9 October 2014
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

Withdrawal assessment report for Oncept Melanoma (EMEA/V/C/003684/0000)

Common name: Plasmid DNA human tyrosinase (pINGhT) vaccine

Procedure No.: (EMEA/V/C/003684/0000)

CVMP Assessment report with all confidential information removed.
Withdrawal at day 180

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Introduction

On 24 April 2013, the applicant MERIAL submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Oncept Melanoma in accordance with Regulation (EC) No. 726/2004.

The eligibility to the centralised procedure was confirmed by the CVMP on 11–13 September 2012 falling within Article 3(1) of Regulation (EC) No 726/2004 as Oncept Melanoma is a product developed by a biotechnological process. The rapporteur appointed was A.-M. Brady and the co-rapporteur was F. Klein.

The application has been submitted in accordance with the requirements under Article 12(3) of Directive 2001/82/EC, as amended.

Oncept Melanoma is a vaccine for dogs, containing melanoma plasmid DNA (deoxyribonucleic acid) pINGhT which encodes the human tyrosinase antigen. The product was to be presented in single dose glass vials (0.4 ml) in outer packs of 1 vial or 4 vials. The vaccine was to be applied transdermally to dogs, using a recommended transdermal, needle-free, medical device.

The applicant initially applied for the following indication: Active immunisation for extending post-surgical survival times of dogs with oral malignant melanoma without pulmonary metastasis, following surgical removal of the primary tumour and achievement of loco-regional disease control (negative local lymph nodes).

On 17 July 2014, Merial withdrew the application at day 180 of the procedure. In its letter notifying the Agency of the withdrawal of application, the applicant stated the reason for the withdrawal: The company's additional investment in research and development required to answer the remaining issues is not justified.

MUMS status:

Upon request by the applicant the CVMP classified the product as minor use or minor species (MUMS) and confirmed that, where appropriate, the data requirements in the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for MUMS/limited markets (EMEA/CVMP/IWP/123243/2006-Rev.2) would apply.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant provided a detailed description of the pharmacovigilance system, which fulfilled the requirements of Directive 2001/82/EC, as amended. Based on the information provided, the applicant would have 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

Oncept Melanoma was to be manufactured by MERIAL in France. Manufacturing authorisations and good manufacturing practice (GMP) certification of the manufacturing sites were provided for all sites to be

involved in the manufacturing process. Additional inspections of any of the proposed sites were not considered necessary.

Overall conclusions on administrative particulars

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

Part 2 – Quality

Composition

Oncept Melanoma vaccine consists of one active ingredient: DNA plasmid expressing the gene coding for the human tyrosinase: plasmid pINGhT, in suspension.

The excipients in the vaccine are a saline-based buffer.

Container

The product was to be presented in type I glass vials (which comply with the current European Pharmacopoeia (Ph. Eur.)) sealed with butyl-elastomer closures and aluminium caps. Each vial would have included one dose of the product (0.4 ml).

The vials and the closures were sterilised in compliance with current Ph. Eur. methods. Further clarification has been requested to confirm that these are the only methods used.

Development pharmaceutics

Development of the vaccine

The original vaccine construct, pINGhT plasmid, is the same as that which was tested on dogs affected by oral melanoma (Bergman, 2003).

The target titre was defined so as to ensure that it was within the specification range determined by the minimum and maximum release titres.

The container constituents were selected for their compatibility with the vaccine, high pharmaceutical quality and compliance with the requirements of the current Ph. Eur.

The vaccine construct included a kanamycin resistance gene as a selection marker. In line with the CVMP Note for guidance: DNA vaccines non-amplifiable in eukaryotic cells for veterinary use (CVMP/IWP/07/98-FINAL), the applicant justified the use of an antibiotic marker and supported this with a suitable environmental risk assessment.

Needle-free device for transdermal administration

The vaccine was to be administered transdermally using a needle-free transdermal device. This delivery method overcomes the natural absence of plasmid DNA infectiousness targeting the transdermal compartment, known to be enriched with antigen presenting cells.

The recommended medical device for this vaccine application ('VetJet') was developed by MERIAL for the transdermal administration of medicines to small animals. The device works by forcing liquid medication through a small nozzle that is held against the skin, creating a very fine, high-pressure stream of medication that penetrates the animal's skin. There are two different nozzle sizes designed for use with the delivery device in different sizes of dogs: transdermal administration in small dogs less than 15 kg bodyweight (bw) using a small nozzle and transdermal administration in large dogs of 15 kg bw and overusing a larger nozzle size.

The applicant provided a description of the development of the device to be used for transdermal delivery of the Oncept Melanoma vaccine in dogs.

Several devices with differences in nozzles size and spring force have succeeded one another over the product development. The modification of the spring power and the nozzles sizes took account of safety concerns (pain, bruising and/or haematoma at the site of administration), particularly in smaller dog breeds. The injection distribution patterns of the devices were validated in a series of studies and were found to be equivalent.

The CVMP agreed that satisfactory justification of the specific characteristics of the recommended administration device had been provided. The CVMP noted, however, that concerns had been expressed by the U.S. Food and Drug Administration (FDA) about a possible fracture risk associated with use of the device in kittens. The applicant was requested to present a strategy to ensure that the recommended device would be readily supplied to the user on any potential future market throughout the EU, and what training materials were intended to train the relevant veterinarians how to use the designated device (VetJet) correctly.

Method of manufacture

E. coli bacteria were used to produce plasmids. The latter were extracted and concentrated.

Detailed descriptions of the production steps were provided and the storage conditions for intermediate products were adequately supported.

The product was developed in the USA and the manufacturing process and control analytical tools were transferred to the French site, and equivalence of the American and European products was shown.

Control of starting materials

Active substance

The original bacteria containing the pINGhT plasmid were supplied to MERIAL. This pre-master seed was the basis for the subsequent master seed bacteria. The history of the strain was well documented.

The master seed bacteria was tested appropriately and the results were acceptable.

The working seed bacteria was also tested appropriately and the results were also acceptable.

Sequence data of the parental plasmid were provided in accordance with the requirements of the CVMP Note for guidance: DNA vaccines non-amplifiable in eukaryotic cells for veterinary use (CVMP/IWP/07/98-FINAL).

Data were provided that confirm the genetic stability of the finished product plasmid. Full sequence data were provided for the master seed, active ingredient and batches of finished product which showed 100% agreement in the sequence. Phenotypic stability was confirmed.

Stability of the active ingredient

Stability of the active ingredient was studied on three batches of pINGhT manufactured in two different manufacturing sites in the USA and Europe. Data was provided to confirm the equivalence of production and testing at the two different active substance sites. Further clarification about equivalence for endotoxin testing has been required.

Excipients

The only excipients in the proposed vaccine were the components of the saline-based buffer, for which a satisfactory in house standard was provided.

All of the buffer components complied with the requirements of the relevant Ph. Eur. monographs.

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

The product did not contain any materials of animal origin.

Control tests during production

The proposed tests during production on both the active ingredient and the filled product were described.

The CVMP considered that the controls implemented during the production were in the main considered adequate to ensure consistency and homogeneity of the production process, however some further information was required before they could be accepted.

Control tests on the finished product

Overall, the controls performed on the finished product were considered sufficient to ensure the safety and efficacy of the vaccine batches to be released. However, there were some points, in particular regarding the specifications set for some of the tests that need to be clarified before a final conclusion could be made.

