Universiteit Utrecht

Evaluation of Oncept® Melanoma vaccine in Dutch dogs with malignant melanoma: 45 cases (2011-2015)

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Abstract

Background – Canine malignant melanoma (CMM) is a spontaneously occurring, highly aggressive tumour that has the propensity to metastasize. Active immunotherapy in the form of vaccines represents one potential therapeutic strategy for melanomas.

Aim of study – To evaluate the influence of Oncept® Melanoma vaccine on the median survival time and quality of life in dogs with canine malignant melanoma (CMM), treated according to the standard procedure as formulated by the University Clinic for Companion Animals in Utrecht, The Netherlands.

Animals – Forty-five dogs with malignant melanoma and a historical control group of 91 dogs with oral melanoma.

Methods – All 45 patients were treated according to the standard procedure as formulated by the University Clinic for Companion Animals in Utrecht. A retrospective control group consisting of 91 dogs with oral CMM was used to compare the effects of the Oncept Melanoma Vaccine on MST.

Results – For all 42 cases (excluding 3 dogs for various reasons), the median survival time (MST) was 282 days. The MST for dogs with oral melanoma was 242 days, that of the historical control group is 174 days. Vaccinated dogs did not have a significant longer survival time than non-vaccinated dogs ($P = .057$). Significant variables with an influence on survival time (ST) within the vaccinated dogs are: oral versus cutaneous/digit melanomas ($P = .032$), lymph node involvement ($P = .012$), radiation therapy ($P = .023$) and the number of doses ($P = .014$). Significant variables with an influence on ST within the vaccinated dogs with oral melanoma are: lymph node involvement ($P = .000$) and disease stage ($P = .035$).

Conclusion – No significant longer ST was found in dogs with CMM treated with the Oncept® Melanoma vaccine in the present study. More recent studies have implicated that the vaccine does not significantly improve survival time and therefore will not become available on the European veterinary market for commercial purposes. CMM, however, still is an aggressive tumour with a high propensity to metastasize and therefore research has to be continued. For future studies to adjuvant therapies of this disease, a recommendation is to set up a prospective, randomized and standardized clinical trial with, perhaps, a whole new and different melanoma vaccine.

Contents

1. INTRODUCTION

Canine malignant melanoma (CMM) is a spontaneously occurring, highly aggressive tumour that has the propensity to metastasize (**Bergman, 2003; Bergman, 2006; Vail, 2013**). Melanomas arise from melanocytes. The exact risk factors for CMM are not well established (**Vail, 2013**). Sites most commonly affected by melanomas include the oral cavity, mucocutaneous junction, nail bed and footpad (**Grosenbaugh, 2011**).

CMM varies widely in biological behaviour (**Smedley, 2011**). The most important prognostic factors in canine melanocytic neoplasms are anatomic site, size, stage and histologic parameters (**Smedley, 2011; Vail, 2013**). Small, cutaneous melanomas that are located away from mucosal margins often behave in a benign manner and therefore have a relative good prognosis (**Manley, 2011; Vail, 2013**). In contrast, a 5.0 cm high-grade oral melanoma has a poor-to-grave prognosis (**Vail, 2013**). However, the prognosis is always uncertain due to the unpredictability of these melanomas and the wide variability in survival times.

Many characteristics of CMM are comparable with human malignant melanoma (HMM), including histological phenotype, tumour genetics and clinical biological behaviour as well as the development of recurrent or resistant disease and metastasis. Both CMM and HMM are resistant to chemotherapy and occur spontaneously in outbred, immune-competent patients (**Bergman, 2008**). CMM therefore represents as an attractive translational model for the assessment of the efficacy of future immunotherapies for the treatment of HMM (**Riccardo, 2014**).

1.1. TREATMENT OF CMM

Surgical removal of melanomas remains the most effective local treatment (**Grosenbaugh, 2011; Vail, 2013**). Canine patients with stage II, III and IV melanoma have a median survival time (ST) of less than 5 months with aggressive local treatment (surgery) (**Bergman, 2003**). An additional systemic therapy used in dogs with CMM is chemotherapy. Unfortunately, low response rates and little evidence for increasing ST make this therapy less popular in the treatment of CMM and in many countries chemotherapy is no longer used in dogs with melanoma (**Bergman, 2003; Vail, 2013**).

1.1.1. ACTIVE IMMUNOTHERAPY

Active immunotherapy in the form of vaccines represents one potential therapeutic strategy for melanomas. This is a xenogeneic DNA vaccine with genes encoding tyrosinase family members (**Bergman, 2003; Liao, 2006; Manley, 2011**). Tyrosinase is a copper-containing enzyme essential for melanin synthesis. This type I membrane glycoprotein is the rate-limiting step in controlling the production of melanin (**Phillips, 2012**). Phillips et al. have showed that all canine melanocytic tumours, including oral, non-oral, benign, malign, pigmented and amelanotic tumours, have high relative tyrosinase expression. In contrast to control tissues, which show a low expression, regardless of degree of pigmentation or anatomic location (**Phillips, 2012**). Tyrosinase is therefore a suitable target for immunotherapy because of its restricted, tissue-specific expression (**Liao, 2006**).

