28 August 2020
EMA/457918/2020
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Innovax-ND-ILT (EMEA/V/C/005190/0000)

Vaccine common name: Marek's disease vaccine, Newcastle disease vaccine & infectious laryngotracheitis vaccine (live recombinant)

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

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Introduction

The applicant Intervet International B.V. submitted on 18 July 2019 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Innovax-ND-ILT, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 11 October 2018 as Innovax-ND-ILT has been developed by means of a biotechnological process.

The applicant applied for the following indications: for active immunisation of one-day-old chicks and embryonated chicken eggs:

- to reduce mortality and clinical signs caused by Newcastle disease (ND) virus
- to reduce mortality, clinical signs and lesions caused by avian infectious laryngotracheitis (ILT) virus and Marek's disease (MD) virus.

The active substance of Innovax-ND-ILT is a live recombinant turkey herpesvirus (HVT) expressing the F protein of Newcastle disease virus and the gD and gI glycoproteins of infectious laryngotracheitis virus. The product is intended for *in ovo* or subcutaneous use.

Furthermore, the CVMP considers that the live recombinant turkey herpesvirus (HVT) expressing the F protein of Newcastle disease virus and the gD and gI glycoproteins of infectious laryngotracheitis virus is a new active substance, as claimed by Intervet International B.V..

Innovax-ND-ILT is a frozen cell suspension stored in liquid nitrogen. It is presented in 2 ml sealed glass ampoules containing 2,000 or 4,000 doses of Marek's disease vaccine, Newcastle disease vaccine & infectious laryngotracheitis vaccine (live recombinant). The solvent is presented in plastic bags containing 400 ml and 800 ml respectively. Dilution with the solvent is required prior to injection into chickens or embryonated chicken eggs.

The rapporteur appointed is Jacqueline Poot and the co-rapporteur is Esther Werner.

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

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (dated 1 July 2018) which fulfils the requirements of Directive 2001/82/EC was provided. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

The active substance and (frozen) vaccine are manufactured either at the Intervet Inc. site in Millsboro US or at the Intervet International site in de Bilt, the Netherlands.

Batch release for the EU will be performed at the Intervet International site in Boxmeer, the Netherlands.

Solvent batch release is performed at the Intervet International site in Boxmeer in the Netherlands.

For the sites listed above, appropriate and valid Good Manufacturing Practice (GMP) certificates were presented. Specific inspections are currently not required.

Overall conclusions on administrative particulars

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

The GMP status of all manufacturing sites has been satisfactorily established and is in line with legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The vaccine consists of a deep frozen suspension of cell-associated recombinant Herpes Virus of Turkey (HVT) containing the F gene from NDV and the gD and gI glycoproteins of infectious laryngotracheitis virus, at a titre between $10^{3.3}$ and $10^{4.3}$ plaque forming units (PFU) per dose. Stabilisers (bovine serum, veggie medium) and a cryoprotectant (DMSO) are included in the formulation.

The solvent is a sterile, watery solution which contains stabilisers (sucrose, NZ-amine), a buffering agent (potassium dihydrogen phosphate) and a colouring agent (phenol red). The vaccine is mixed with the solvent prior to subcutaneous injection into chickens or *in ovo* vaccination of embryonated chicken eggs.

Container and closure

The vaccine is filled in 2 ml heat-sealed type I sterile glass ampoules in accordance with European Pharmacopoeia (Ph. Eur. 3.2.1). The solvent is filled in 400 and 800 ml multilayer plastic (MLP) bags (in accordance with European Pharmacopoeia (Ph. Eur. 3.2.2.1). Forming, filling, closing/sealing and terminal heat sterilisation of the bags is performed in a continuous process. Specifications and certificates demonstrating Ph. Eur. compliance were provided for the ampoules and bags.

Product development

Innovax-ND-ILT is a frozen cell-associated live virus vaccine containing a recombinant Herpes Virus

of Turkey (HVT) with genes from Newcastle Disease Virus (NDV) and Infectious Laryngotracheitis Virus (ILT). The use of a vector vaccine has the potential to circumvent safety issues in young chicks as well as efficacy problems encountered with (live) vaccines applied in young birds with maternal derived immunity. HVT is an apathogenic (serotype 3) virus related to MDV and is widely used as MD vaccine strain. Since HVT hardly spreads, is fully apathogenic to all avian species and not infectious to any other species, it forms an appropriate vector species. The same vector (HVT FC-126) is already used in other vaccines from the applicant (Innovax-ILT, Innovax-ND-IBD).

The production system and pharmaceutical form is the same as for other Marek vaccines routinely manufactured by the applicant. The solvent used with the vaccine is the same as for the other live Marek vaccines manufactured by the applicant. An indicator (phenol red) is included to enable a check on filling of the automated vaccination equipment in the field.

The vaccine was originally developed for the US market. The production process of the US registered product is identical to the product intended for the EU market as described in this registration dossier. The only difference is that gentamicin (0.5 mg/ml) is added to the final product intended for the US market. Gentamicin is not added to the product for the EU market. The use of US batches for the clinical studies in considered acceptable.

Tests for titration of the vaccine strain, sterility of the finished product and absence of mycoplasma in the finished product were appropriately described and validated.

Description of the manufacturing method

Primary chicken embryo fibroblast (CEF) cell suspensions in veggie medium are prepared from SPF eggs. The HVT/NDV/ILT virus seed is passaged on CEF monolayers to manufacture finished product. Cells are harvested using papain, counted, cell concentration is adjusted and stabiliser is added. The cell suspension is then filled in sterile 2 ml glass ampoules by automated filling and flame sealing. After labelling the product is frozen in a program freezer and stored in liquid nitrogen.

The solvent used is routinely supplied with existing MD vaccines from the applicant. The solvent is prepared by a simple mixing process and sterilised filtered into a sterile bulk vessel. After interim storage the solution is filled into MLP bags.

The manufacturing of the active substance and the finished product are described satisfactorily.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Certificates of analysis have been provided for the following starting materials: SPF eggs, bovine serum, DMSO, Sucrose, Pancreatic digest of casein (USP/NF), Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, Phenolsulfonphthalein and water for injections. All conform to specifications in the respective Ph. Eur. monographs.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

A Turkey Herpesvirus vaccine strain (HVT FC-126) was genetically modified by insertion of the NDV F gene and the ILT gD and gI genes and regulatory sequences, to generate HVT/NDV/ILT. The

HVT/NDV/ILT virus was multiplied on CEF cells, plaque purifications were performed, expression of NDV F and ILT gD and gI proteins was verified during passaging. One batch was selected to prepare the pre-master. This pre-master seed was used to establish the master seed.

The Master Seed Virus (MSV) was produced in CEF cells, supplemented with bovine serum and DMSO, filled in ampoules and stored in liquid nitrogen. Sterility, absence of mycoplasma and extraneous agents were tested in accordance with relevant Ph. Eur. monographs (resp. 2.6.1, 2.6.7 and 2.6.24). Identity was confirmed.

Working Seed Virus (WSV) is prepared as described for the finished product. WSV is tested for Sterility, absence of Mycoplasma and Extraneous agents. In the production facilities in the US, WSV is tested in accordance with 9CFR requirements, while in the EU facilities this is done in accordance with relevant Ph. Eur. monographs.

Starting materials not listed in pharmacopoeia include veggie medium, veggie protease and leupeptin; example certificates of analysis are provided.

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

Information regarding the qualitative and quantitative composition of all culture media, their treatment processes and their storage conditions is provided in the dossier. All components are either tested for or treated to ensure that there are no contaminants; no materials of animal origin are used.

The maximum residual amount of antibiotics in the finished product was calculated and shown to be several orders of magnitude below the MRL limit (neomycin) or levels allowed in vaccines (polymyxin B).

The applicant has provided a risk assessment regarding TSE, in accordance with commission directive 199/104/EC and note for guidance EMEA/410/01 REV 2. Starting materials of animal origin include the HVT/NDV/ILT Seed Virus, SPF chicken eggs or CEF cells and Bovine serum. Materials of avian origin are not considered a risk for TSE contamination. For the materials of bovine origin (serum), only materials for which an EDQM Certificate of Suitability has been issues are used (CoS are provided). The risk of transmission of TSE is estimated to be zero based on the starting materials. Moreover, the product is intended for poultry, a non TSE susceptible species.

Control tests during the manufacturing process

The applicant presented in-process data for the manufacture of three consecutive antigen bulks. During the manufacture of the antigen the following tests are carried out: Cytopathic effect (CPE) before harvest, cell count after harvest, filling volume during filling. Test descriptions and the limits of acceptance were presented. The in-process tests are deemed to be adequate to control all the critical steps in the manufacturing.

