovigilance data have been provided for a related product (APEX-IHN) not authorised in the European Union, which has the same plasmid backbone and manufacturing process. The product has been licensed for use in Canada since 2005. Pharmacovigilance data covering the period 01 January 2005 to 06 February 2013, during which more than 83 million fish were vaccinated with APEX-IHN, reports a single accidental injection of a human. No signs resulting from injection could be seen one day after exposure, and no long term adverse reactions were reported. The minimum weight indicated in the SPC has been increased to 25 g, which reduces the potential risk of self-injection associated with vaccination of large numbers of small fish. A QRA has been conducted and concluded that the risk to humans is negligible.

Potential risks to the environment include:

Spillage of the vaccine would expose humans possibly other animals and also the environment to the product. The absence of live organisms and the innocuousness of the components of the phosphate buffered saline solvent indicate that the risk from these factors is likely to be low. A QRA has been conducted on a worst case and concluded that the risk to the environment from spillage is negligible.

Some of the vaccine could leak out from the injection site of the fish and get into the surrounding water which may affect the environment. The quantity of product that might leak from a single injection site is small and would be greatly diluted by the surrounding water A QRA has been conducted based on a worst case and found the risks associated with product leakage are negligible.

The water surrounding the vaccinated fish will be released into the sea with the fish when they are transferred to sea from the freshwater hatchery site and any remaining DNA plasmid or parts of it may contaminate the environment (other fish, sea water, sediments).

An ERA has been conducted based on a worst case and allows to conclude that the risks associated with product contamination of the environment are negligible.

Fish may escape from the containment tanks or nets in seawater and come into contact with wild fish. While measures are generally in place to prevent this happening some fish on occasions do escape and have been known to breed with wild salmon. There would be a risk of propagating the plasmid if it became integrated into the salmon genome. A study to investigate this possibility has been carried out using state of the art methods and found no evidence of integration. Current scientific knowledge is that integration and transfer of this integrated DNA to future generations of fish is a theoretical rather than a real risk. A QRA of the risk of transfer of integrated vaccine plasmid into the wild salmon population has been conducted using extreme worst case scenarios and concluded that this risk to the environment is effectively zero.

No specific safety studies in broodstock have been provided. However, use in broodstock is contraindicated on the SPC, therefore this risk is adequately mitigated.

Potential risks for the consumer include:

Humans or animals eating the vaccinated fish may be exposed to the DNA of the plasmid or parts of it, which is comparable to the potential risks associated with consumption of recombinant plant DNA in either animals or the human gastrointestinal tract which has been considered by the European Food Safety Authority (EFSA). The conclusion was that after digestion biologically active genes and proteins are rapidly degraded into short DNA or peptide fragments. It is considered that the risk from consumption of residual vaccine is not a major safety concern as the vaccine DNA will be digested as would any DNA found within the normal diet. A QRA has been conducted and concluded that the risk to humans is negligible.

Given that there are no components of the vaccine for which an MRL would be required, a withdrawal period of zero degree days was acceptable.

Risk management or mitigation measures

A warning in case of self-injection when the vaccine is being administered has been included. The SPC will include the standard warning: 'In case of accidental self-injection seek medical advice immediately and show the package leaflet or the label to the physician.' which is appropriate.

The vaccine is contraindicated for use in broodstock. This is not intended to mitigate the potential risk of transfer of integrated plasmid DNA into the wild salmon genome. This risk has been considered above and found to be effectively zero.

Appropriate information has been included in the SPC and other product information 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 vaccine induces an immune response in Atlantic salmon capable of reducing impaired daily weight gain, and reducing mortality and cardiac, pancreatic and skeletal muscle lesions caused by PD following infection with SAV3.

The protection provided is clinically relevant and provides direct benefit to the target species in terms of improved health and welfare.

The benefit-risk balance is between demonstrated significant benefits to the target species and the end user, compared to a theoretical risk to the environment.

The vaccine is not adjuvanted and is formulated in buffered saline and is administered by the i.m. route. There are no risks expected of longer term lesion development in fish and down grading at slaughter which has both welfare and production benefits.

The vaccine does not contain any adjuvant and therefore has an improved user safety profile because the risk frequently associated with accidental self-injection with adjuvants are absent with the use of this vaccine.

The vaccine uses an *in vitro* potency test for batch control of potency which has benefits for 3Rs (replacement, reduction, refinement) through the reduced use of experimental fish on a routine basis for batch release typically associated with fish vaccines. This confers a positive 3Rs aspect to this vaccine. The *in vitro* test also eliminates biological variation leading to a more robust batch test.

The vaccine has been shown to cause no specific local reactions in the target species other than macroscopic needle damage at the site of injection. The risks to the end user and the consumer have been assessed and shown to be negligible.

The vaccine has shown transient systemic reactions such as changes in the swimming behaviour, pigment changes and inappetence, which are very commonly observed in vaccinated fish for a few days after vaccination. Similar reactions were also seen in control fish.

The most significant risk considered is whether the DNA of the plasmid would become integrated and passed to future generations of fish. This has been investigated in specific studies and the risks assessed and quantified and been shown to be effectively zero, therefore a theoretical risk rather than a real risk.

The formulation and manufacture of CLYNAV is adequately described and set specifications will ensure that a finished product of consistent quality will be produced. CLYNAV is well tolerated by the target animals and presents an acceptable risk for users when used as recommended and the environment and appropriate warnings have been included in the SPC. The withdrawal period is set at zero degree days.

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

Conclusion on benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall. The product has been shown to be efficacious for the indication "For the active immunisation of Atlantic salmon to reduce impaired daily weight gain and reduce mortality, and cardiac, pancreatic and skeletal muscle lesions caused by pancreas disease following infection with salmonid alphavirus subtype 3 (SAV3)".

The product is unadjuvanted purified DNA plasmid in buffered saline and therefore has significant benefits for safety of the target species (injection site reactions) and end user (accidental self-injection) in terms of lower severity of reactions.

The product has animal welfare benefits since potency is controlled by an *in vitro* assay rather than a more typical *in vivo* assay. The formulation and manufacture of CLYNAV is well described and specifications set will ensure that product of consistent quality will be produced.

It is well tolerated by the target animals and presents a low risk for users and a negligible risk to the environment and appropriate warnings have been included in the SPC. The withdrawal period is set at zero degree days.

Overall conclusion on the application

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for CLYNAV is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned medicinal product.