Canine tyrosinase and human tyrosinase are much alike but vary enough for human tyrosinase to be used in active immunotherapy in dogs (**Liao, 2006**). Injections of xenogeneic tyrosinase DNA may overcome canine immune tolerance to self-tyrosinase because of the antigen being transcribed and translated in the canine host followed by recognition and processing by major histocompatibility complex (MHC) and associated co-stimulatory molecules (**Liao, 2006; Grosenbaugh, 2011**). Normal cutaneous melanocytes do not express class II MHC, however the expression of class II MHC in malignant melanocytes is upregulated. This explains why the immune response is preferentially directed towards the tumour cells (**Wang, 1999; Grosenbaugh, 2011**).

Research has shown that DNA vaccines only induce very modest plasmid uptake by relevant cell types *in vivo*. Therefore, Oncept® Melanoma vaccine is administered using a transdermal needle-free device to deliver the plasmid into both the dermis and the muscle. This method induces a significant specific cell-based immune response (human tyrosinase-specific IFNγ T cell response) compared

to a conventional intramuscular (IM) injection. One hypothesis for this is a synergism between plasmid expression from the muscle and the dermis that allows for a better immune response (**Goubier, 2008**).

1.2. AIM OF THE STUDY

The aim of this study is to evaluate the influence of Oncept® Melanoma vaccine on the median survival time and quality of life in dogs with CMM, treated according to the standard procedure as formulated by the University Clinic for Companion Animals in Utrecht, the Netherlands.

2. MATERIALS AND METHODS

2.1. ANIMALS

Between February 2011, and May 2015, 45 dogs were treated with the Oncept® Melanoma Vaccine at the University Clinic for Companion Animals in Utrecht, the Netherlands. All dogs with malignant melanoma were considered eligible for inclusion in this trial.

Clinical disease staging (stage, I to IV) was performed for all dogs according to the World Health Organization (WHO) TNM-based scheme for dogs with oral melanoma (see table 1). Even though different types of melanomas were included, all melanomas were staged according to this staging guideline to prevent bias and to better compare effects of vaccine treatment.

Due to the lack of a control group, a retrospective control group consisting of 91 dogs with oral CMM was used to compare the effects of the Oncept Melanoma Vaccine on MST. Dr. Sarah Boston collected the data for this historical control group through submissions of dogs by members of the Veterinary Society of Surgical Oncology. Inclusion criteria for this group of dogs were histological confirmed diagnosis of oral malignant melanoma and were treated with tumour excision in the period from 2001 to 2012.

2.2. TREATMENT PROTOCOL

All patients were treated according to the standard procedure as formulated by the University Clinic for Companion Animals in Utrecht. Before dogs were included in the clinical trial, they underwent a thorough physical examination. The sizes of tumours were measured when possible or measurements were estimated from medical records. By fine-needle aspirate biopsy (FNAB) or histology the diagnosis of CMM was confirmed. In most cases blood was collected for full blood work analysis to exclude any possible underlying diseases. Dogs were screened for metastasis to local lymph nodes by palpation, FNAB or histology and for distant metastatic disease by computed tomography (CT). If possible, the macroscopic tumour was surgically removed; any positive lymph nodes were excised as well. In some cases, the tumour was too advanced to completely remove and debulking of the primary tumour was initiated. Within 7 to 10 days' post-surgery, dogs underwent radiation therapy (6x6Gy). Radiation therapy was given twice a week, for up to 3 weeks. Most initial vaccination series started during radiation therapy; however, the interval between surgery and administration of the first dose varied among patients. Follow-up took place 1 month, 3 months and 6 months after completion of de initial vaccination series. At 6 months, the owners were given the possibility to start a second vaccination series.

Table 1: 'World Health Organization' (WHO) TNM-Based Staging Scheme for Dogs with Oral Melanoma (*Vail, 2013*)

T: Primary Tumour	
T1	Tumour ≤2 cm in diameter
T2	Tumour 2-4 cm in diameter
T3	Tumour >4 cm in diameter
N: Regional Lymph Nodes	
N0	No evidence of regional node involvement
N1	Histologic/cytologic evidence of regional node involvement
N2	Fixed nodes
M: Distant Metastasis	
M0	No evidence of distant metastasis
M1	Evidence of distant metastasis
Stage I	T1N0M0
Stage II	T2N0M0
Stage III	T2N1M0 or T3N0M0
Stage IV	Any T, any N, and M1

The historical control group consists of dogs from various clinics in the United States of America, and therefore did not have a standardized protocol as described above.

2.2.1. Surgery and radiation therapy

Most dogs underwent surgery at the University Clinic in Utrecht, other dogs were treated by their own veterinarians and referred to the University Clinic for adjunctive therapy. The extent of surgical margins was based on the available histopathology reports and classified into four categories: 0 for no surgery (or debulking), 1 for complete removal, 2 for marginal removal (< 5 mm) and 3 for dirty margins (> 5 mm).