Control tests on the finished product

General tests are not performed on the finished product since this is stored in liquid nitrogen. General tests on the solvent consist of clarity (Ph. Eur. 2.2.1) and appearance (colour). Physicochemical tests on the solvent are: pH (Ph. Eur. 2.2.3), sucrose and potassium phosphate (Ph. Eur. 2.3.1) content, and filling volume. Sterility is tested in accordance with Ph. Eur. 2.6.1.

Tests performed on the finished product are: batch potency (virus titre) including identity, sterility (Ph. Eur. 2.6.1), absence of mycoplasma (Ph. Eur. 2.6.7) and absence of extraneous agents (2.6.25).

The description of the methods used for the control of the finished product and the specifications were provided. Virus titration and identity testing was appropriately validated. Suitability of the Ph. Eur. tests for sterility, mycoplasma and extraneous agents was confirmed.

The PCR test for detection of mycoplasma was appropriately validated and may be used alternatively to the culture method.

Batch-to-batch consistency

Results of finished product tests for 3 consecutive batches manufactured at the site in Millsboro and 3 batches manufactured at the site in de Bilt are provided, all batches conform to the release requirements. Results of finished product tests for 3 consecutive batches of the solvent filled in MLP bags are presented. All batches conform to the release requirements.

Stability

The finished product is stored in liquid nitrogen (or in the gas phase) for a maximum of 36 months. The results of a 39 months stability study were provided. The product was tested for HVT/NDV/ILT titres; tests for integrity of the closure (sterility) are not required in airtight sealed glass ampoules. Results up to 39 months (3 batches) show no decrease of titre.

In-use stability was investigated for the diluted vaccine suspension. Results show no change in titre after 2 hours at 15-25 °C. A two-hour in-use period is considered justified.

A stability study was performed using solvent batches filled in MLP bags (200 and 1000 ml). Sucrose, pH, clarity, appearance and sterility were tested at regular intervals. Bags were stored at 25 °C for a maximum of 24 months. All parameters remained within specifications throughout the storage period. The proposed shelf life of 24 months for solvent in MLP bags is considered justified.

Overall conclusions on quality

Innovax-ND-ILT is a live recombinant vaccine for active immunisation of chickens against Marek's Disease, Newcastle disease and Infectious Laryngotracheitis. The vaccine is available in ampoules containing 2000 or 4000 doses and is diluted before use in solvent supplied in plastic bags containing 400 or 800 ml.

One dose of vaccine contains ≥ $10^{3.3}$ and ≤ $10^{4.3}$ PFU* of Innovax-ND-ILT virus strain HVT/NDV/ILT as active ingredient. The virus is grown on chicken embryo fibroblast (CEF) cells produced from embryos obtained from SPF chicken flocks. The manufacturing method can be considered as standard for this type of vaccine. Cells containing the virus are harvested and combined with bovine serum and a cryoprotectant (DMSO) to allow storage in liquid nitrogen.

Appropriate procedures have been implemented to ensure the absence of extraneous agents in starting materials of animal origin. A TSE risk assessment for the starting materials used is provided. The risk that the final product may transmit TSE to the target animal is considered negligible.

The production method, including appropriate in-process controls and quality controls on the finished product together with control of the starting materials, ensure a consistent quality of batches of vaccine. The whole production process was evaluated at production scale and shown to

be consistent.

Results of the stability tests for final product showed no loss in infectivity titre during a 39 months storage period in liquid nitrogen. The data support the proposed 36 months shelf life. Stability data of reconstituted product show that the vaccine remains stable at room temperature for 3 hours; the proposed 2 hours in-use shelf life is sufficiently supported.

The proposed shelf life of 24 months for solvent in MLP bags is considered justified.

In conclusion, the production process is adequately described and controls in place are appropriate to ensure the quality of the product at release and throughout the shelf life.

Part 3 – Safety

Introduction and general requirements

The active substance of Marek's disease vaccine (MD), Newcastle disease vaccine (ND) and infectious laryngotracheitis (ILT) vaccine (live recombinant) of Innovax-ND-ILT, a recombinant HVT virus, is a new active substance not authorised for a veterinary medicinal product in the EU before. A full safety file in accordance with Article 12(3)(j) has been provided.

The indication for use of Innovax-ND-ILT is the active immunisation of poultry against MD, ND and ILT. The vaccine is intended for chickens for subcutaneous (s.c.) administration at one day of age or *in ovo* vaccination in embryonated chicken eggs. The volume of one dose is 0.2 ml for s.c. administration and 0.05 ml for *in ovo* vaccination. Innovax-ND-ILT contains at least $10^{3.3}$ but not more than $10^{4.3}$ PFU recombinant HVT per dose as active ingredient. Before use, the vaccine is diluted in solvent for cell-associated poultry vaccines (previously known as Nobilis Diluent CA).

Innovax-ND-ILT was developed first in the US and therefore part of the safety studies were conducted at US research facilities. These studies do not have a Good Laboratory Practice (GLP) certification but were performed to the same standards as those conducted at EU (GLP) facilities. The critical overdose safety study was repeated and performed under GLP-compliant conditions. The quality of the studies is considered acceptable.

All batches used in the safety studies were prepared according to the current manufacturing process described in part 2 of the dossier. The only exception is that, during the blending of several batches, gentamicin sulfate was added in line with the registration of the product in the US. The addition of gentamycin does not change the safety properties of the vaccine strain when applied to chickens and therefore these studies are regarded as representative of the final product to which no gentamycin is added.

For the evaluation of the laboratory safety studies, an Innovax-ND-ILT batch at the lowest passage level was used. Batches were used at overdose in most studies. For the associated use studies, representative batches with standard dose were used in line with the guideline on association of IVMPs. For the mixed use with Nobilis Rismavac, representative batches with maximum dose were used in line with the guideline. In the field trials, representative batches with standard dose were used to evaluate both safety and efficacy in the same field studies, in line with Directive 2009/09/EC as amended.

Safety documentation

Eight safety studies were conducted to investigate the safety of the product. This included one laboratory study investigating the safety of the administration of a 10-fold overdose and two field

trials. The vaccine was administered by the *in ovo* and subcutaneous routes, as recommended. Laboratory studies were carried out using SPF chickens of the minimum age recommended for vaccination, using pilot batches diluted to contain the maximum titre of vaccine virus. Production batches were used in the field trials.

Studies applicable to live vaccines and genetically modified organism (GMO) products were conducted to investigate the dissemination of the vaccine strain, the spread from vaccinated animals to non-vaccinated in-contact animals and reversion to virulence.

Laboratory tests

Safety of the administration of one dose

Overdose safety testing was performed, which is considered to encompass the safety of the administration of one dose.

Safety of one administration of an overdose

The overdose safety study was appropriately designed, in accordance with Ph. Eur. monograph 0589 (Marek's disease vaccine, live), and performed under GLP-compliant conditions. Two groups were vaccinated with a tenfold maximum dose of Innovax-ND-ILT, either subcutaneously (day-old SPF chicks) or *in ovo* (19-day embryonated SPF eggs). An additional group of eggs served as hatchability control and chicks were kept as non-vaccinated controls. A fourth group served as susceptibility control and was challenged at 8 days of age with virulent MDV. All chicks were observed daily for general health, clinical signs and mortality for 123 days after vaccination or 70 days after challenge. *Post- mortem* examination was performed on all chicks. Results show that hatchability was not affected by *in ovo* vaccination. No clinical abnormalities that could be attributed to vaccination were observed in either of the vaccinated groups. Non-vaccinated controls remained in good health throughout the study, chicks that had received the challenge were euthanized at various timepoints due to severe clinical signs and were all found to be MD positive. The test complied with the validity criteria in Ph. Eur. monograph 0589. It could be concluded that a tenfold maximum dose of the vaccine is safe in chickens from one day of age by subcutaneous route and in embryonated chicken eggs by the *in ovo* route.

Safety of the repeated administration of one dose

Innovax-ND-ILT is to be administered once either via *in ovo* vaccination or at one day of age via the s.c. route. In accordance with Directive 2001/82/EC as amended, a study to test the safety of the repeated administration of one dose therefore is not required.

Examination of reproductive performance

Studies on reproductive performance safety have not been conducted. Layers and breeders are routinely vaccinated against Marek's Disease at one day of age, often using HVT strains; there are no data to suggest negative effects on the reproductive tract. This is confirmed by experience with licensed HVT vaccines such as Innovax-ILT, Innovax-ND, Innovax-ND-IBD. Moreover, HVT FC-126-vaccinated flocks lay more eggs on average in comparison to flocks not vaccinated against Marek's Disease (Maas, 1982). A relevant warning is included in the SPC: "The safety of the veterinary medicinal product has not been established during lay".