Due to the fundamental importance of the exact identity of the vaccine construct to its function, it was considered important that the control tests at release were capable of confirming that the product is as expected. The applicant was requested to address this.

The applicant provided data on two consecutive production scale batches of the active ingredient and finished product which demonstrated consistency of production. This application was classified as MUMS/limited market and therefore batch data from two (production scale) batches was considered to be acceptable.

The results of finished product testing were within the proposed specifications. Certificates of analysis were provided for both batches and were acceptable.

Stability

Stability of the finished product was studied with batches of vaccine manufactured in two different manufacturing sites, one in the USA and one in Europe. All batches were tested according to the control tests proposed by the applicant.

The applicant's claimed shelf life for the finished product was based on the results from the two sets of stability studies described above. Data were provided to confirm equivalence of the manufacturing process and control tests at both sites, and therefore the longest stability data from batches produced at one site can be used to establish the shelf life of the product.

Overall conclusions on quality

The analytical part of the application was well documented. The active substance is DNA plasmid pINGhT, which encodes the human tyrosinase antigen. Oncept Melanoma vaccine was intended to be injected transdermally using the recommended device in dogs, targeting the muscle of the medial thigh.

A specific delivery device was developed and designed to be used with two nozzle sizes, for use in either small or large dogs.

The rationale for product development was well documented, and the inclusion of an antibiotic (kanamycin) resistance gene (KanR) as a selection marker was adequately justified.

The manufacturing method was adequately described. The active ingredient, DNA plasmid pINGhT was cultured in *E. coli* and was subjected to various sequential steps of downprocessing.

Details of the production steps, manufacturing parameters were provided. Details of the storage conditions for intermediate products were also provided and supported by data.

Starting materials were well defined and controlled. The product did not contain any materials of animal origin.

The controls implemented during the production were mostly considered adequate to ensure consistency and homogeneity of the production process; however, further information would have been required on some test methods. Consistency batches for active ingredient and finished product have been provided to support the consistency of production.

Overall, the controls performed on the finished product were considered sufficient to ensure the safety and efficacy of the vaccine batches to be released. However, there were some points, in particular regarding the specifications set for some of the tests that would have needed to be clarified before the product could have been authorised. Due to the fundamental importance of the exact identity of the vaccine construct to its function, it was considered important that the control tests at release were capable of confirming that the product is as expected. At the time of withdrawal of the application, the applicant was requested to address this.

The stability of the active ingredient and finished product had been supported with batches produced in two different manufacturing sites. Data had been provided to confirm that the production process and testing performed at both sites were equivalent. The shelf life for the product was established.

Part 3 – Safety

Oncept Melanoma contains a plasmid DNA expressing the gene coding for the human tyrosinase, pINGhT, in an aqueous solution. The principle of xenogeneic DNA vaccination was applied with Oncept Melanoma to overcome immunological tolerance to canine tyrosinase in dogs with melanoma. The xenogeneic (human) tyrosinase protein is foreseen to stimulate in dogs an immune response, cross-reacting with canine tyrosinase protein and canine melanoma cells expressing the canine tyrosinase.

The vaccine intended for use in Europe originated from the same master seed bulk as the same vaccine authorised in the USA. Some safety studies carried out for the initial development of the USA product (using the US batches) were presented to support the safety part of the dossier. The applicant stated that the vaccine batches used to conduct these safety studies were produced according to the manufacturing process described in Part 2, except for the formulation titre of plasmid pINGhT that could be higher on purpose. This was acceptable for the CVMP.

Laboratory tests

Safety of the administration of one dose

No single dose studies were provided, but the safety of the administration of a single dose has been demonstrated in safety studies of repeated administration of one dose according to the recommend schedule. This was considered acceptable by the CVMP.

Safety of one administration of an overdose and repeated administration of one dose

The safety of repeated doses was shown in a pivotal study using the pINGhT plasmid, and was further supported by several studies not conforming to good laboratory practice (GLP) using either the pINGhT plasmid or the final canine melanoma vaccine.

Safety of the pINGhT plasmid in dogs (overdose and repeated administrations)

The pivotal safety study was designed to evaluate simultaneously the safety of an overdose (10 doses of the maximum dose) followed by 4 repeated single doses of pINGhT (above the maximum dose, 0.4 ml).

Clinically healthy dogs (Beagles; equal numbers of males and females), at 4–5 months of age at the start of the study were either injected with the test vaccine (n=9) or with water for injections (n=3) at 2-week intervals at the inside of the hind leg. All the dogs were injected using the recommended transdermal vaccination system (fitted with a small nozzle size as proposed for dogs of less than 15 kg bw; or a larger nozzle size for dogs of 15 kg bw or more). Animals were monitored daily for the duration of the study, for local and general reactions, including rectal temperature. In addition, a specific observation was done at 3-5 hours after each vaccination. After euthanasia of the animals on Day 70, a sample was taken from the last (Day 56) injection site.

In general, the vaccine was well tolerated. Transient fever up to 40.7 °C, and lasting up to 10 days was observed post-vaccination in some dogs. Minor transient local reactions were observed in some dogs, i.e. red skin marks, local swelling, redness in the injection site. It was considered that some of the local

reactions were related to the route of administration rather than to the vaccine, and particularly to the device (pressure spring) used for the administration of the product.

Other supportive studies

In support of the safety of repeated administrations of the vaccine, the applicant also provided several early development non-GLP studies using either the pINGhT plasmid or the final canine melanoma vaccine. While the non-GLP status might have still been acceptable in view of the confirmed MUMS status of the application, due to other shortcomings in study design, the studies could only be regarded as supportive in regard to the safety of the vaccine, confirming the observation from the pivotal study, i.e. minor systemic reactions (transient hyperthermia) in vaccinated dogs, and local reactions at the site of administration. The CVMP agreed that, in general, the vaccine was well tolerated and that adverse reactions observed (hyperthermia and local reactions) should be included in the summary of product characteristics (SPC) and package leaflet.

However, as local reactions were mainly attributed to the needle-free transdermal administration device (not to the vaccine), the supportive studies were used to refine the design of the needle-free transdermal administration device for its use in dogs, as outlined below.

Safety of the needle-free transdermal device

The device used in some development studies was an earlier design (with a single nozzle size and two different spring forces, one for dogs up to 9 kg bw, and a higher-pressure spring in dogs over 9 kg bw). The recommended VetJet device has two different nozzle sizes (a smaller one for dogs up to 15 kg bw; and a larger one for dogs from 15 kg bw and more, using the same spring force). The devices were validated in a series of studies and found to be equivalent.

Safety in small dogs using the recommended device

The design and objectives of this study were the same as for the pivotal safety study, i.e. an overdose of the vaccine followed by 4 single dose vaccinations according to the recommended regimen, 2-weeks apart.

Twelve clinically healthy Beagle dogs, aged 8 months, were vaccinated using the recommended VetJet transdermal vaccination system. Injection site reactions were limited to redness, and the presence of blood droplets or crusts at the injection sites. Bruising and/or haematoma (as observed with earlier designs of the delivery device trials using a larger size nozzle) were not observed. The applicant has been asked to clarify the weights of the dogs at the time of vaccinations.