Radiation therapy was advised in dogs that had histologic findings suggestive of marginal removal, dirty margins or in dogs in which, due to the size of the tumour or the location, no complete removal could be achieved. Dogs that had already been diagnosed with local lymph node involvement were also candidates for radiation therapy. The standardized radiation protocol consisted of 6 twice-weekly 6 Gy fractions.

2.2.2. Vaccinations

The initial vaccination series started during radiation therapy. Some dogs have had radiation therapy at their own veterinarians and therefore vaccination started after the radiation therapy. All dogs received an initial series of 4 injections of the Oncept® Melanoma Vaccine, one vaccination every 2 weeks (with minor variations due to client scheduling needs). At 6 months after the initial vaccination series, owners were given the possibility to start a second vaccination series. One dog, Dino, was given 5 vaccination series in total.

The Oncept® melanoma vaccine is packaged in single-dose vials; these contain 0.4 mL dose volume. The vaccine is administered by using the VET JET® transdermal vaccination system (needle-free). The site used for injection was on the medial aspect of the thigh, in muscle just caudal of the femur. Prior to administration of the vaccine, the injection site was shaved and cleaned with alcohol.

2.2.3. Follow-up

The first follow-up took place approximately 30 days after the fourth vaccination was given. Dogs were evaluated by physical examination to rule out local recurrence of the tumour, regional lymph node enlargement (possible metastasis) and any side effects from the vaccination. Three months' post vaccination; thoracic radiographs were taken for detection of possible distant metastasis. And at 6 months, owners were given the choice to begin with a new vaccination series, starting with a new CT scan to check for distant metastasis.

All dogs were followed until death. Some dogs currently are still alive and, unfortunately, some dogs were lost to follow-up.

2.3 STATISTICAL ANALYSIS

All statistical analyses were performed by use of the SPSS 22 statistical software package. Values of $P < 0.05$ were considered significant; values of $P \geq 0.051$ were left out in the results.

Survival time (ST; in days) was defined as the time from date of diagnosis until date of death or last contact (for dogs lost to follow-up). STs were evaluated by Kaplan-Meier (KM) product survival analyses and followed by a Log Rank test.

Age of the dogs (in years) was defined as the time from date of birth until date of diagnosis.

3. RESULTS

Of the 45 dogs that received the Oncept® melanoma vaccine, three dogs were removed from the study for variable reasons and are not included in the results.

Signalment – 42 dogs with CMM were considered eligible for inclusion in this trial. There were 24 breeds represented in the group of vaccinates (which included purebreds and crossbreeds). No breed was significantly more common than others. The dogs ranged in age from 5.6 to 13.3 years, with a median age of 9.3 years and a mean age of 9.8 years. Nineteen female dogs (of which 15 spayed) and 23 male dogs (of which 8 castrated) were included. The weight of the dogs ranged from 6.2 kg to 62.6 kg, with a median

weight of 25.3 kg. Table 2 presents an overview of the patient characteristics. Of 42 dogs, 18 dogs were still alive in May 2015. Fourteen out of 24 deceased dogs had died by CMM. Vaccinates that died of causes other than CMM, such as other cancer diseases, only two had evidence of CMM recurrence or metastasis at the time of death.

Table 2: Patient characteristics

Variable	Vaccinates
No. of dogs	42
No. of males (castrated)	23 (8)
No. of females (spayed)	19 (15)
Age (y)	.
Median (mean ± SD)	9.3 (9.8 ± 2.3)
Range	5.6 – 13.3
Weight (kg)	.
Median (mean ± SD)	25.3 (26.7 ± 12.7)
Range	6.2 – 62.6
Stage I disease (No. of dogs)	15
Stage II disease (No. of dogs)	10
Stage III disease (No. of dogs)	13
Stage IV disease (No. of dogs)	4
Regional lymph node involvement	11 (26.2%)
Distant metastasis	4 (9.5%)

Tumour characteristics – Among the 42 dogs, tumour location was primarily oral (n=23 [54.8%]), followed by cutaneous (n=10 [23.8%]) and digit (n=8 [19.0%]). One dog had ocular melanoma. Of the 23 oral malignant melanomas, 11 were found on the maxilla and 7 were found on the mandibula. Six CMM were considered amelanotic (14.3%).

Tumour staging – The regional lymph nodes were evaluated for involvement. In 11 dogs (26.2%), the regional lymph nodes were found positive (see table 2). Thoracic CT to screen for distant metastasis was executed in 40 dogs before surgery, in two dogs only thoracic radiography was performed due to limited costs. Findings were considered positive in 4 dogs (9.5%). Taking all this in consideration (based on the WHO staging scheme): 15 dogs had stage I disease, 10 dogs had stage II disease, 13 dogs had stage III disease and 4 dogs were diagnosed with stage IV disease.

Surgery and radiation therapy – In 6 dogs no surgery or only debulking was performed. Nine excisions had dirty margins based on histopathologic findings. In 14 dogs the tumour was completely removed and in 13 dogs only marginal removal was achieved. In addition, 23 dogs underwent radiation therapy (radiation protocol as mentioned above).