Examination of immunological functions

The parent strain HVT is apathogenic and it is not known to be immunosuppressive in chickens. This was further confirmed in a study using Innovax-ILT, which is considered relevant for Innovax-ND-ILT since the vaccines were constructed using the same HVT parent backbone. In this study, chicks were vaccinated at day old with a tenfold overdose of Innovax-ILT and at two weeks of age with a commercial Newcastle Disease (ND) vaccine; no evidence of reduced response to ND vaccination was observed.

In the dissemination study, the HVT/NDV/ILT strain showed a slightly reduced level of replication compared to the parent HVT FC126 strain, indicating that the insertion of genes has not resulted in an increase in virulence. The combined data are therefore considered adequate to support the absence of effects on immunological functions.

Special requirements for live vaccines

Spread of the vaccine strain

The spread of the vaccine strain from vaccinated to unvaccinated animals was investigated in a study in which day-old SPF chicks were vaccinated according to the recommended vaccination schedule by the recommended route and left in contact with unvaccinated sentinels, a group of hatch mates and a group of two-day old commercial turkey poults, for up to 56 days. The vaccine strain was not isolated from any of the sentinels at any time point. However, sentinel turkeys were found positive for HVT wild-type virus from day 14 whereas sentinel chicks were positive for HVT wild type virus on day 56.

Although no evidence of spread of the vaccine strain was obtained, it is unclear whether the sentinel turkeys and chickens were fully susceptible to the vaccine virus due to the circulation of the wild type HVT. Based on the results of various PCR tests, it can be concluded that the vaccine virus was not contaminated with wild type HVT or that it became defective (i.e. loss of insert): the wild type HVT must have been introduced with the turkey poults.

Spread of the vaccine strain between non-target animals was investigated in a study in 12-day-old commercial turkeys and 18-day-old commercial quail. Groups of turkey and quail were vaccinated with HVT/ND/ILT and housed with unvaccinated hatch-mates. Similarly, groups of turkeys and quail were vaccinated with HVT FC-126 (parent strain) and housed with unvaccinated hatch mates for a maximum of 28 days. The results show the HVT/NDV/ILT did not spread to in-contact birds in either turkey or quail. The HVT parent strain did spread in turkeys but did not spread in quail. From this data it appears the HVT/NDV/ILT strain is less likely to spread than the parent strain.

The risk of spread to non-avian species is considered negligible. There is no known infection of mammals with the parent virus (HVT) and this was confirmed by a study with Innovax-ILT in mice.

Dissemination in the vaccinated animal

Dissemination of the vaccine strain in vaccinated animals was also investigated in a study in SPF chicks vaccinated with HVT/NDV/ILT or HVT FC-126 parent strain. Samples of bursa, spleen, lung, peripheral blood lymphocytes, feather follicle epithelium and tracheal wash were analysed by virus titration on cells and PCR analysis. Both viruses were recovered from all but the tracheal wash samples up to 5 weeks post vaccination. In conclusion, the recombinant virus tissue tropism has not changed compared to the parent strain, although the parent appeared to replicate slightly better in most tissues. The applicant has justified the use of day-old chicks (instead of embryonated eggs) by

showing that the parent HVT FC126 strain has comparable dissemination after either s.c. or *in ovo* application.

Although Innovax-ND-ILT is a live vaccine, the active ingredient is non-pathogenic and unable to colonise non-target species, including humans; therefore, studies to determine the persistence of the organism at the injection site are not necessary.

Reversion to virulence of attenuated vaccines

HVT is a naturally apathogenic virus and thus was not obtained by attenuation. Ph. Eur. monograph 0589 on Marek's disease vaccine (live) stipulates that test 2-4-2 "increase of virulence" is not required for turkey herpesvirus vaccine strains. This is however not considered fully applicable to a recombinant virus. A reversion to virulence study was performed in accordance with Veterinary Service Memorandum 800.201, the design and performance of the study was adequate.

Sequential passage of vaccine strain through 5 groups of day-old SPF chicks was investigated; a single dose of test vaccine was administered to 25 chicks in the first group by the intraperitoneal route and, at passages 2, 3, 4 and 5, 1.6×10^7 spleen cells harvested on day 7 from birds of the preceding passage were administered to each animal by the same route. Each passage group consisted of 10 animals and the last passage was performed in 35 animals. No clinical abnormalities were observed during the study. The passage 5 chickens showed no gross lesions or tumours at necropsy. Phenotypic stability of the vaccine virus was confirmed by detection of recombinant proteins in MSV and passage 5-infected cells. Genotypic stability of the vaccine virus was confirmed by sequencing of PCR products amplified from the inserted genes and flanking regions of MSV and BP5.

It is concluded that no reversion to virulence was observed following five passages *in vivo.*

Biological properties of the vaccine strain

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strain. The HVT/NDV/ILT strain expresses NDV F and ILT gD and gI proteins. Based on the results of studies described in sections B.2 and B.6, it is concluded that the biological properties of the parent HVT FC-126 strain have not otherwise changed.

Recombination or genomic reassortment of the strains

The applicant has sufficiently addressed the risk of recombination or genomic reassortment occurring. Recombination has never been reported for MDV. The risk assessment for Innovax-ILT as presented is considered to be relevant for Innovax-ND-ILT. It can be agreed that the risk of recombination and/or reassortment is effectively zero. It is noted that several similar recombinant HVT vaccines are already on the market in the EU and are used extensively.

User safety

The applicant has presented a user safety risk assessment, which has been conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006 (and EMEA/CVMP/543/03-Rev.1).

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely routes are accidental self-injection and dermal and/or oral exposure. The active substance is not pathogenic for humans and therefore does not pose a risk for the user.

The excipients are commonly used in other vaccines and do not pose a risk for the user.

Glass ampoules may rarely explode when thawed, and the consequences of this are estimated to be medium (skin cuts). The overall risk for the user is therefore considered to be medium/low.

As a result of the user safety assessment, the following advice to users/warnings for the user are considered appropriate:

- The handling of liquid nitrogen should take place in a well-ventilated area.
- Innovax-ND-ILT is a virus suspension packed in glass ampoules and stored in liquid nitrogen. Before withdrawing ampoules from the liquid nitrogen canister, protective equipment consisting of gloves, long sleeves and a facemask or goggles should be worn.
- In case of an accident, to prevent serious wounds by either the liquid nitrogen or the ampoules when removing an ampoule from the canister, hold palm of gloved hand away from body and face.
- Care should be exercised to prevent contaminating your hands, eyes and clothing with the suspension.
- CAUTION: Ampoules have been known to explode on sudden temperature changes. Do not thaw in hot or ice-cold water. Thaw the ampoules in clean water at 25–27 °C.

Based on the above risk assessment, the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

MRLs/Study of residues

The active substances being of biological origin intended to produce active immunity are not within the scope of Regulation (EC) No 470/2009.

The excipients listed in section 6.1 of the SPC either are 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.

Residue studies are not required.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has provided data concerning the safety of mixed use with Nobilis Rismavac and associated use with Nobilis ND Clone 30, Nobilis ND C2, Nobilis IB 4-91 and Nobilis IB Ma5 vaccines.

The safety of mixed use with Nobilis Rismavac was investigated. Nobilis Rismavac is a live vaccine containing Marek Disease virus serotype 1, strain CVI-988. The *in ovo* route was chosen as the most sensitive route. The first group of embryonated eggs was vaccinated with an overdose of Nobilis Rismavac, the second group with a maximum dose of Innovax-ND-IBD mixed with a maximum dose of Nobilis Rismavac and the third group with a maximum dose of Innovax-ND-ILT mixed with a maximum dose of Nobilis Rismavac, a non-vaccinated control group and a challenge control group were shared with another study. There was no difference in hatchability between the groups. All chickens in the challenge control group were MD positive, indicating the birds were susceptible. Clinical or post-mortem macroscopic abnormalities due to MD were not observed in the vaccinated and control groups indicating the safety of mixed use.

The safety of associated use of Innovax-ND-ILT with Nobilis Clone 30 and Nobilis ND C2 is

supported by the results of a study investigating compatibility of Innovax-ND-IBD and Nobilis Clone 30 or Nobilis ND C2. In this study, which was assessed in the Innovax-ND-IBD dossier, the subcutaneous application of a standard dose of Innovax-ND-IBD at the same time as a standard ocular dose of either of the ND vaccines was found to be safe. Based on the biological properties of the Innovax-ND-ILT vaccine strain (refer to part 3.B.2 and 3.B.6), it is considered that the safety profile of this strain is highly similar to the Innovax-ND-IBD strain that was derived from the same HVT vector using the same genetic manipulation strategy and partially the same inserted genes (NDV F). Both vaccine strains show a dissemination and safety profile that is comparable to the HVT parent strain. Based on these biological properties, there is no reason to expect that associated use of Innovax-ND-ILT with Nobilis Clone 30 or Nobilis ND C2 would lead to safety issues. The results of the efficacy studies on concurrent use of Innovax-ND-ILT with Nobilis ND Clone 30 and ND C2 further support the safety of concurrent use.