Safety study in healthy dogs, using another device than the recommended one

Fifty clinically healthy dogs (Beagles and mini-mongrels) aged between 3–14 months were vaccinated with the Canine Melanoma Vaccine, DNA. The vaccine was administered as recommended, i.e. in the proximal half of the medial thigh every 2 weeks, using alternate legs. On the day of vaccination, all dogs were observed for acute general and local reactions. Each dog was then observed 3 times per week for two weeks after each vaccination for chronic reactions and also monitored daily for general clinical condition. Apart from moderate fever (40.3–40.7 °C in 7 different dogs up to two days), some per-acute

local reactions at the injection site were observed: response to a painful stimulus, formation of raised red skin marks and droplet of blood in 27% of vaccinations.

The device used to administer the vaccine was an earlier design, using two different models of a needle-free injection device with a slightly larger nozzle size than currently recommended with two different spring forces.

Safety study in small dogs (with a needle-free vaccination device different to the recommended one)

The aim of the study was to demonstrate the safety of transdermal administration of the vaccine using an earlier design of the needle-free transdermal administration device with a high-pressure spring, and slightly larger nozzle size than the recommended one in small dogs.

Ten clinically healthy dogs (Beagles), males and females, with weights between 2.3 kg and 4.5 kg were vaccinated twice and monitored as described in the previous study. No statement of GLP compliance has been provided for this study. No control group was included in the study. The study report provided was not comprehensive for this small in-house study, and this limits the usefulness of these data.

Pain was observed at the injection site in five out the 20 vaccination events (40% of animals) either after the first or the second vaccination. A total of five dogs (50%) were affected and in one case, the pain was so severe that it questions whether the administration of the vaccine using this specific preliminary transdermal device is safe in small dogs.

High dose safety study in healthy dogs (with a needle-free vaccination device different to the recommended one)

A study was conducted to evaluate the safety of the canine melanoma DNA vaccine at high levels of impurities, and plasmid DNA, when administered to 25 clinically healthy dogs (Beagles and mongrels), aged between 6 months and 11 months. The vaccine was administered as recommended, in the proximal half of the medial thigh of alternate legs, every 2 weeks, using a needle-free injection device different from the one that is recommended, i.e. with a single nozzle size and different spring force for dogs ≤ 9 kg bw and > 9 kg bw.

The vaccine batch used was produced in the US manufacturing site. The dogs were monitored as described in the previous study. No statement of GLP compliance has been provided for this study. No control group was included in this study.

Temperature increases were less marked and only two dogs had rectal temperatures in the range of 40.3–40.7 °C that lasted for a maximum of two days. Wheal formation was observed on 17 occasions. The only chronic reactions observed were small encrustations (< 1 mm) in 9 dogs following 10 vaccination events).

The CVMP agreed that most of the safety concerns in regard to the recommended model of the needle-free transdermal administration device had been addressed. However, the device is currently not commercially available in EU, and the applicant was requested to present a strategy to ensure that the recommended device would be readily available to the user on any potential future market throughout EU, and also what training materials would be provided to train the relevant veterinarians on the use of the device.

The CVMP also noted that the administration method is unique as it requires the recommended device for the transdermal administration. As this device is not supplied with the vaccine or currently commercially available in EU, there remain concerns that instead of using the recommended device for vaccine administration, a different, non-assessed method of administration might be chosen by the veterinarian. The risk arising from such use is that the safety (and efficacy) of the product could be different from that observed in the studies carried out.

Examination of reproductive performance

No specific reproductive safety test was performed in dogs, which is acceptable under the MUMS considerations: omission of studies of the effect on reproduction will be accepted – if not performed, relevant warnings should be given in the SPC. The SPC also mentions that the "safety of the veterinary medicinal product has not been established during pregnancy and lactation".

Examination of immunological functions

Oncept Melanoma is an immunological veterinary medicinal product containing plasmid DNA expressing the gene coding for the human tyrosinase, pINGhT, in an aqueous solution. Because Oncept Melanoma vaccine is based on xenogeneic DNA vaccination, several specific potential risks are addressed below.

Auto-immunity

Tyrosinase antigens are differentiation antigens which are expressed on both cancer cells and normal cells. For this reason, immunity to self-antigens on cancer cells is constrained by immune tolerance/ignorance. Weber et al. (1998) demonstrated in a mouse model of melanoma that using a plasmid expressing an evolutionary conserved, xenogeneic melanosomal differentiation antigen, tolerance to differentiation antigens can be overcome, establishing the principle of xenogeneic DNA vaccination for cancer immunotherapy.

The principle of xenogeneic DNA vaccination was applied for the Oncept Melanoma vaccine. The resulting human tyrosinase protein would stimulate an immune response in dogs, cross-reacting with canine tyrosinase protein and canine melanoma cells expressing the canine tyrosinase.

No auto-immune sequelae have been reported in the submitted data in dogs with oral melanoma treated with Oncept Melanoma. The applicant has adequately considered the possibility of potential occurrence of autoimmune side effects in the target species with a vaccine intended for immunisation against a self-protein (tyrosinase). The CVMP noted that no such side effects have been observed in the field safety studies or included in the pharmacovigilance data available from the use of the product in authorised markets. The CVMP therefore agreed that specific warnings to veterinarians or information on such specific effects are not needed on the SPC. Tyrosinase-related pathologies would be expected to be picked up as part of routine safety surveillance of the product and adverse events would be reported in accordance with the DDPS and regulatory requirements.

Tolerance

It has been hypothesised that due to the low but possible long term expression of the transgene, DNA vaccination could induce tolerance more easily than other vaccine technologies. A single publication was provided reporting the induction of tolerance with a specific malaria antigen in newborn mice (Mor, 1997) however this concern had not been confirmed in any other models. The CVMP accepted the argumentation that since Oncept Melanoma was expected to be used in adult/elderly dogs, the risk of tolerance induction appeared to be very low and that any consequences should be very limited.

Special requirements for live vaccines

Oncept Melanoma contained as active ingredient plasmid DNA, which is not a live organism, so the special requirements for live vaccines do not apply to this product. However, specific studies to document plasmid DNA safety were submitted, and they are described in this section due to the similarity of the design to those of live organisms.

Spread of the vaccine strain to in-contact dogs

Diffusibility of DNA plasmid pINGhT coding for human tyrosinase after injection to dogs

To assess the diffusibility into the environment, a high dose of plasmid DNA pINGhT vaccine was administered to 3–4 month-old healthy Beagle dogs (n=6) by the transdermal route into the left thigh (0.4 ml). Two further dogs were non vaccinated in-contact controls. To detect plasmid pINGhT or fragments thereof in biological samples, DNA was extracted from blood and from various tissues (urine, nasal and rectal swabs) of the dogs on D-1, D1, D3 and D8and was subsequently amplified using a PCR technique.

The plasmid DNA was not shed in urine but was detected, in order of frequency, in nasal swabs, rectal swabs and blood at least during 3 days after vaccination (see also below: recombination or genomic re-assortment of the strains). Shedding of plasmid DNA did not result in transmission to in-contact dogs, a finding which was stated to be expected given the non-infectious character of the vector system and the difficulty of achieving efficient DNA uptake following natural routes.

PCR validation data were limited to the techniques performed on skin and muscle samples. The relevance of the batch of vaccine used in this study was justified. The PCR used to detect plasmid has not been validated for the other tissues tested, therefore it was not possible to establish a meaningful assessment of the risk of integration, and the applicant was asked to provide appropriate validation data. Consequently, it was not possible for the CVMP to reach a definitive conclusion on the dissemination of the vaccine construct, although it was possible to use the data supplied in association with the applicant's risk assessment to formulate a rationale to take account of the limitations of the studies presented.