Vaccination – All 42 dogs received vaccinations. Thirty-one dogs underwent only one vaccination series (4 doses), six dogs received 8 doses (two vaccination series) and one dog even underwent 5 vaccination series with a total of 20 doses. One dog did not complete the initial vaccination series and 3 dogs died during their second vaccination series.

Retrospective control group – In the group of patients as collected by Dr. Sarah Boston, 91 dogs did not receive the melanoma vaccine and therefore were considered to be of use in a retrospective control group. Because of the heterogeneity (and lacking information) of this group, only survival time was used for statistical analysis.

3.1 STATISTICAL ANALYSIS

More than 50% of the dogs died and therefore a median survival time (MST) could be calculated. For all 42 cases, the median survival time (MST) was 282 days and a 95% confidence interval of 310 – 526 days with a mean of 418 days. The Kaplan-Meier survival plot for this study group can be seen in figure 1.

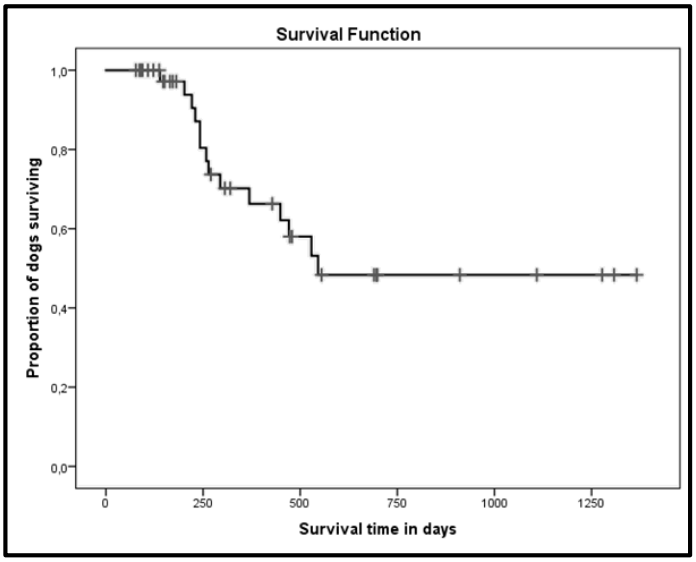

Figure 1 - Kaplan-Meier survival plot for the vaccinated dogs in the present study (n = 42). The MST is 282 days and a 95% confidence interval of 310 – 526 days with a mean of 418 days.

To better compare the results of the present study with previous studies, the MST was calculated for dogs with oral melanoma. The MST was 242 days and a 95% confidence interval of 228 – 520 days with a mean of 374 days.

For the control group, a MST was calculated of 174 days and a 95% confidence interval of 209 – 345 days with a mean of 276 days.

3.1.1. Kaplan-Meier Analysis

To compare ST of the vaccinated and the non-vaccinated retrospective control group, a Kaplan-Meier analysis with Log Rank test was performed. Dogs died of other causes than CMM and which were lost on follow-up were censored. Vaccinated dogs did not have a significant longer survival time than non-vaccinated dogs (*P* = .057) (see figure 2).

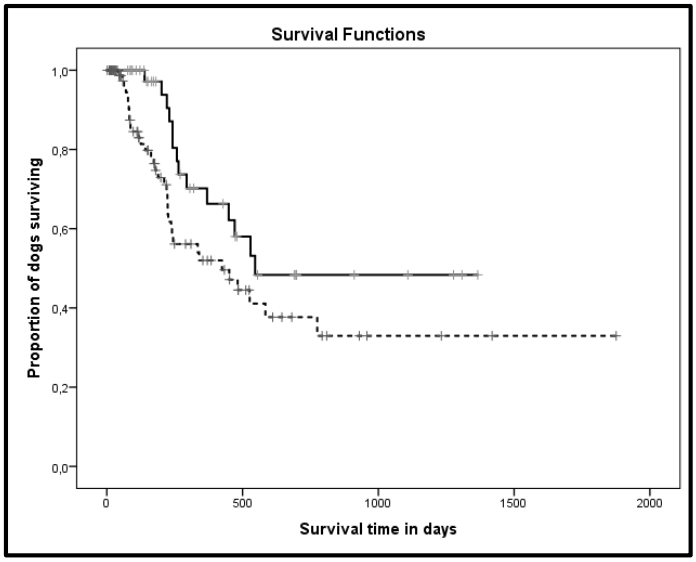

Figure 2 - Kaplan-Meier survival plot for vaccinated (n=42; solid line) and non-vaccinated (n=91; dashed line) dogs. There was no significant difference between the two groups (P = .057); the MST is 282 and 174 days for the vaccinated group and for the non-vaccinated group, respectively. Dogs died of other causes than CMM and which were lost on follow-up were censored.

Within the vaccinated group, several factors were taken in consideration for influencing the ST. Various Kaplan-Meier analyses were performed, *P*-values and MST can be seen in appendix I. In all analyses, dogs that died of other causes than CMM and which were lost on follow-up were censored.