Similarly, safety of associated use of Innovax-ND-ILT with Nobilis IB Ma5 mixed with Nobilis IB 4-91 is supported by a study performed with Innovax-ND-IBD. This study was assessed when it was presented in the Innovax-ND-IBD dossier. The study was performed using standard doses of each vaccine and appropriate control groups were included. No clinical signs were observed after vaccination and the safety requirements of Ph. Eur. 0442 (avian infectious bronchitis vaccine, live) were met. Based on the biological properties of the Innovax-ND-ILT and Innovax-ND-IBD strains, the data are considered relevant. There is no reason to expect that associated use of Innovax-ND-ILT with Nobilis IB 4-91 mixed with Nobilis IB Ma5 would lead to safety issues. The results of the semi-field study provide additional evidence of the safety of associated use of these vaccines.

Field studies

Two controlled GCP-compliant field trials were performed, one safety field trial in the US and one safety/efficacy semi-field trial in the UK. Study results are summarised below.

The UK field trial evaluated the safety and efficacy of Innovax-ND-ILT when mixed with Nobilis Rismavac which is considered a worst-case scenario with respect to safety. Commercial pullets were vaccinated at the hatchery with Nobilis IB Ma5 and IB 4-91. Upon arrival at the containment facilities, chicks were vaccinated with 1) Innovax-ND-ILT mixed with Nobilis Rismavac (test) or 2) Nobilis Rismavac+CA126 (control) and placed in floor pens divided over two rooms for each group. For these groups, live weight, clinical observations, local reactions, mortality and adverse events were monitored during the trial period. Local reactions were not observed between day 0 and 14. Control birds consumed around 5% more feed and live weights were slightly higher in the controls throughout the study. The average weight gain was the same for the two groups throughout the study. With the exception of some higher mortality in the control group during the first 28 days, the percentage of mortality was similar across both groups. No adverse events occurred. Overall, the field data support the results of the laboratory safety studies. The vaccine is safe when applied (mixed with Nobilis Rismavac) via s.c. injection in day-old commercial layer pullets.

The US field trial evaluated the safety of Innovax-ND-ILT when used under field conditions and involved a total of four study sites. Commercial broilers were used at all test sites. At each site, one test group (vaccination with Innovax-ND-ILT) and one control group (vaccination with commercially licensed Marek's disease vaccine) of approximately 21,000–25,000 chicks was included. The study was blinded. Vaccination was performed either on day-old chicks by subcutaneous route or by *in ovo* route to 18–19 days-old embryonated eggs. Chicks were subsequently vaccinated with a number of commercial vaccines against coccidiosis, IBV, NDV, IBDV. For these groups the following safety data were collected: Percent hatchability, performance parameters, clinical assessment, local reactions, mortality and disease history for the first 21 days. There were no differences in hatchability between

vaccinated and control groups. Mortality ranges in the first 21 days were within the acceptable range for these flocks. Clinical assessment of the birds' health was performed 3 times in the first 21 days, by a veterinarian. All birds were scored 'healthy'. Local reactions were scored in 25 birds, 3 times during the first 21 days: all observations were normal. The condemnation rates were accepted as normal for the type of bird/flock/management. No adverse events occurred.

The study was appropriately designed and executed to an acceptable standard (GCP, blinded). The husbandry practises for broilers in the US are not considered different from EU systems to the extent that this would affect the relevance of safety outcomes. The results give no indication of safety issues after application under field conditions of use, when applied via either of the proposed routes in commercial broilers.

Both studies (UK and US) were well designed and conducted. The US field study confirmed that the product is safe for use in embryonated chicken eggs and in day-old chicks under field conditions. In the semi-field trial, the product was applied to day-old chicks mixed with Nobilis Rismavac, which was found to be safe.

Environmental risk assessment

The applicant provided an environmental risk assessment in accordance with the note for guidance EMA/CVMP/074/95. Spread of the HVT/NDV/ILT vaccine strain was not observed in the studies but is considered to be possible based on literature data on HVT. The vaccine virus may survive for months in feather dust from vaccinated animals. The HVT FC-126 parent virus host range is limited to avian species. HVT virus is non-pathogenic for the target species and other avian non-target species. Based on study results, the insertion of NDV F and ILT gD and gI protein genes has not altered the host range, pathogenicity or spreading capacity of the HVT FC-126 parent strain. The vaccine preparation does not contain any toxic or pharmacologically active components.

Based on the data provided, the ERA can stop at phase I. Innovax-ND-ILT is not expected to pose a risk for the environment when used according to the SPC.

The SPC contains a warning sentence that is considered to mitigate the risk of spreading of the vaccine strain: "As a live vaccine, the vaccine strain is excreted from vaccinated birds and may spread to turkeys. Safety trials have shown that the strain is safe for turkeys. However, precautionary measures have to be followed in order to avoid direct or indirect contact between vaccinated chickens and turkeys."

Environmental risk assessment for products containing or consisting of genetically modified organisms

A technical dossier containing the information required by Annex IIIA of Directive 2001/18/EC is provided in part 3E, including a copy of the written consent of the Dutch authorities for the deliberate release in the environment as this vaccine is a GMO. Information on the origin, method of construction, stability, biological properties and genomic sequence of the vaccine strain is provided in this part. An assessment of risks based on the detailed information provided for the genetically modified organism was performed.

The HVT/NDV/ILT strain was shown not to contain vector sequences but some remnants of cloning vectors and linker sequences remain. Both *in vivo* and *in vitro* studies did not show any tendency for genetic instability.

HVT/NDV/ILT does not infect humans or other mammals; the host range is restricted to avian

species.

Spread of the HVT/NDV/ILT vaccine strain was not observed in the studies but is considered to be possible based on literature data on HVT. The vaccine virus may survive for months in feather dust from vaccinated animals. HVT virus is non-pathogenic for the target species and other avian non-target species. Based on study results, the insertion of NDV F and ILT gD and gI protein genes has not altered the host range, pathogenicity or spreading capacity of the HVT FC-126 parent strain.

Taken together, any risk emerging from the use of the Innovax-ND-ILT vaccine virus is expected to be negligible for humans and for the environment.

Overall conclusions on the safety documentation

The safety of application of a tenfold overdose of Innovax-ND-ILT administered subcutaneously to chickens of the youngest age to be treated and to 18-day old embryonated chicken eggs was investigated in accordance with regulatory requirements. Based on results obtained, it was concluded that the safety of the targeted animals is acceptable when the vaccine is administered according to the recommended schedule and via the recommended route.

Examination of reproductive performance was not performed; this is considered acceptable based on the safety profile of the parent HVT strain. A warning concerning the absence of data is included in the SPC.

As this is a live vaccine, the applicant also conducted studies to establish the potential for spread and dissemination of the vaccine strain. No evidence of spread was obtained. However, based on the properties of the parent strain, spread of the vaccine strain cannot be excluded and an appropriate warning is included in the SPC. An assessment of the risk of genomic reassortment was provided and the risk is found to be very low and acceptable. Reversion to virulence of the strain was also investigated and results showed that the potential risk is low and acceptable. The biological properties of the vaccine strain were described adequately and found to be acceptable.

The product is not expected to adversely affect the immune response of the target animals or of its progeny. A study performed with the related vaccine Innovax-ILT showed no effect of vaccination on the response to ND vaccine. This is acceptable based on the highly similar biological properties and known safety profile of the parent and related recombinant strains.

The data presented are considered adequate to characterise the safety profile of the vaccine as acceptable.

Based on the assessment presented, the product does not pose an unacceptable risk to the user when used in accordance with the SPC. The appropriate warnings for the user have been included in the product literature.

An appropriate environmental risk assessment was provided. The product is not expected to pose a risk for the environment when used according to the SPC.

The vaccine virus strains were shown to be genetically and phenotypically stable in both *in vitro* and *in vivo* studies. There is no risk of reversion to virulence since the parent strain is non-pathogenic. A recombination event between the vaccine virus strain and a field virus is highly unlikely.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is indicated for active immunisation of chickens by *in ovo* application in embryonated chicken eggs or via subcutaneous administration of day-old chicks using a minimum dose of $10^{3.3}$ PFU:

- to reduce mortality and clinical signs caused by Newcastle disease (ND) virus

- to reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus

- to reduce mortality, clinical signs and lesions caused by Infectious Laryngotracheitis (ILT) virus

After single vaccination, immunity is intended to be established after 9 days for MD, 5 weeks for ND and 4 weeks for ILT. The immunity is intended to last for the entire risk period for MD, 62 weeks for ND and 62 weeks for ILT.