Spread of the vaccine strain to other species and/or environmental bacteria

The vaccine construct includes an antimicrobial (kanamycin) resistance gene (KanR) as a selection marker, and the potential transmission of the kanamycin resistance gene to other species and/or environmental bacteria was addressed through the environmental risk assessment, which concludes that the risk is very low (see below).

Dissemination in the vaccinated animal

Biodistribution and persistence of DNA sequences related to plasmid pINGhT at the injection site of dogs

To assess the biodistribution and persistence at the injection site of DNA sequences related to plasmid pINGhT, a high dose of plasmid pINGhT, was administered to 3–4 month-old healthy Beagle dogs (n=6) by the transdermal route. Two further dogs were non vaccinated in-contact controls. To detect plasmid pINGhT or fragments thereof in samples, DNA was extracted from various tissues (blood, bone marrow, gonad, liver, lung, injection site - muscle and skin-) at D59, and was subsequently amplified using a PCR technique.

On the sampling day all dogs were anaesthetised. Before anaesthesia, a blood sample was drawn from the jugular vein. After anaesthesia, a bone marrow sample was taken at the sternum. Dogs were then euthanised. Samples of the liver, gonad, and lung and from the injection sites (skin and the adjacent muscle) were obtained. The applicant justified the batch of pINGhT used in the study. The results of this study indicated the absence of pINGhT DNA or related DNA sequences 59 days after transdermal administration.

However, the study had a number of limitations, in particular as for the study above, the applicant was requested to provide appropriate validation data for all tissues tested.

Reversion to virulence of attenuated vaccines

The vaccine is not infectious and cannot replicate in eukaryotic cells, thus reversion to virulence is not a relevant issue.

Recombination or genomic re-assortment of the strains

Because plasmid DNA may penetrate into the cellular nucleus, there is a potential risk of genomic integration into the genomic DNA of treated canine melanoma patients. A risk assessment for a potential chromosomal integration of vaccine DNA based on existing literature was provided by the applicant. Data from a number of different mammalian species to support the safety profile of DNA vaccines and a suitable risk assessment were provided. Data were also provided which confirm that detectable levels of plasmid were present in the blood of vaccinated animals 1 day after administration, although results were negative by the next time point (3 days) The CVMP considered that it was not possible to make a complete assessment of the risk taking into account that the PCR results obtained from other tissues were not fully understood in lack of method validation for all relevant tissues. The applicant was asked to provide PCR validation data for the other tissues tested.

It was acknowledged that the data presented have a number of weaknesses, which preclude the precise risk from being quantified. However the data, in association with the risk assessment submitted, the current understanding about DNA vaccines and the natural barriers in place to reduce the risk of integration, indicate that the risk may be very low. In addition to this, and in view of the circumstances of use (relatively small numbers of dogs, under close care and supervision, administered under controlled conditions, dogs are not food producing species, most are elderly and past breeding age, life span is relatively short) the risk from the potential for integration is considered to be very low, and it was accepted that a study to investigate integration is not necessary.

Field studies

Evaluation of the safety of “Canine Melanoma Vaccine, DNA” in dogs diagnosed with oral melanoma

The safety of the canine melanoma vaccine was investigated in 2006–2007 in 60 dogs diagnosed with oral melanoma (WHO stage 2 or 3) based upon post-vaccination systemic and injection site reactions. This safety trial ran concurrently with the field efficacy study, i.e. the same dogs were used to assess both safety and efficacy. Dogs of various breeds (28 females and 32 males) were included that ranged in age from 5 to 16 years and weight from 1.4 kg to 46.7 kg.

Dogs were treated as recommended, i.e. four single doses (0.4 ml) at 2-week intervals, administered transdermally into the proximal half of the medial aspect of the thigh, caudal to the femur, using a needle-free device. On the day of each vaccination, physical examination and clinical observation was done by a veterinarian 30 minutes post-vaccination. Daily owner examinations occurred for 13 days after each vaccination. On day 14 after each vaccination, physical examination and evaluation of the injection site was performed by a veterinarian. Vaccination site reactions were documented.

The results of this field safety trial indicate that the profile of Oncept Melanoma in dogs with melanoma in the field is similar to that observed during the safety laboratory studies. Only transient acute reactions were observed after each injection. The most frequent and very common (i.e. observed in more than 10% of treated animals) acute adverse reactions were pain on palpation of the injection site (mild to moderate) and leakage from the injection site in the form of serum droplet in most of the cases. Wheal formation was common (i.e. observed in 1% – 10% of treated animals). Encrustations were only reported in two cases.

A new finding was the observation of 16 cases of bruising in 11 dogs which resulted in haematoma formation in 3 of the cases, all of them in small dogs (weighing 5–6 kg). All but one resolved within 2 weeks. Increased body temperature was not recorded post-vaccination; however, in the field study animals were returned to their owners post vaccination and therefore body temperatures were not systematically monitored unless the animals were returned with adverse reactions. Whilst not ideal in the design of a field study, the lack of temperature recording can be accepted. Oncept Melanoma is classified as MUMS / limited market and data requirements allow omission of field studies if laboratory studies sufficiently show no safety risk. However, one of the laboratory studies showed that transient hyperthermia (up to a maximum of 40.7 °C) may occur after vaccination of clinically healthy dogs, and a warning to that effect was proposed to be placed on the SPC.

The medical device used for transdermal administration in the field study was an earlier design to the currently recommended device (VetJet) device, which was modified due to tolerance problems at the site of administration.

Pharmacovigilance data

In addition to the field study, the applicant also provided pharmacovigilance data from the use of the vaccine in USA and Canada collected from 2007 to 2012; i.e. since the beginning of the commercialization. The data support the observations in the safety study, i.e. transient hyperthermia and minor reactions at the site of injection.

User safety

The user safety assessment was based on the CVMP Guideline on user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006) and adequately considered its principles of assessment. Potential exposure of humans to the plasmid DNA expressing human tyrosinase (pINGhT) could be following accidental self-injection or oral uptake, or via direct contact to a vaccinated animal.

The argumentation provided for assessment was that the worst potential outcome of human exposure would be the development of an immune response to the tyrosinase protein, which could theoretically lead to depigmentation of skin and hair. No other reactions were expected as the parental plasmid pING is not pathogenic in humans. The potential for local reactions (in the user) was not discussed. Immune responses against self-antigens (the antigen encoded by the pINGhT plasmid is the human tyrosinase)

were considered unlikely to occur, as the likelihood to overcome tolerance from a single, accidental exposure of humans was considered to be low.

A risk assessment was provided which adequately considered the potential risk to the user considering accidental self-injection (1) with a DNA vaccine expressing human tyrosinase, but also people in contact (2) with a vaccinated dog, as the plasmid has been found in the secretions and blood of vaccinated animals. Based on this risk assessment it was considered that the risk is very low.

In the first scenario, the likelihood of an accidental self-administration of a full dose of plasmid DNA using the recommended transdermal device by veterinarians and specialized staff trained to use it, was considered to be very small. Shedding of vaccine plasmid DNA in secretions and/or blood of vaccinated dogs is transient. Also, the amount of DNA concerned was expected to be low and the plasmid DNA is not expected to persist for long in the environment. Furthermore, accidental contact of plasmid in secretions and/or blood from vaccinated dogs with human skin was expected to be without consequence as plasmid DNA is not known to be taken up when applied on the skin without any enabling technology. The worst case scenario of oral intake (linked to licking of contaminated hands) was also considered to be of very low risk, as DNA plasmid is known to be sensitive to gastric fluids.