Oral melanoma is associated with a significantly shorter ST compared to cutaneous and digit melanoma (*P* = .032; see figure 3). Also positive lymph node involvement decreased ST significantly (*P* = .012; see figure 4). Dogs that underwent radiation therapy survived significantly less than dogs who did not undergo radiation therapy (*P* = .023; see figure 5). And the number of doses was of significance for longer survival (*P* = .014; see figure 6).

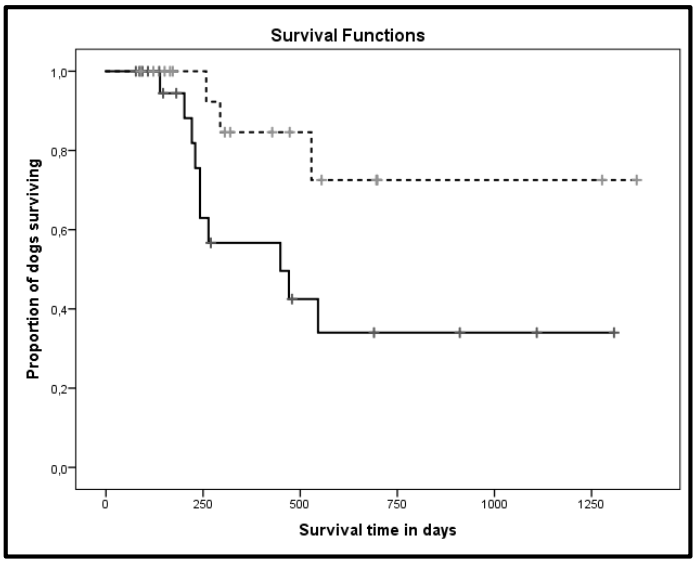

Figure 3 - Kaplan-Meier survival plot for oral melanomas (n = 23; solid line) and other melanomas (cutaneous or digit; n = 18; dashed line). There was a significant difference between the groups (P = .032); the MST is 242 and 374 days for the oral melanomas and for the other melanomas, respectively. Dogs died of other causes than CMM and which were lost on follow-up were censored.

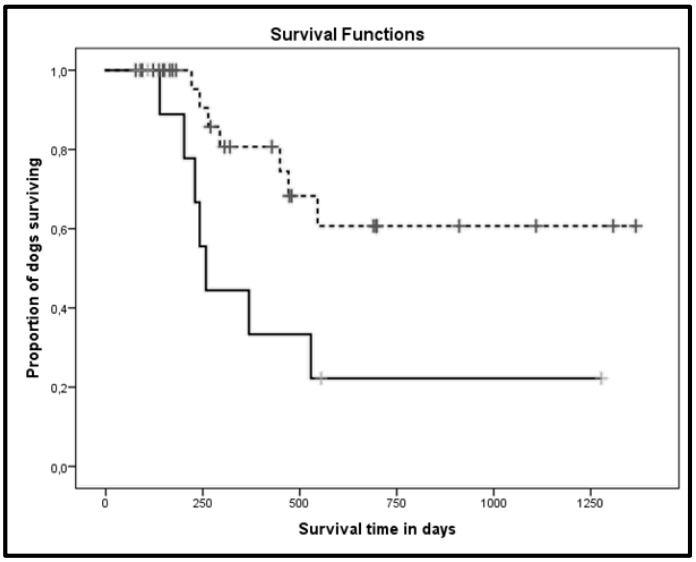

Figure 4 – Kaplan-Meier survival plot for lymph node involvement in all vaccinated dogs. There was a significant difference in survival time between dogs that had positive regional lymph nodes (n = 11; solid line) versus dogs without positive regional lymph nodes (n = 31; dashed line) (*P* = .012); the MST is 242 and 306 days for positive lymph nodes and negative lymph nodes, respectively. Dogs died of other causes than CMM and which were lost on follow-up were censored.

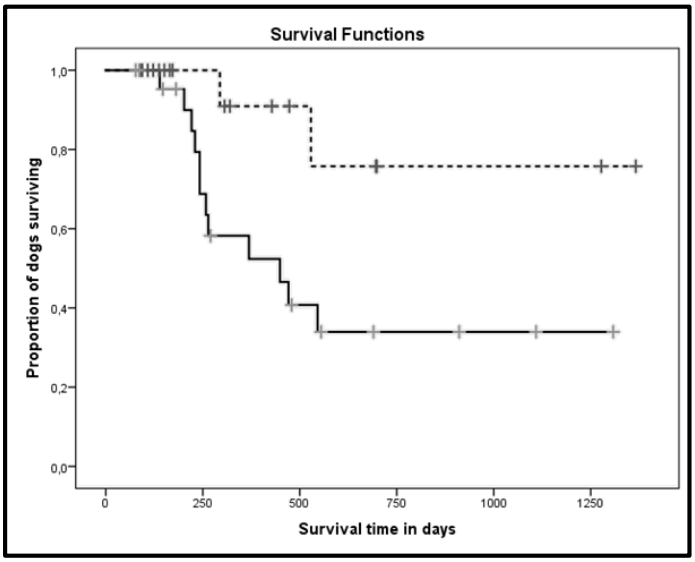

Figure 5 – Kaplan-Meier survival plot for dogs that underwent radiation therapy (n = 23; solid line) versus dogs without radiation therapy (n = 19; dashed line). There is a significant difference in survival time between the two groups (P = .023); the MST is 264 and 306 days for the radiation therapy group and for the non-radiation therapy group, respectively. Dogs died of other causes than CMM and which were lost on follow-up were censored.