The vaccine can be mixed with Nobilis Rismavac and administered by subcutaneous route (onset of immunity for MD at 5 days).

The vaccine can be used on the same day (at different administration sites) with Nobilis ND Clone 30 or Nobilis ND C2 (onset of immunity for ND at 2 weeks), Nobilis IB 4-91 or Nobilis IB Ma5.

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC (as amended by 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7. as well as monograph 0589 (Marek's disease vaccine (live)), monograph 0450 (Newcastle disease vaccine (live)) and monograph 1068 (Avian Infectious Laryngotracheitis vaccine (live)).

Challenge model:

Studies were designed in line with respective Ph. Eur. monographs (or 9CFR) for MDV, NDV, ILTV and IB vaccines. Appropriate challenge strains or controls to ensure a sufficiently strong challenge were therefore used.

Efficacy parameters and tests:

The efficacy parameters investigated in the efficacy studies are clinical signs, mortality and lesions due to MDV, clinical signs and mortality due to NDV and clinical signs, mortality and lesions due to ILTV.
The parameters chosen are considered appropriate for evaluating the efficacy of the product.

Efficacy documentation

Twenty-one studies were conducted to investigate the efficacy of the product and included twenty laboratory studies and one field trial. Laboratory studies were well documented and carried out in chickens of the minimum age recommended for vaccination, using pilot batches of the maximum passage level reconstituted to contain a minimum dose. Pilot batches were used in the field trial.

Laboratory trials

Onset of immunity

In total, 5 studies were performed to evaluate onset of immunity (OOI) against MD.

The first study was performed in accordance with Ph. Eur. monograph 0589 and was valid with respect to the number of animals, the percent of disease in control animals and the percentage of surviving chickens in the period between vaccination and challenge. A group of embryonated eggs and a group of day-old chicks was vaccinated with a minimum potency dose of vaccine ($10^{3.3}$ PFU) at the highest passage number. Challenge was performed with very virulent strain RB1B. Hatchability was normal in the vaccinated group. The calculated relative protection percentage (RPP) was 88.9% in the *in ovo* group and 74.1% in the subcutaneous group. Only the *in ovo* vaccinated group meets the required minimum of 80% RPP.

A second study was performed in day-old chicks in accordance with 9CFR 113.330 requirements, which is largely in accordance with Ph. Eur. Monograph 0589 requirements. The follow up period was shorter: 44 days post challenge, rather than 70 days. The actual dose of vaccine applied was $10^{3.3}$ pfu. Challenge was performed with a virulent strain: GA5 at 5 days of age. The observation period was sufficient to observe development of disease in non-protected animals. The study was valid, the RPP was 88%.

As part of a larger study, the efficacy in embryonated eggs was investigated. This study was designed in accordance with 9CFR 113.330 requirements (shorter follow up). Challenge was performed with the virulent strain GA5 at 5 days of age. Hatchability was not affected by vaccination. The study was valid, and RPP of 78% was achieved for the group vaccinated with $10^{3.3}$ PFU and 97% in the group vaccinated with $10^{3.4}$ PFU. Thus, the higher dose group met the requirement of at least 80% RPP.

In the fourth study, the efficacy of mixed use of the vaccine with Nobilis Rismavac in day-old chicks was evaluated. The study was designed and valid in accordance with Ph. Eur. monograph 0589 requirements. Animals were challenged with very virulent strain RB1B at 5 days of age. An RPP of 100% was achieved.

In the fifth study, designed in accordance with Ph. Eur. 0589 requirements, two groups of 33-day-old SPF birds were included. One group was vaccinated at day old with a minimum dose of vaccine and challenge was performed with virulent GA5 strain at 7 days of age. The study was valid, an RPP of 96.7% was achieved in the vaccinates.

In conclusion, despite some variability in the level of protection, which is expected for the type of study, the minimum requirement of OOI at 9 days could be shown for both routes of application using vvMDV or vMDV challenges.

Eight studies were performed to determine OOI for ND.

Three studies for onset of protection against ND were performed using the challenge strain Texas GB at four weeks of age. The studies were otherwise in accordance with Ph. Eur. monograph 0450 and valid with respect to the number of animals, the percent mortality in control animals and the percentage of surviving chickens between vaccination and challenge. The vaccine batches used in the first two studies were diluted to below minimum potency ($10^{3.3}$pfu) and were at maximum passage level. In the first study, 100% of day-old SPF chicks were protected; in the second study, 95% of embryonated eggs were protected. In the third study, a dose of $10^{3.36}$ PFU was used either alone or mixed with Nobilis Rismavac. This resulted in 100% protection from challenge at 3 and 4 weeks for the single vaccine and 95% and 100% protection respectively after challenge at 3 and 4

weeks for the mixed use. It can be concluded from these studies that the vaccine at lowest potency confers protection against challenge with velogenic Texas GB strain with an OOI of 4 weeks and that there is no indication of interference when used mixed with Nobilis Rismavac.

Five studies were performed with the challenge strain Herts 33/56, largely in accordance with Ph. Eur. monograph 0450 requirements with the exception of the time of challenge. All studies were valid with respect to the number of animals, the percent mortality in the controls and the percentage of surviving chicks between vaccination and challenge. In the first study, SPF chicks were vaccinated with a minimum dose and challenged at 4 weeks; 81% protection was achieved. In the second study, both embryonated eggs and day-old chicks were vaccinated with a minimum dose and at 4 weeks 80% (eggs) and 85% (chicks) protection was achieved. In the third study, one group of eggs and two groups of chicks were vaccinated with a dose of $10^{3.4}$ PFU, at 4 weeks 85% of eggs and 70% and 80% of chicks were protected. An additional group of chicks was vaccinated with a minimum dose and 90% protection was achieved when challenged at 4 weeks. In the fourth study, one group of eggs and two groups of chicks were vaccinated with a minimum dose and challenge was performed slightly later, at 30 days: in this study 90% of eggs and 83 and 100% of chicks were protected. In the last study, eggs and chicks were vaccinated with a minimum dose and challenge was performed at 5 weeks, the protection achieved was 100% in eggs and 93% in chicks, which is in accordance with Ph. Eur. monograph 0450 requirements.

In conclusion, the studies with the Texas GB strain challenge (95% - 100% protection at 4 weeks post vaccination) are considered supportive evidence only. Although it is acknowledged that the product is not a live NDV vaccine, the Ph. Eur. 0450 requirements with respect to study set up, challenge strain and outcome (protection percentage) are considered to be applicable nevertheless. When animals were challenged with NDV Herts 33/56 strain at 35 days of age good protection was achieved. The claimed OOI of 5 weeks is adequately supported by the data presented.

One study was performed to determine OOI for ILT.

Two groups of embryonated eggs and two groups of day-old SPF chicks were included in one study, for each category of animals one group was vaccinated with a minimum dose of vaccine and one group was vaccinated with the vaccine at minimum dose mixed with a minimum dose of Nobilis Rismavac. One additional group of chicks was kept as non-vaccinated controls and one group served as non-vaccinated, non-challenged controls. The study was performed in accordance with Ph. Eur. monograph 1068 requirements with the exception of the challenge, which was performed at 4 weeks. The vaccine was shown to be efficacious (reduction of mortality, clinical signs and lesions) with an OOI at 4 weeks of age. The protection achieved after mixed use was 96% in the s.c. vaccinated chicks but did not meet the requirement (≥ 90%) when applied *in ovo* (86%). It is considered that there was no clear indication of interference.

Duration of immunity

DOI against MD

No studies were performed to determine the duration of immunity of Innovax-ND-ILT against Marek's Disease. HVT causes a persistent infection in chickens. The virus remains present lifelong (Witter and Offenbecker, 1978) and therefore protection against Marek's Disease by HVT is generally accepted to be lifelong.

DOI against ND

Two studies were performed in SPF chicks to investigate the duration of immunity against Newcastle Disease. The first study included two groups of day-old SPF chicks vaccinated with a below-

minimum dose ($10^{3.20}$ or $10^{3.25}$ PFU) and challenged with Texas GB strain at 10, 23, 36 weeks ($10^{3.20}$ PFU) or at 10, 23, 36, 47, 53 and 62 weeks of age. The NDV challenge was valid at each time point (≥ 90% of controls exhibiting signs of ND) except for time point 53 weeks (80%). The $10^{3.20}$ PFU dose was protective (100%, 100%, and 100% protection) at the 10, 23, and 36 weeks challenge of the study. The $10^{3.25}$ PFU dose was protective (100%, 100%, 100%, 93%, 100% and 100%) at the 10, 23, 36,47, 53 and 62 weeks challenge of the study.