Adequate general warnings were requested by the CVMP for inclusion in the SPC to mitigate any potential user safety risk.

In conclusion, the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the proposed SPC, taking into account the requested modifications.

Environmental risk assessment

A comprehensive environmental risk assessment (ERA) was provided in line with CVMP Note for guidance: environmental risk assessment for immunological veterinary medicinal products (EMEA/CVMP/074/95), i.e. Phase I assessment, focussing on the transmission of plasmid-related DNA to the environment in the context of canine vaccination with Oncept Melanoma, with specific focus on the transmission of a DNA fragment harbouring the antimicrobial (kanamycin) resistance gene (KanR). The ERA was supported by extensive literature. In the assessment the applicant also considered Guidance on environmental risk assessment for veterinary medicinal products consisting of or containing GMOs as or in products. It is concluded that the risk is low and therefore Phase II assessment not needed. The vaccine is not a GMO and assessment in accordance with Annex 1, Part 3.E is not needed.

Natural gene transfer to bacteria is known to exist (transformation) and to contribute to evolution in aquatic and/or terrestrial environments. Because the pINGhT plasmid harbours KanR there is a theoretical risk that this gene could be transferred into bacteria of the environment. However, the risk was considered to be very remote. A possible gene transfer would occur at an extremely low frequency and would have a very limited impact, and an increase in the level of already existing kanamycin resistance is not expected. In conclusion, adverse effects on human health and to the environment resulting from the transfer of DNA sequence from a canine plasmid DNA pINGhT vaccine encompassing KanR to bacteria are considered unlikely.

The CVMP agreed that the risks from KanR transfer could be considered very low, based on the current state of knowledge. Adverse effects on human health and to the environment resulting from the transfer of DNA sequence from a canine DNA vaccine pINGhT encompassing a kanamycin resistance gene to bacteria were considered unlikely.

On the basis that a comprehensive ERA was provided which has established the risks from KanR transfer being very low, and taking into account the circumstances in which the product would be used, the CVMP considered that an additional study to investigate the transfer of KanR to environmental bacteria was not necessary.

Based on the data provided Oncept Melanoma is not expected to pose a risk for the environment when used according to the proposed SPC.

Overall conclusion on safety

Based on an assessment of the data and information provided by the applicant the safety of the product was considered acceptable subject to satisfactory resolution of a number of remaining concerns. The applicant was asked to clarify the weights of dogs at time of vaccination to ensure safety of administration in smaller dogs. It was considered that validation of the analytical method for use on the other tissues tested (apart from muscle and skin) was necessary before a conclusion can be made concerning the persistence of the plasmid vaccine in the animal. Warnings on the SPC need to be revised to detail hyperthermia observed following vaccination (i.e. fever (maximum 40.7 °C) may occur, lasting up to 10 days), the formation of raised red skin marks (wheals) at the site of administration, and the frequencies of the various adverse effects observed.

Part 4 – Efficacy

Introduction and general requirements

Clinical features of canine malignant melanoma of the oral cavity

Oral canine malignant melanoma (CMM) is a rapidly progressive, locally invasive and metastatic neoplasm of dogs' mouths. Oral tumour locations include the gingival, lingual, buccal, pharyngeal, tonsillar and palatine mucosal epithelium. Local invasion and metastasis are common and CMM is generally associated with a poor prognosis. Prognostic indicators include tumour stage (WHO staging criteria based on TNM classification (4 stages): tumour (T), lymph nodes (N), metastasis (M), histological classification, location in mouth (lingual/buccal mucosa and rostral location are associated with improved prognosis), clean/dirty surgical margins and presence/absence of bone lysis. Survival time reported in the literature (all WHO stages) ranges from approx. 7–24 months in dogs which had undergone surgical excision with or without radiation therapy. Survival time for dogs with stage II and III tumours appears to be shorter where reported (< 5 months to approximately 12 months following aggressive local excision).

DNA vaccination and mechanism of delivery

DNA vaccination is an innovative recombinant vaccine technology, based on purified bacterial plasmids engineered to express recombinant antigens following *in vivo* administration. Upon injection to the target species, vaccine plasmids need to gain access to cellular cytoplasms and nuclei to induce *in vivo* and *de novo* expression of a recombinant antigen and to elicit the desired immune response. The proposed hypotheses for mechanism of action detailed by the applicant are 1) transfection of myocytes leading to major histocompatibility complex (MHC) class I antigen presentation; 2) phagocytosis of transfected myocyte and/or recombinant antigen by antigen presenting cell (APC) leading to MHC class II antigen presentation; 3) direct transfection of APC and consequent MHC class I antigen presentation.

Superior transfection is reported in the literature for transdermal needle-free plasmid delivery compared to intramuscular and intradermal delivery, and on this basis transdermal injection was taken forward in the development of this product (see also Part 2).

Selection of pINGhT plasmid DNA and target antigen

See Part 2 for quality aspects on choice of the plasmid.

Oncept Melanoma contains a DNA plasmid (pING) encoding the human tyrosinase antigen (pINGhT). The enzyme tyrosinase is one of a group of differentiation antigens and is a melanosomal membrane glycoprotein expressed by normal melanocytes but over-expressed by neoplastic melanocytes. Whilst dogs show immune tolerance towards the canine tyrosinase, human tyrosinase induces a strong active immune response in dogs, cross-reacting with canine tyrosinase. By using the human tyrosinase antigen (xenogeneic vaccination principle) which shows significant homology with the canine molecule, the intention was to overcome this immune tolerance.

Laboratory trials

No laboratory trials have been submitted; this is justified by the absence of an experimental model of oral melanoma in dogs. Demonstration of efficacy of the product rests on data submitted under 'field trials'.

Field trials

In support of the efficacy of the DNA vaccination with Oncept Melanoma and the proposed dose and dosing regimen, the applicant provided one pivotal field study using the product in the proposed posology and target population, as well as several supportive studies obtained from published literature.

These published reports were used to support the principle of DNA vaccination of melanoma dogs with human tyrosinase (Bergman et al., 2003; Liao et al., 2006), and as historical negative control group (MacEwen et al., 1999) for the pivotal field study. It should be noted that the pivotal clinical study ran concurrently with the field safety study, i.e. the same dogs were used to assess both safety and efficacy. In addition, a retrospective, independently conducted US study (Ottnod et al., 2013) was taken into consideration during the assessment. This paper was published after the assessment of the dossier had already started.

DNA vaccination of dogs with xenogeneic human tyrosinase

Two published papers (Bergman et al., 2003; Liao et al., 2006) reported data from a study conducted in the USA involving nine dogs with advanced (stage II to IV) malignant melanoma, not restricted to the oral cavity. A human tyrosinase DNA vaccine pINGhT was administered transdermally to the caudal thigh at four doses of either 100, 500 or 1,500 µg DNA at fortnightly intervals. Median age of the dogs was 13 years (range 9–14); and median bodyweight was 13 kg (range 3.6–61 kg bw). Three were female, five male; four males were entire and all other animals were neutered. All dogs had undergone surgery prior to enrolment and three dogs had also received radiation therapy previously. Median survival time was 389 days (range 57 to > 588) which the authors compared favourably with median survival times reported in the literature for stage II–IV dogs (reported to be ≤ 2 – 5 months). Measurable recombinant human tyrosinase-specific antibodies were detected in three dogs. In two of these dogs cross-reactivity against syngeneic canine tyrosinase was also demonstrated.