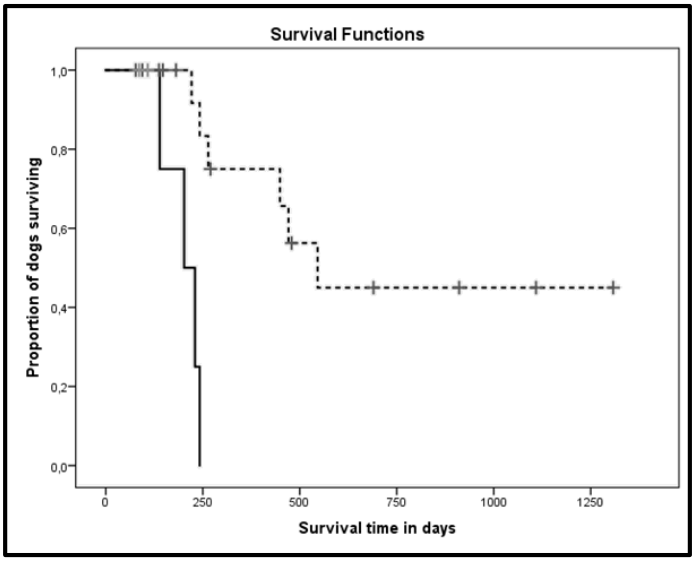

Figure 7 – Kaplan-Meier survival plot for lymph node involvement in dogs with oral melanoma. There was a significant difference in survival time between dogs with oral melanoma that had positive regional lymph nodes (n = 6; solid line) versus dogs without positive regional lymph nodes (n = 17; dashed line) (*P* = .000); the MST is 170 and 270 days for positive lymph nodes and negative lymph nodes, respectively. Dogs died of other causes than CMM and which were lost on follow-up were censored.

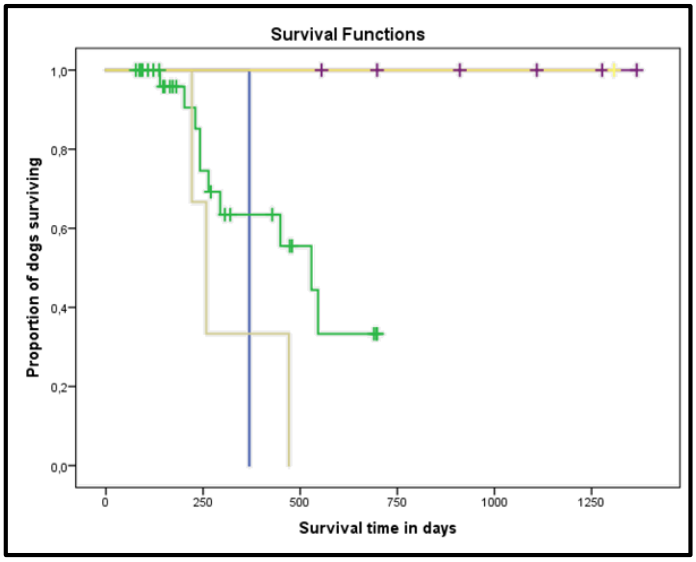

Figure 6 – Kaplan-Meier survival plot for number of doses the vaccinated dogs got. There was a significant difference in survival time between the groups (*P* = .014). The MST for the dog that got less than 4 doses was 369 days (n =1, blue line) MST for dogs that got 4 doses was 242 days (n = 31, green line), MST for dogs that got 5-8 doses was 258 days (n = 3, gray line), MST for dogs that got 8 doses was 1010 days (n = 6, purple line) and the ST for the dog that got 20 doses was 1308 days (n = 1, yellow line). Dogs died of other causes than CMM and which were lost on follow-up were censored.