In the second study, one group of day-old SPF chicks was vaccinated with a minimum dose mixed with a minimum dose of Nobilis Rismavac and one group was kept as placebo controls. Challenge with Texas GB strain was performed at 9, 50 and 60 weeks. In the controls, 92% was ND positive at week 9, 82% at week 50 and 80% at week 60. In the vaccinates, 100% was protected in week 9, 97% in week 50 and 100% in week 60.

Conclusion: since the Ph. Eur. monograph 0450 requirements do not strictly apply for DOI studies, the data are considered to support the claimed DOI for NDV. Mixed use resulted in a similar level of protection compared to the single vaccine and the data are therefore considered to support the claimed DOI after mixed use.

DOI against ILT

One study was performed that included two groups of day-old SPF chicks vaccinated with a below-minimum dose ($10^{3.20}$ or $10^{3.25}$ PFU) and challenged intratracheally with ILTV challenge virus at 10, 23, 36, 47 and 62 weeks. After challenge, chickens were observed daily and clinical signs were scored. After one week the remaining birds were euthanised and at necropsy macroscopic lesion scoring was performed. The study included a placebo control group (hatch mates) and a challenge control group (birds 4 weeks of age at the time of each challenge). The challenge induced clinical signs and lesions in 60% of challenge controls in week 10, 80% in week 23 and 90-100% in weeks 36, 47 and 62 indicating adequate challenge at least at the last three time points. The percentage of protection in the age-matched placebo group was 20, 80, 60, 36 and 54% respectively. Protection in the two vaccinated groups was between 90 and 100% at all time points (93% in both groups in week 62).

No laboratory study was performed to investigate directly the DOI against ILT after mixed use with Nobilis Rismavac. Mixed use was investigated in the field study and a challenge revealed 70-80% protection at 6 weeks, which is slightly lower than the protection achieved in the DOI study. The absence of interference on the DOI against ILT after mixed use was further justified based on the results of NDV challenge for up to 60 weeks after mixed use; the protection achieved was 100% at 60 weeks. Since the NDV gene is present in the same construct as the ILTV genes and this result for NDV supports continued presence of the recombinant virus as well as continued expression of the inserted genes this also supports the DOI for ILTV after mixed use. Moreover, serological responses to NDV and ILTV are detectable between 9 and 58 weeks post mixed vaccination, indicative of a continued immune response.

Maternally derived antibodies (MDA)

In order to support the efficacy against MD, ND and ILT in maternally derived antibody (MDA) positive birds the results of a number of studies were presented. In two studies, MDA positive (MD MDA+) birds were vaccinated with Innovax-ND-ILT and challenged with NDV and ILTV respectively. In the first study 95% of vaccinates were protected from NDV challenge while in the second study 70-80% of vaccinates was protected from challenge with ILTV. These results support the 'take' of the vaccine in day old, (MD MDA+) commercial birds. Further supportive evidence of protection against MDV in MD MDA+ birds comes from studies performed with related HVT-backbone vaccines

(Innovax-ND-IBD and Innovax-ILT). Although these are only related vaccines, containing different gene inserts in the same HVT backbone, some support for the 'take' of these HVT recombinant vaccines and the subsequent protection against MDV challenge in MDA+ birds can be derived from them. In conclusion, protection against MD afforded by Innovax-ND-ILT is not expected to be significantly reduced by the presence of MD MDA.

One study was performed in MD and ND MDA+ birds to support efficacy against NDV. The study was performed in commercial birds in accordance with Ph. Eur. 0450 requirements and included a group of day-old SPF chicks vaccinated with a minimum dose and a control group. A vaccinated MDA- control group was not included in the study. However, since the set up and requirements of Ph. Eur. 0450 for efficacy evaluation were followed, an additional group for validation of the challenge is not considered necessary. All animals were NDV and MDV seropositive at Day 0, antibody levels decreased gradually thereafter in both groups. Challenge was performed at 42 days using NDV Herts 33/56. All controls succumbed to the disease while 95% of vaccinates were protected.

The efficacy against ILT in MDA+ birds is supported by the results of the semi-field study in which vaccinated commercial ILT MDA+ birds were challenged with ILTV. The results of the study indicate that significant reduction of clinical signs, lesions and mortality due to ILT was achieved after challenge at 41 days of age in commercial birds vaccinated with Innovax-ND-ILT mixed with Nobilis Rismavac.

Interactions

Innovax-ND-ILT can be administered on the same day but via different administration routes with Nobilis ND C2, Nobilis ND Clone 30, Nobilis IB Ma5 and Nobilis IB 4-91. The efficacy of concurrent application of these vaccines was evaluated by a study of viraemia and by challenge with NDV, ILT and IBV in 4 studies in total.

A study was designed to quantify the viraemia of the vaccine strain after single and associated use. Six groups of day-old SPF chicks were included. The first five groups were vaccinated with a standard dose of Innovax-ND-ILT ($10^{4.09}$pfu) either singly (group 1) or in combination (via ocular application) with Nobilis ND C2 (group 2), Nobilis ND Clone 30 (group 3), Nobilis IB Ma5 (group 4) or Nobilis IB 4-91 (group 5). Group 6 was kept as non-vaccinated control. At 8 days post vaccination, spleens were harvested and analysed for virus content by titration on CEF cells and confirmation of virus identity was done by using specific antibodies. All samples of groups 1-5 were positive, with similar levels of virus detected. The OOI for MD at 9 days post vaccination is based on the replication of the vaccine strain and therefore viraemia, which is an indicator of virus replication, can be used to evaluate interference. Since no difference in frequency of viraemic birds was observed between the groups, it can be concluded no evidence of interference was found.

Associated use with Nobilis ND C2 and Nobilis ND Clone 30 was investigated in one study. Groups of day-old SPF birds were vaccinated with a standard dose of Innovax-ND-ILT and a standard dose of Nobilis ND C2 (group 1) or Nobilis ND Clone 30 (group 2) a third group was left as unvaccinated control. No control groups for single use of the ND vaccines were included in the study. However, since efficacy was tested in accordance with Ph. Eur. 0450 requirements, this is acceptable. Challenge was performed after 14 days with NDV Herts 33/56. All controls died or were euthanised due to severe ND, protection in group 1 was 91%, in group 2 100%. No indication of interference due to associated use of Nobilis ND Clone 30 or Nobilis ND C2 with Innovax-ND-ILT was observed; the OOI of 2 weeks as registered for Nobilis ND Clone 30 and ND C2 vaccines was confirmed.

With respect to the effect of concurrent use of IB vaccines on the efficacy against ND the following justification was provided: The vaccine strain and IB vaccine viruses replicate in different cell types

and thus no interference is expected. After concurrent use of IB vaccines equivalent vaccine take was supported by data on viraemia. Lastly, in one study after concurrent vaccination with either ND or IB vaccines, the Ph. Eur. requirements for OOI against ILT were met, therefore no evidence of interference was obtained. Taken together, the occurrence of interference with immunity against NDV after concurrent use with IB vaccines is considered unlikely.

The above-mentioned study was performed to investigate efficacy against ILT after associated use with ND and IB vaccines. Four groups of day-old SPF chicks were treated as follows: all were vaccinated with a standard ($10^{4.1}$ PFU) dose of Innovax-ND-ILT and at the same time with a standard dose (ocular) of Nobilis ND C2 (group 1), Nobilis ND Clone 30 (group 2), Nobilis IB Ma5 (group 3) or Nobilis IB 4-91 (group 4); group 5 was left unvaccinated; group 6 served as non-vaccinated, non-challenged control. A single use control group was not included in the study, however since the study was designed and valid in accordance with Ph. Eur. 1068 this can be accepted. Groups 1-5 were challenged with ILTV strain LT-96-3 intratracheally at 4 weeks post vaccination and monitored for 7 days and necropsied thereafter. For all four combinations of vaccines, the criteria of the Ph. Eur. monograph 1068 (≥ 90% protection) were met; therefore, no indication of interference by IB and ND vaccines was obtained for immunity against ILT.