Bergman et al. (2003) concluded that their results indicated that the product was potentially efficacious based on clinical antitumour responses and prolonged median survival times. Liao et al. (2006) further

noted that the three dogs with measurable tyrosinase antibody responses had a higher median survival time than the six dogs without a detectable antibody response although this was not statistically significant, possibly due to the very small numbers of animals. A final observation made by both author teams was that in clinical trials, humoral response can take 4–8 weeks to be induced and it may be months before clinical response is seen.

Supportive study (MacEwen et al., 1999): "negative control group"

The second study was presented as a peer reviewed paper by MacEwen et al. (1999) who reported the results of two linked clinical trials conducted in the USA in dogs with oral malignant melanoma. The objectives of these trials were unrelated to DNA vaccination against the neoplasm; the importance of this paper for the proposed product application was principally as a source of historical control dogs for the vaccinated population described in the applicant's pivotal field study.

The objective of these trials was evaluation of the parameters "disease free survival" (DFS) and "survival time" (ST) of four different therapeutic strategies: liposome-encapsulated muramyl tripeptide (L-MTP-PE) administration vs. placebo; L-MTP-PE once weekly vs. twice weekly; L-MTP-PE alone vs. L-MTP-PE + granulocyte macrophage colony-stimulating factor (GM-CSF); and finally, the effect, if any, of simple vs. radical surgical resection of the melanoma.

Eligibility criteria included dogs with spontaneously occurring, histologically confirmed oral melanoma; no previous treatment of the melanoma; no evidence of distant metastasis on thoracic radiography; tumour and any regionally affected lymph nodes amenable to surgical excision; overall good health.

Ninety eight dogs were involved across both studies, and 53 of these dogs presented with stage II or III oral malignant melanoma. The analysis by MacEwen et al. (1999) indicated that a statistically significant increase in survival time was only detected in dogs with stage I melanoma which received L-MTP-PE compared with placebo. No significant treatment effect was seen for any analysis for stage II and III dogs, and the survival times for these dogs are numerically fairly similar (median 243–306 days). The median disease free survival was 90–156 days. However, regarding the use of these stage II and III dogs as historical controls, the CVMP considered that it is not possible to fully rule out that L-MTP-PE could have a beneficial therapeutic effect, although it would be logical to conclude that any such effect would make demonstration of efficacy of Oncept Melanoma more difficult. It might be possible that there are other effects of the test therapies administered by MacEwen et al. which are unknown, and could include safety implications. While these are unquantified, administration of these test therapies must– in principle at least – be considered a potential confounding factor.

Pivotal field study:
Evaluation of the efficacy of canine melanoma vaccine in dogs diagnosed with oral melanoma

This was a non-GCP (good clinical practice) multi-centre, single-group, non-randomised, non-blinded prospective study conducted over 2 years in specialist veterinary oncology centres in the USA (2006–2008). The test product was 'Canine Melanoma Vaccine, DNA' which held a conditional USDA licence at the time of the study.

The dose regimen tested was the one recommended by the applicant: a primary course of a single minimum doses (0.4 ml) of plasmid DNA administered four times at two-week intervals, followed by single boosters every six months thereafter. Route of administration was transdermal needle-free injection to medial thigh caudal to femur using an earlier model of the recommended needle-free device.

The study included 58 client-owned dogs (only 53 were included in the survival analysis), and results were compared to 53 dogs ("historical controls") from the population reported by MacEwen et al.

(described above). Vaccinated dogs and controls were aged 5–16 years and 1–20 years, respectively; 28 vaccinated dogs were males vs. 25 females; 23 controls were males vs. 19 females.

Broad anatomical tumour location was given for vaccinated dogs and controls. Blood samples were collected for a complete blood count (CBC) and serum chemistry. CBC and serum chemistry results were however not presented by the applicant. Blood samples for immunological assay were taken and archived, but these immunological analyses were not carried out. A physical examination was performed on each dog prior to inclusion in the study and at each vaccination event. Examinations included determination of rectal temperature, abdominal palpation, thoracic auscultation, and evaluation of peripheral lymphnodes, eyes, ears, oral cavity and digits. Follow-up examinations were regularly performed, monthly for the first year of post-vaccination survival, and at 1–3-month intervals thereafter.

Inclusion criteria: all dogs had to fulfil the following eligibility criteria:

• Melanoma of the oral cavity (diagnosed histologically), and

• WHO stage II or III (as described in MacEwen et al., 1986: modified WHO criteria), and

• Surgical removal of the primary tumour, and

• Achievement of "local disease control" defined as:

  - no gross disease of the oral cavity, and

  - absence of regional lymph node metastasis based upon bilateral fine needle aspirates of submandibular lymph nodes, or surgical removal or radiation of regional lymph nodes, and absence of pulmonary metastasis based upon three dimensional radiographic view of the thorax.

The primary efficacy variable was “survival time (ST) to event” (death due to melanoma) measured from date of surgery to date of death or study termination; acceptance criterion was statistically significant ($p<0.05$) increase in survival time for vaccinates compared with historical controls.

Dogs which died due to causes other than CMM, were lost to follow-up or were alive at the end of the study were censored. Dogs which received radiation treatment or chemotherapy post-vaccination were also censored, although post-vaccination surgical resection of the primary tumour site/lymph nodes was permitted). The primary variable was analysed using Kaplan-Meier analysis and log-rank test. For analysis of the effect of covariates on survival, Cox proportional hazard model was used. Results showed that out of the 53 dogs in each group, 13 of the vaccinated dogs died (versus 34 controls), while the number of dogs alive at the end of the study was 19 (compared to 11 in the control group). Death unrelated to disease occurred in 9 vaccinated dogs (7 controls), 7 dogs in the vaccinated group were removed post-inclusion (none in the control), and 5 (versus 1) dogs were lost during follow-up.

Of the five vaccinated dogs lost to follow-up, two were reclassified as having died from melanoma (one left to pursue radiation therapy and one died but no necropsy was performed). This change was taken into account in a sensitivity analysis.

Analysis of primary efficacy variable (Kaplan-Meier survival analysis for the outcome of death from canine melanoma)

The results of a Kaplan-Meier survival analysis for the outcome of death due to canine melanoma showed that there was a significant group difference ($p=0.0003$) with the vaccine group showing better survival. Point estimates with 95% confidence intervals (unadjusted) showed that the median survival time for death due to canine melanoma was more than 19 months for the vaccine group (> 583 days, could not be calculated since less than 50% of the deaths can be attributed to canine melanoma), versus 10 months

(324 days) in controls. If the 25% percentile was considered, survival time was 464 days in vaccinates, versus 156 days in controls. However, recalculating the figures of the point estimates (95% confidence intervals) with sensitivity analysis and amendments due to errors showed shorter survival rates, i.e. > 411 days (50%) and 181 days (25%) in vaccinated dogs versus 324 and 156 in the controls, respectively. Ancillary analyses comparing effect of stage in vaccinated and control dogs and length of follow-up in censored animals found no significant difference between the two groups.

From the data analysis alone, the statistically significant difference between groups implied that the vaccine, when used as an adjunct to surgical removal of oral canine melanoma primary tumours, was effective in extending the mean survival time in treated dogs beyond that achieved in a population of historical controls. However, the CVMP considered that the problems with study design and concerns over the validity of comparing the two populations meant that this result was not reliable.