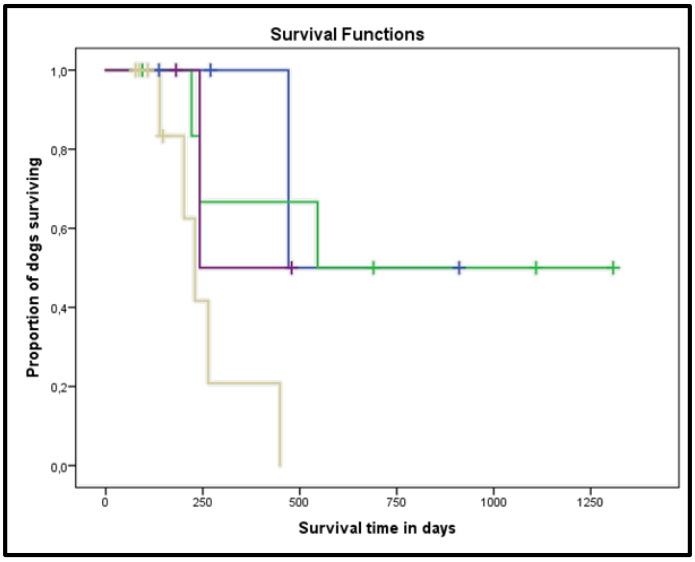

Figure 8 – Kaplan-Meier survival plot for disease stage in dogs with oral melanoma. There was a significant difference in survival time between the disease stages (*P* = .035). The MST for stage I was 370 days (n = 4; blue line), the MST for stage II was 546 days (n = 7; green line), the MST for stage III was 147 days (n = 9; yellow line) and the MST for stage IV was 242 days (n = 3; purple line). Dogs died of other causes than CMM and which were lost on follow-up were censored.

Other studies describing and evaluating Oncept® Melanoma vaccine, only use dogs with oral malignant melanoma. To better compare the results, Kaplan-Meier analysis with Log Rank test was performed for only the dogs with oral malignant melanoma within the present group of dogs. *P*-values and MST of the variables within the oral melanomas with possible influence on the ST can be found in appendix II.

Vaccinated dogs with oral melanoma did not have a significant longer ST in comparison with non-vaccinated dogs with oral melanoma (*P* = .555).

Within the vaccinated group, dogs with oral melanoma and positive regional lymph nodes had a significant shorter ST than dogs with negative regional lymph nodes (P = .000; see figure 7). Disease stage had a significant influence on ST for oral melanomas (P = .035; see figure 8).

4. DISCUSSION AND CONCLUSIONS

The purpose of this study was to evaluate the efficacy of the ONCEPT Melanoma Vaccine as adjunctive treatment for CMM in dogs.

Almost all 42 dogs conducted in this study followed a standardized protocol. Unfortunately, the group size is small and, when divided in subgroups, the statistical power is low. Although it was tried to follow the protocol as good as possible, due to financial constraints a few dogs were treated only with the vaccine on the Faculty of Veterinary Medicine in Utrecht, The Netherlands, and had their surgery and even their radiation therapy elsewhere.

No breed predilection was seen in the present study. A wide variety of breeds was represented, 24 in total, which suggests that all dogs, purebreds and crossbreeds, are at risk of developing CMM. Smith et al. described that melanoma incidence not only varies with site but also with breed. For example, the miniature schnauzer, standard schnauzer and the Scottish terrier are at increased risk of developing a cutaneous melanoma (**Smith, 2002**). Oral melanoma on the other hand is more commonly seen in golden retrievers, poodles, dachshunds and the Scottish terrier as well (**Vail, 2013**). A 2011 melanoma vaccine study by Manley et al. evaluated 58 dogs and some of the predisposed breeds were commonly seen (10 golden retrievers and 5 Scottish terriers) (**Manley, 2011**). This group of dogs was larger than the group in the present study, implying that when the group is bigger, breed predisposition is more seen.

An increasing risk for melanomas in male dogs has been described (**Smith, 2002**), but in this study the number of males (n = 23) was not that different from the number of females (n = 19). Also no significant sex differences were seen in terms of survival. This is consistent with the findings reported by **Smedley et al.** (**2011**).

Lymph node involvement is an important factor in assessing disease stage in CMM. Two previous studies have shown that lymph node involvement did not have prognostic value in dogs with CMM (**Smedley, 2011**). The present study shows a significant shorter survival time when positive regional lymph nodes are found and, therefore, suggesting lymph node staging in dogs with CMM worthwhile.

No significant difference in survival time was found in dogs that did not undergo surgery compared to dogs that did undergo surgery. This was not expected, as no surgery (or debulking) means macroscopic disease and is, therefore, considered palliative. It can be biased because of the number of dogs which did not undergo surgery or debulking (n = 6). Furthermore, most dogs in this group did, however, receive radiotherapy and this could explain the lack of significant difference in survival time. Other studies, for example **Boston et al.** (**2014**), have shown that no surgery or debulking significantly decreased survival time. Following previous studies, it still is recommended to aim for complete removal of both macroscopically and microscopically diseased tissue. Debulking may not seemingly increase survival time but may improve the patients' quality of life and therefore always needs to be considered when removal cannot be achieved. (**Boston et al., 2014**).

Comparing MST between vaccinated and non-vaccinated dogs, gave a near-significant result in which vaccinated dogs would have a longer survival time than non-vaccinated dogs (P = .057). As this was the main aim of the study, the focus lay on this result and could be promising. Previous studies, however, have shown that the Oncept® Melanoma vaccine did not make a difference in the course of this disease (**Ottnod et al., 2013**). As the non-vaccinated group only consisted of dogs with oral melanoma, comparison with the vaccinated group of dogs with oral melanomas was made but no significant difference in ST was found. This is a remarkable finding as the previous mentioned difference was near-significant.