The study was performed to investigate efficacy against IB after associated use with IB vaccines. Four groups of day-old SPF birds were vaccinated with a standard dose of Innovax-ND-ILT and a standard dose (oculonasal) of Nobilis IB Ma5 (group 1 and 3) or Nobilis IB 4-91 (group 2 and 4). Three non-vaccinated control groups were included. At 3 weeks, groups 1 and 2 and two of the control groups were challenged, with the respective virulent IBV (M41 or 4-91) strains. At 5 and 6 days, chickens were euthanised and ciliary activity was scored. The study was valid in accordance with Ph. Eur. 0442 requirements: 100% of control birds showed extreme loss of vigour of ciliary activity. Protection was 86% in group 1 and 100% in group 2. The results show that for both combinations of vaccines the criteria of the Ph. Eur. monograph 0442 (≥ 80% protection) were met; therefore, no indication of interference was obtained for immunity to IB.

Field trials

Efficacy against Marek's disease

One safety/efficacy semi-field trial was performed in the UK. The study design is described in part 3. The results relevant to efficacy are two laboratory challenge studies that were performed on birds taken from the semi-field study.

A study included two groups of 60-day-old commercial birds sourced from the field trial. Group 1 (control) was vaccinated on Day 0 (at the hatchery) with Nobilis IB Ma5 mixed with Nobilis IB 4-91. Group 2 was vaccinated on Day 0 (at the hatchery) with Nobilis IB Ma5 mixed with Nobilis IB 4-91 followed (at the trial facilities) by vaccination with Innovax-ND-ILT mixed with Nobilis Rismavac, s.c. Challenge was performed at 9 days with MDV RB1B strain. All chicks were observed for 70 days following challenge for clinical signs or Marek's disease. Thereafter all remaining birds were euthanised and necropsied, macroscopic and histological lesions were scored. In group 1, 80% were MD positive, in group 2 all birds survived to the end of the study and no macroscopic lesions were found at autopsy (100% RPP).

Efficacy against Infectious Laryngotracheitis

A study included two groups of 60-day-old commercial birds sourced from the field trial. Group 1 was vaccinated on Day 0 with Nobilis Rismavac +CA126, s.c.

Group 2 was vaccinated on Day 0 with Innovax-ND-ILT mixed with Nobilis Rismavac, s.c. At 41 days of age half of each group was challenged with one of two doses of an ILTV challenge strain, intratracheally. Birds were observed for 7 days following challenge for clinical signs or mortality due to ILT. Thereafter all remaining birds were euthanised and necropsied, macroscopic lesions were scored. A chick was considered ILT positive if it died (reached humane endpoint) due to ILT, or severe/combined signs or ILT were observed for more than one day or if macroscopic lesions were observed. In the control group, 62% of the high-challenge birds were ILT positive while in the low-challenge group 80% was ILT positive. In the vaccinated group, 30% of birds receiving the high-challenge and 20% of birds receiving the low-challenge were ILT positive.

Conclusion

The MD challenge study can be considered valid and 100% RPP was achieved. Therefore, the results support efficacy of mixed use of Nobilis Rimavac with Innovax-ND-ILT in MDA+ birds and there was no indication of interference of Innovax-ND-ILT mixed with Nobilis Rismavac when applied on the same day with both IB vaccines (Nobilis IB Ma5 mixed with Nobilis IB 4-91).

The ILT challenge was not valid in accordance with Ph. Eur. monograph 1068 since fewer than 90% of the control chickens were ILT positive and fewer than 90% of vaccinated chickens were protected. However, the criteria of the Ph. Eur. monograph are considered to be applicable only to pivotal efficacy studies in SPF birds. The results indicate that significant reduction of clinical signs, lesions and mortality due to ILT was achieved after vaccination with Innovax-ND-ILT mixed with Nobilis Rismavac when applied in birds vaccinated under (semi) field conditions. Whether or not interference occurred cannot be concluded from this study. The MDA levels found in this study can be regarded representative for MDA levels seen in the field.

For efficacy of Innovax-ND-ILT against challenge with ND under field conditions, reference is made to the vaccination challenge study conducted in MDA positive animals (discussed under part 4. Laboratory studies). This can be accepted since vaccination was performed by s.c. injection (not mass application) which makes laboratory studies more easily comparable to field studies. The data provided on ILTV challenge of birds from the field efficacy study provides additional indication that

the vaccine is efficacious under field conditions of use.

Overall conclusion on efficacy

In total 20 laboratory studies and one field study were performed to evaluate efficacy. The laboratory efficacy studies were performed largely in accordance with the immunogenicity tests described in the following monographs: Ph. Eur. 0589: Marek's disease vaccine (live), Ph. Eur. 0450: Newcastle disease vaccine (live) and Ph. Eur. 1068: Avian infectious laryngotracheitis vaccine (live). A number of studies investigating efficacy against MDV and NDV were however performed in accordance with requirements set by 9CFR. Onset and duration of immunity studies in SPF chickens and the influence of MDA were investigated using vaccine batches at or below the minimum dose stated on the label.

Onset of immunity

Reduction of mortality, clinical signs and lesions caused by Marek's Disease Virus was demonstrated. Despite some variability in the level of protection, which is expected for the type of study, OOI at 9 days could be shown for both routes of application using vvMDV or vMDV challenges.

The claimed onset of immunity of 5 weeks for NDV is sufficiently demonstrated by the data provided. The required level of protection was achieved when birds were challenged in accordance with Ph.Eur. 0450 requirements at 5 weeks of age.

The study for onset of protection against Infectious Laryngotracheitis was performed in accordance with Ph. Eur. 1068. Vaccination of embryonated eggs or day-old birds resulted in adequate protection from clinical signs, lesions and mortality when challenged at 4 weeks of age.

Duration of immunity

No studies were performed to determine duration of immunity against MD since HVT causes a persistent infection, which is considered to provide protection for the entire risk period. Duration of immunity against ND was demonstrated at 62 weeks post vaccination, with 100% of birds protected from challenge with NDV Texas GB strain. With 93% protection from clinical signs, duration of immunity against ILTV was demonstrated at 62 weeks of age.

Influence of MDA

The influence of maternal antibodies on the efficacy of the vaccine against MDV was studied. Protection against NDV was studied using commercial broiler chicks with confirmed levels of MDA against NDV and MDV. After challenge of MDA+ birds with NDV Herts strain, significant reduction of clinical signs and mortality was demonstrated, no indication of interference of MDA with development of immunity against NDV was observed. The efficacy of the vaccine against ILTV was studied in MDA+ commercial birds (vaccinated as well with Nobilis Rismavac). No indication of interference was found. The MDA levels found in the study can be regarded as representative for MDA levels seen in the field.

Studies on Interactions

Interactions with Nobilis Rismavac

Efficacy regarding MDV after simultaneous (mixed) use of Innovax-ND-ILT and Nobilis Rismavac was investigated in two studies. The OOI of 2 weeks as registered for Nobilis Rismavac was confirmed in day-old chicks. Efficacy against MDV after *in ovo* use was not studied as mixed use with Nobilis Rismavac is only claimed in day-old chicks.

Onset of immunity against NDV was investigated after mixed application of both vaccines in day-old birds, efficacy against NDV was adequate and similar for Innovax-ND-ILT single use and mixed use with Nobilis Rismavac.

In a further study, the duration of immunity against NDV after mixed use was confirmed.

Protection against ILTV after mixed use was studied in chicks vaccinated *in ovo* or at day-old. No clear evidence of inference was obtained. In the field study in MDA+ animals, after mixed use with Nobilis Rismavac, 70-80% protection against ILTV challenge at 6 weeks was found. The absence of interference on the DOI against ILTV after mixed use was further justified based on the protection against NDV challenge at 60 weeks after mixed use. Since the NDV gene is present in the same construct as the ILTV genes this result for NDV supports continued presence of the Innovax-ND-ILT vaccine strain as well as continued expression of the inserted genes. Moreover, serological responses to NDV and ILTV were detectable between 9 and 58 weeks post mixed vaccination, indicative of a continued immune response.

Interactions with ND or IB vaccines

Concurrent use with Nobilis ND C2, Nobilis Clone 30, Nobilis IB Ma5 or Nobilis IB 4-91 does not affect the viraemia of the Innovax-ND-ILT vaccine. This is considered to support OOI against MDV since the viraemia indicates replication and thus establishment of the vaccine strain which subsequently confers protection. OOI against NDV was demonstrated at 2 weeks after vaccination with Innovax-ND-ILT and Nobilis ND C2 or Nobilis Clone 30. Interference of concurrent use of IB vaccines on efficacy against ND is considered unlikely based on the supportive data provided. OOI against ILTV after concurrent use with the ND and IB vaccines was confirmed. This is considered to also support the OOI against NDV after concurrent use since genes for both viruses are included in the same construct. OOI against IB was supported by results of a study on simultaneous use of Innovax-ND-ILT with Nobilis IB Ma5 and Nobilis IB 4-91. The omission of studies on DOI for ND, ILT and IB after concurrent use with Innovax ND-ILT was appropriately justified. Since the DOI depends on persistence of vaccine virus in the host it is considered that when there is similar and adequate OOI after mixed use, the DOI after mixed use will also be similar.