The amount of information available for control animals was very sparse. This is a major deficiency and a recognised limiting factor in the use of historical controls. In the vaccinated dogs, the protocols implemented for loco-regional control and specifically radiotherapy were not harmonized over the 8 investigators. The applicant considered that pre-treatment biases were compensated by the large number of dogs enrolled and balanced across treatment and historical control populations; this was the case for tumour stage and duration of follow-up, but could not be evaluated for a number of other factors which influence prognosis. Important detail on those parameters with prognostic significance, such as histological classification of the tumours, was unavailable for both vaccinates and control dogs. The proposed justification for use of historical controls on the basis of it being unethical to enrol negative controls was questioned by CVMP given the lack of efficacy of the test product found in a published field study (Ottnod et al., 2013, see below). As a result of using a historical control population with incomplete information the similarities and differences between vaccinated and control dog populations could not be adequately identified. This compromised the validity of comparing the two populations, which consequently severely limited the scope for drawing conclusions on the efficacy of the product on the basis of this study.

Regarding analysis of the primary efficacy variable, the Kaplan-Meier survival curve, log rank p value and Cox proportional hazards analysis indicated a statistically significant increase in survival time for vaccinated dogs compared to controls. However, due to the deficiencies of the study design, this study could only be plausibly viewed as a descriptive case series of 53 vaccinates.

Therefore, from the data available on the pivotal study and due to the deficiencies described above it was not possible to draw conclusions on the efficacy of the vaccine.

The CVMP also noted that the disease-free interval (DFI) was not evaluated in this study, although it is considered a clinically relevant endpoint as discussed in the CVMP Guideline on dossier requirements for anticancer medicinal products for dogs and cats (EMEA/CVMP/28510/2008); however, the committee agreed that DFI would not be a good indicator of immunotherapy vaccine efficacy due to the fact that there is often a lack of significant improvement in DFI despite a significant improvement in overall survival time, since the effect of vaccination is a reduction in tumour growth and increased time to reach a critical tumour burden with death. This approach was supported by two peer-reviewed publications. Nevertheless, the SPC would need to include clear information that the efficacy of the product was assessed based on overall survival time only, and that other parameters such as the disease free interval were not considered, i.e. the use of the product may not reduce the time to disease progression (e.g. melanoma recurrence, metastasis) and effective loco-regional control would be critical to ensure that a clinically significant therapeutic effect could be realised.

Retrospective analysis of efficacy data using Oncept Melanoma vaccine (Ottnod et al., 2013)

During the assessment phase of this application, a paper was published (Ottnod et al., 2013) reporting the findings of a retrospective, independently conducted US study investigating whether the use of Oncept Melanoma as an adjunct therapy affected the outcome of dogs with loco-regionally controlled oral malignant melanoma. Controls were also based on historic data, but in this study the control dogs had been treated earlier at the same centre. The review covered the period 1998–2012 and included 45 dogs, 22 vaccinated with Oncept Melanoma and 23 non-vaccinated, all with stage I–III oral melanoma tumours (30 dogs with stage II–III). The main finding of this study was that the data did not show a statistical difference between vaccinated and non-vaccinated dogs for the endpoints progression-free survival (PFS), disease-free interval (DSI) or median survival time (MST).

A comparative critique was provided by the applicant for the pivotal study and the study reported by Ottnod et al. (2013). Within this critique the applicant put forward the view that although the reasonable expectation of success of treatment with the proposed product is challenged by Ottnod et al. in their published study, the data available from the Ottnod paper are still insufficient to reach conclusive certainty for treatment effect or lack of thereof. The smaller sample size and the inclusion of patients receiving second line therapies from progression are considered by CVMP to be valid criticisms of the Ottnod study.

However, the CVMP compared further the design and conduct of the pivotal study with that of Ottnod et al. (2013), and considered that the greater standardisation, greater effort to show groups to be representative of each other, inclusion of disease free interval (DFI) and progression free survival (PFS) endpoints, and the much smaller temporal window between control and vaccinated dog enrolments made the Ottnod study superior to the applicant's pivotal study in a number of ways, and its results consequently more reliable.

The CVMP considered therefore that the results of the Ottnod et al. study added to the uncertainty over the efficacy of the product.

The CVMP concluded that a prospective, randomised, blinded, contemporaneously controlled study data was needed in order to reliably characterise and evaluate the efficacy for this product. This is consistent with MUMS guidance, which allows for deviations from standard data requirements when assessing minor use product applications but which nevertheless upholds that studies should be adequate for demonstration of their objectives.

Overall conclusion on efficacy

No experimental model exists for CMM and therefore the efficacy studies relating to this product were conducted in clinical cases only.

An overview of the clinical manifestation of CMM was provided in the application, and the disease is well characterised.

An overview of the principles of DNA vaccination and the rationale for use of the transdermal delivery device, as well as their reasons for selection of the target antigen have been provided and are acceptable. Human tyrosinase is considered different enough to induce an immune response in dogs, yet similar enough for induction of cross-reaction against canine tyrosinase so that the treatment is directed against the canine melanocyte.

Proof of concept for the use of DNA vaccination using plasmid DNA (pING) encoding human tyrosinase (pINGhT) was taken forward by Bergman et al. (2003), who showed encouraging results in nine dogs with severe (stage II–IV) canine melanoma (not restricted to the oral cavity). Further support for the concept was provided by Liao et al. (2006) who found recombinant human tyrosinase antibodies in three of the nine dogs post-vaccination, with some correlation between antibody response and clinical picture.

The plasmid dose, initial vaccination course, and the six-month interval between repeated booster vaccinations were all taken forward on the basis of the preliminary proof of concept data from the Bergman et al. (2003) study, with the aim of confirming the dose in the pivotal field study.

The pivotal field study (05-171) failed to provide valid results. While results appear supportive of efficacy when looking at the statistical analysis for survival time between vaccinated dogs and historic controls, due to extensive missing information (including on details relevant to prognosis) it is not possible to make a valid comparison between the vaccinated dogs and the historical control dogs. Therefore, it is not possible to conclude from this study that the vaccine is efficacious.

The most recent study by Ottnod et al. (2013), which indicated no statistically significant differences in vaccinated and non-vaccinated dogs, further strengthened the CVMP conclusion that there is insufficient evidence available at this time to support a conclusion that the Oncept melanoma vaccine is efficacious. Finally, the proposed dose/dosing regimen have not been sufficiently substantiated.

In summary, the efficacy of the product is inconclusive. A prospective, randomised, blinded, contemporaneously controlled study data is needed in order to reliably characterise and evaluate the efficacy for this product. This requirement is consistent with MUMS guidance, which gives allows for deviations from standard data requirements when assessing minor use product applications but which nevertheless upholds that studies should be adequate for demonstration of their objectives.

Part 5 – Benefit-risk assessment

Introduction

Oncept Melanoma suspension for injection for dogs is a DNA vaccine presented as a suspension containing plasmid DNA encoding the human tyrosinase enzyme (pINGhT). The product was to be administered via transdermal 'needle-free injection' using a specified device in a primary course with four single doses given at 2-week intervals, followed by 6 monthly boosters. The proposed product is classified as MUMS and was therefore assessed in line with the recommendations in the CVMP Guideline on data requirements for immunological veterinary medicinal products intended for MUMS/limited markets (EMA/CVMP/IWP/123243/2006-Rev.2).