The mean ST of the non-vaccinated dogs is long (276 days) in contrast to the study of **Bergman et al.** (**2003**), who describes a less than 5-month survival period for dogs with CMM with only radical surgery. **Grosenbaugh et al.** (**2011**)

also had a historical control group in which the MST was long (324 days). As **Boston et al.** (**2014**) already suggested, maybe a prospective clinical trial with a placebo versus treatment is not as non-ethical as was suspected.

A small clinical trial by Liao et al., consisting of 9 vaccinated dogs, showed that the level of tyrosinase-specific antibody response in 3 dogs ranged from two- to four-fold higher in the post-vaccination sera than in the pre-immune sera or in the serum of a normal, healthy dog used as a control. The humoral responses were sustained 3 – 9 months after the final vaccination. In 1 dog the antibody-titer gradually decreased to a level comparable to that of a pre-immune or normal canine sera. This suggests that further vaccine boosts may be needed in order to maintain, if not increase, tyrosinase-specific antibodies (**Liao, 2006**). Unfortunately, the present study did not measure antibody titers. It would be interesting to investigate the humoral response induced by the Oncept® Melanoma vaccine in a much larger group of outbred dogs and see if the results can be compared to the clinical trial of **Liao et al**.

Within the vaccinated group, radiation therapy had a negative influence on ST, which is surprising, as melanomas are considered sensitive to radiation therapy. This negative influence can be due to the used protocol, as not all cases underwent radiation therapy. Cases that did undergo radiation therapy, where cases that had advanced disease stage, macroscopic disease or already visible metastases.

To better compare the results of the present study to other studies evaluating Oncept® melanoma vaccine, statistical analysis was performed on the vaccinated dogs with oral melanoma. Only lymph node involvement and disease stage were of significant influence on ST within this group of patients. This result underlines the aforementioned suggestion that lymph node involvement is likely to be of prognostic value for dogs with (oral) melanoma.

A major limitation of the present study is not containing a control group and making the results therefore less powerful. An attempt to use a retrospective control group had to obviated this, however, due to not standardized diagnostic protocols for this group and the fact that a lot of information was missing, made it suboptimal to statistically compare the groups.

Oncept® Melanoma vaccine is officially registered for use in WHO stage II and III oral canine malignant melanomas against micrometastases. This study did not exclude dogs depending on their stage or tumour location. The only inclusion criteria for the present study was diagnosis of CMM and vaccinated with the Oncept® Melanoma vaccine at least once. The stage I and IV dogs were included as well, as this represents a significant group of patients with CMM.

More recent studies have implicated that Oncept® Melanoma vaccine does not significantly improve survival time and therefore will not become available on the European veterinary market for commercial purposes (**Henry, 2016**). CMM, however, still is an aggressive tumour with a high propensity to metastasize and therefore research has to be continued. For future studies to adjuvant therapies of this disease, a recommendation is to set up a prospective, randomized and standardized clinical trial with, perhaps, a whole new and different melanoma vaccine.

5. REFERENCES

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Appendix I

Table 3: Variables within the vaccinated dogs with possible influence on the ST, evaluated with KM analyses followed by Log Rank Test. In bold the variables which had a significant influence on ST.

Variable	*P*-value	MST (in days)
F/FC/M/MC	.914	397/181/306/502
Castrated/non-castrated	.652	258/320
F/M	.551	242/428
Tumour location		
Oral/cutaneous/digit	.099	242/501/313
Oral vs. cutaneous/digit	**.032**	242/374
Amelanotic/melanotic	.321	698/261
Lymph node involvement	**.012**	
Positive lymph node(s)		242
Negative lymph nodes		306
Distant metastasis	.418	
Distant metastasis present		306
No distant metastasis		282
Disease stage	.064	
Stage I		306
Stage II		487
Stage III		230
Stage IV		305
Surgery		
Surgical margins	.066	
Complete		156
No surgery/debulking		243
Marginal		529
Dirty		369
Surgery/no surgery	.266	243/300
Radiation therapy	**.023**	
Radiation therapy		264
No radiation therapy		306
Number of doses	**.014**	
< 4 doses		369
4 doses		242
5-8 doses		258
8 doses		1010
20 doses		1308

Appendix II

Table 4: Variables within the oral melanomas with possible influence on the ST, evaluated with KM analyses followed by Log Rank Test. In bold the variables which had a significant influence on ST.

Variable	*P*-value	MST (in days)
F/FC/M/MC	.051	230/181/475/221
Castrated/non-castrated	.386	211/471
F/M	.979	206/449
Amelanotic/melanotic	.419	827/230
Lymph node involvement	**.000**	
Positive lymph node(s)		170
Negative lymph nodes		270
Distant metastasis	.775	
Distant metastasis present		242
No distant metastasis		236
Disease stage	**.035**	
Stage I		370
Stage II		546
Stage III		147
Stage IV		242
Surgery		
Surgical margins	.500	
Complete		122
No surgery/debulking		221
Marginal		449
Dirty		471
Surgery/no surgery	.212	242/221
Radiation therapy	**-***	
Radiation therapy		253
No radiation therapy		108
Number of doses	.161	
< 4 doses		-[#]
4 doses		216
5-8 doses		346
8 doses		1010
20 doses		1308