A semi-field study, in which birds were kept under containment, was performed in the UK. Birds taken from the field study were challenged in the laboratory with MDV and ILTV. Chicks were vaccinated with Nobilis IB Ma5 mixed with Nobilis IB 4-91 and with Innovax-ND-ILT mixed with Nobilis Rismavac. After challenge at 9 days of age, adequate protection was achieved against MDV; however, this protection cannot be attributed to either MDV vaccine due to the mixed use. At 41 days of age, another group of birds was challenged with ILTV. Significant protection from clinical signs, lesions and mortality due to ILTV was found. The level of MDA in the birds is representative for MDA levels found in the field.

Part 5 – Benefit-risk assessment

Introduction

Innovax-ND-ILT is a live recombinant Herpes Virus of Turkeys (HVT) strain containing the F gene from Newcastle Disease Virus and the gD and gI genes form Infectious Laryngotracheitis Virus. The vaccine is intended for protection of chickens from Marek's Disease (MD), Newcastle Disease (ND) and Infectious Laryngotracheitis (ILT).

MD is a highly contagious lympho-proliferative disease in chickens, characterized by mononuclear cellular infiltrates in peripheral nerves and various organs and tissues. Several clinical and pathological syndromes have been associated with MDV infection. Syndromes that are described are

lympho-proliferative syndromes (lymphoma's, paralysis, skin leucosis and ocular lesions), lympho-degenerative syndromes (early mortality syndrome, cytolytic infection of lymphoid cells, immuno-depression) or CNS syndromes (paralysis and neurological disease). In the classical form (involvement of nerves) mortality rarely exceeds 10-15%. The acute form (visceral tumours, most prevalent) can have disease incidences from 10-30% with outbreaks of 70% while mortality can reach 50%. Chickens become infected at early age via the respiratory route as the virus is shed via the feather follicles. MDV infected birds can be carriers and shedders of the virus for life, feather dust from MDV-infected chickens can remain infectious for several months in the environment.

Newcastle disease (ND) is a highly contagious disease of major economic significance. NDV strains show great variation in pathogenicity and are classified as velogenic, mesogenic and lentogenic in decreasing order of virulence. Besides other factors such as age and management, clinical observations depend largely on the pathotype and virulence of the causative NDV and may vary from 100% mortality to only a mild respiratory infection. The most prevalent loss amongst layers results from reduced egg production and impairment of eggshell and albumen quality. Reduction of fertility and hatchability of eggs has also been reported.

Infectious laryngotracheitis (ILT) is a viral respiratory tract infection in chickens which is caused by infectious laryngotracheitis virus (ILTV) or Gallid herpesvirus 1. Two forms of ILT are recognised: the mild and the severe ILT forms. Clinical signs associated with the mild form include (haemorrhagic) conjunctivitis, nasal discharge and mild tracheitis, with morbidity as low as 5% and very low mortality (0.1-2%). Clinical signs associated with the severe form include coughing, gasping, marked dyspnoea, respiratory depression and expectorations of bloody stained mucus. The severe form causes high morbidity (90-100%) and mortality usually in the range of 10-20%. The disease often persists for two to six weeks and the virus can be latently present in birds of an infected flock and might reappear after a stress period.

Innovax-ND-ILT was initially developed for and is currently licensed in the US market.

The dossier was submitted in line with requirements of Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

The benefit of Innovax-ND-ILT is intended to be its efficacy for active immunisation of one-day-old chicks or embryonated chicken eggs:

- to reduce mortality and clinical signs caused by Newcastle disease (ND) virus,
- to reduce mortality, clinical signs and lesions caused by Marek's disease (MD) virus,
- to reduce mortality, clinical signs and lesions caused by Infectious Laryngotracheitis (ILT) virus,

which was shown in a number of appropriately designed and well executed laboratory and field studies.

Onset of immunity was established at 9 days post vaccination for MDV, at 4 weeks of age for ILTV and at 5 weeks of age for NDV. A duration of immunity of 62 weeks was established for NDV and ILTV. No data are provided for the duration of immunity against MDV infection and this is acceptable as the HVT virus produces a persistent infection providing a lifelong immunity.

The influence of maternally derived antibodies on the efficacy of the vaccine against NDV was confirmed by challenge in NDV and MDV MDA+ birds. Efficacy against ILT in MDA+ birds is

supported by the results of the semi-field study. Efficacy against MDV and ILTV was sufficiently justified and supportive data was provided.

Innovax-ND-ILT was shown to be efficacious against MDV, NDV (if used in day old chicks) and ILT (if used in day old chicks and embryonated eggs) when used mixed with Nobilis Rismavac. Data and justification in support of the DOI after mixed use was provided.

Innovax-ND-ILT was shown to be efficacious when administered with Nobilis ND C2 or Nobilis ND Clone 30 on the same day but by different routes in SPF chickens. The onset of immunity against NDV was shown to be 2 weeks p.v. for the concurrent use. Onset of immunity against ILTV was confirmed after concurrent use with ND vaccines. Administration of Innovax-ND-ILT and Nobilis IB Ma5 mixed with Nobilis IB 4-91 on the same day but via different routes was shown to be efficacious against IB and ILT. The efficacy against ND after concurrent use with IB vaccines was appropriately justified. The data provided on vaccine-strain viraemia after mixed use with ND or IB vaccines are considered to support the efficacy against MDV.

The omission of studies on DOI for ND, ILT and IB after concurrent use with Innovax-ND-ILT was appropriately justified.

Additional benefits

Innovax-ND-ILT combines protection against three important poultry diseases. This limits the number of times the animals are required to be handled.

Innovax-ND-ILT reduces the need for live attenuated ND and ILT vaccinations. With this vaccine, long lasting immunity against ND and ILT can be obtained by vaccination in the hatchery. There is no interference from the presence of MDA. Contrary to the use of live attenuated ILTV vaccines, there is no risk of establishment of latent ILTV carriers or reversion to virulence.

The vaccine strain was shown to be fully apathogenic to other avian species, limiting the risk to the environment.

Risk assessment

The main potential risks are identified as follows:

Quality:

The formulation and manufacture of Innovax-ND-ILT is well described and specifications set will ensure that product of consistent quality will be produced. The claimed shelf life is considered fully supported by the available data.

For the target species:

The product is generally well tolerated in the target animal. No adverse reactions were observed after a tenfold overdose of Innovax-ND-ILT by the subcutaneous or *in ovo* route. The vaccine strain was obtained by insertion of genes into a naturally apathogenic vaccine strain, which is known to be safe for chickens. The biological properties (safety, dissemination, spread) of the original strain were not changed by the genetic modification, reversion to virulence could not be demonstrated. The chance of recombination with other strains or other viruses occurring is considered to be effectively zero.

For the user:

The user safety for this product is acceptable when used as recommended. Appropriate risk

mitigation measures are described in the SPC.

The vaccine is filled in glass ampoules and stored in liquid nitrogen, in exceptional cases ampoules may explode upon heating. Appropriate precautions and warnings for safe handling of the ampoules are included in the SPC.

For the environment:

The vaccine virus is shed with feather dust and can remain infectious in the environment for prolonged periods. Spread to chickens or turkeys was not observed but cannot be excluded. HVT in general can infect avian species only, the related vaccine strain Innovax-ILT was shown to be unable to infect mice.

For the consumer:

A residue study is not required. The withdrawal period is set at zero days.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

The applicant applied for the following indications: "active immunisation of one-day-old chicks or 18-19 day old embryonated chicken eggs to reduce mortality and clinical signs caused by Newcastle disease (ND) virus and to reduce mortality, clinical signs and lesions caused by avian infectious laryngotracheitis (ILT) virus and Marek's disease (MD) virus".

The product has been shown to be efficacious for these indications, and the CVMP accepted the indications as proposed by the applicant.

Information on development, manufacture and control of the active substance and the finished product has been presented and leads to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information. A withdrawal period is not required. Onset of immunity for ND is comparably late, possibly requiring concurrent use with classical ND vaccines for earlier protection.

The influence of maternal antibodies on the efficacy of the vaccine against MDV was adequately justified. Protection against NDV and ILT was studied using commercial broiler chicks with confirmed levels of MDA against NDV and MDV or against ILT.

Based on the data presented, the overall benefit-risk is considered positive.

Conclusion[1]

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 Innovax-ND-ILT is approvable since these data satisfy the requirements for an authorisation set out

[1] It is important that questions are categorised separately as "major objections" and "other concerns" to ensure that applicants have a clear understanding of the implications of such categorisation when preparing their responses.

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 veterinary medicinal product.