Canine malignant melanoma is an aggressive neoplasm and following surgery oral malignant melanoma has a high incidence of local recurrence and distant metastasis. The prognosis for survival using current treatment options (surgery with or without radiation therapy or chemotherapy) remains poor. Oncept Melanoma was intended to be used as an immunotherapy in canine oral melanoma to extend post-surgical survival time. The proposed indication was for the active immunisation for extending post-surgical survival times of dogs with oral malignant melanoma without pulmonary metastasis, following surgical removal of the primary tumour and achievement of loco-regional disease control (negative local lymph nodes).

Benefit assessment

Direct therapeutic benefit

This product represented a novel strategy for the treatment of stage II and III oral canine malignant melanoma in dogs, a neoplasm with a poor prognosis and short survival times under current standard treatment. The proposed therapeutic effect of the product was to extend post-surgical survival times of dogs with oral malignant melanoma without pulmonary metastasis, as an adjunct to surgical management of the disease with or without local radiotherapy.

The mode of action was demonstrated and is based on the principle of xenogeneic DNA vaccination to overcome tolerance in canine melanoma patients. The human tyrosinase protein, expressed in dogs by pINGhT plasmid, stimulates an immune response in dogs, cross-reacting with canine tyrosinase protein and canine melanoma cells expressing canine tyrosinase.

The pivotal field study failed to demonstrate efficacy. In this prospective, non-randomised, non-blinded, single arm, prospective US field study, a significant increase in survival time was seen in a population of 53 dogs with stage II or III oral malignant melanoma treated with the product compared with 53 historical controls ($p=0.0003$; median survival time of vaccinates not reached; median survival time of controls: 324 days). However, the validity of this comparison and therefore the clinical significance of this result were brought into question mainly due to the use of an inadequate control. A historical control population was used which could not be confirmed as being comparable with the vaccinated population. The applicant's pivotal study could therefore only be considered as a descriptive exploratory study, i.e. as an uncontrolled case series. Moreover, a more recent independent study (Ottnod et al., 2013), which also used historical controls but with fewer study design deficiencies than the pivotal field study, observed no significant difference in efficacy between dogs vaccinated with Oncept Melanoma and those treated with conventional means alone.

The efficacy of the product was therefore not considered to be satisfactorily supported, and a prospective, randomised, blinded, contemporaneously controlled study is considered necessary to enable characterisation and evaluation of the efficacy for this product. This requirement is consistent with MUMS guidance, which allows for deviations from standard data requirements when assessing minor use product applications but which nevertheless upholds that studies should be adequate for demonstration of their objectives.

Additional benefits

In general, only a small amount of DNA is required to induce an intended immune response. The expression of the antigenic gene in the target species through eukaryotic systems means that the protein to which the immune response will develop better resembles the native protein, which is a benefit for inducing a relevant immune response.

Since the DNA plasmid is not (usually) in itself immunogenic the risk of developing an immune response that could interfere with the administration of the vaccine in subsequent booster immunisations is potentially minimal, which is a benefit when frequent repeated doses are required to achieve and maintain efficacy.

Risk assessment

Main potential risks have been identified as follows:

Quality:

The formulation and manufacture of the proposed product was reasonably well described and specifications were set, however some further information would have been required concerning some of the tests before the CVMP would have been satisfied that a product of consistent quality would have been produced.

The assessment of quality remains inconclusive as detailed above and further information would have been required to conclude on this part of the application.

For the target animals:

There is a potential risk of integration of the plasmid DNA into the genomic DNA of the target species through homologous recombination. The human tyrosinase gene in the DNA plasmid pINGhT will have high homology with the canine gene encoding tyrosinase. This greatly increases the potential for recombination which would cause a mutation in the host genome and increase the risk for melanoma in the treated animal. In addition, plasmid DNA could integrate into the genome of gametes which would have the potential to pass to subsequent generations and thereby become part of the germline DNA. It is acknowledged that the data presented have a number of weaknesses, which preclude the precise risk from being quantified, however the data, in association with the applicants risk assessment and what is currently understood about DNA vaccines and the natural barriers in place which reduce the risk of integration have the potential (pending adequate validation of the PCR method for use on the full range of tissues tested) to enable the level of risk to be verified as very low.

The product is recommended for administration for the remainder of the dog's life. The potential side effects of DNA vaccines administration over such a long term are yet unknown. On the basis of the mode of action a low risk for development of tolerance and related consequences has been identified.

In general the vaccine was well-tolerated. The adverse reactions observed in the safety studies were minor systemic reactions (slight transient temperature increase) and local reactions at the site of administration. The results from the safety studies indicate a potential risk of enhanced local adverse reactions post vaccination in small dogs, however this risk is managed by the recommendation to use the proposed version of the needle-less transdermal device, which is designed for use with two nozzle sizes to provide better control of safety concerns (pain, bruising and/or haematoma) in smaller breeds: a smaller nozzle size of for dogs of less than 15 kg bw, and a larger nozzle size for dogs of 15 kg bw and above.

However, the administration method is quite specialised and the recommended device for the transdermal administration is not supplied with the vaccine. In addition, the device is currently not commercially available in EU. Consequently, there remain concerns that instead of using the recommended device for vaccine administration, a different, unassessed method of administration might be chosen by the veterinarian. The risk following on such use is that the safety (and efficacy) of the product could be different from that observed in the studies carried out.

The assessment of target animal safety remains inconclusive as detailed above and further information would have been required to conclude on this part of the application.

For the user:

There is a risk of exposure to the DNA plasmid expressing human tyrosinase (pINGhT) from accidental self-injection or contact with excreta of vaccinated dogs. This has the potential to cause depigmentation of skin and hair. However, the likelihood of this was considered very small.

In conclusion, the product would not pose an unacceptable risk to the user when used in accordance with the proposed SPC, taking into account the requested modifications. The applicant has adequately considered the potential risk to the end user from accidental self-injection with a DNA vaccine expressing human tyrosinase and also those in contact with the vaccinated animals as the plasmid has been found in the secretions and blood of vaccinated animals and the risks to the user are considered very low. It was concluded that the risk to user safety is very low.

For the environment:

There is a risk of vaccine persistence at the administration site that would facilitate dissemination into the environment. The DNA plasmid pINGhT was detected in nasal secretions, faeces and in the blood of vaccinated animals. Plasmid DNA will therefore be accessible to environmental bacteria on the skin and in the gut and nasal cavity, all of which are rich in bacteria. The risk of transmission of the kanamycin resistance gene KanR to environmental bacteria has been assessed which concludes that this risk is very low.

Based on the data provided Oncept Melanoma would not be expected to pose a risk for the environment when used according to the proposed SPC.

Risk management or mitigation measures

The identification of necessary risk management or mitigation measures remains outstanding due to the inconclusive assessment.

Evaluation of the benefit-risk balance

Efficacy has not been appropriately demonstrated for Oncept Melanoma.

The assessment of quality and target animal safety remains inconclusive.

Clarification on the commercially availability of the recommended needle-free transdermal device in the EU remains outstanding.

The product would present an acceptable risk for users and the environment when used in accordance with proposed recommendations.

In the presence of outstanding major and other concerns, the benefit-risk balance of the application remains inconclusive.

Conclusion on the benefit-risk balance

The overall benefit-risk evaluation for the product remains inconclusive.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that Oncept Melanoma was not approvable since "major objections" were identified which precluded a recommendation for a marketing authorisation. The details of these major objections were outlined in a list of questions. At the time of withdrawal of the application, responses to the questions remained pending.

No conclusions could be taken on the benefit-risk balance of the application.