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Use of Oncept[TM] melanoma vaccine in 74 canine oral malignant melanomas in the United Kingdom

S. Verganti[1], D. Berlato[1], L. Blackwood[2], I. Amores-Fuster[2], G. A. Polton[3], R. Elders[1], R. Doyle[4] &, A. Taylor[5], S. Murphy[1]

1. Animal Health Trust, Kentford, Newmarket CB8 7UU
2. School of Veterinary Science, University of Liverpool, Liverpool, Merseyside L3 5DA
3. North Downs Specialist Referrals, Bletchingley, Redhill RH1 4QP
4. Davies Veterinary Specialists, Higham Gobion, Hitchin SG5 3HR
5. The Royal Veterinary College, Kings Cross, London NW1 0TU

Corresponding author email: sara.verganti@aht.org.uk

1   Summary

2

3   **Objectives** – Oral malignant melanomas (OMM) carry a poor-to-guarded prognosis, due to local

4   invasiveness and high metastatic propensity. The Oncept[TM] melanoma vaccine is licensed to treat dogs

5   with stage II or III locally-controlled OMM. The aim of this retrospective study was to assess survival of

6   dogs with OMM treated with the vaccine in the UK.

7

8   **Methods** – Medical records of dogs with histopathologically-confirmed OMM that received at least four

9   doses of the vaccine were evaluated. Survival analyses for potential prognostic factors were performed.

10

11   **Results** – Seventy-four dogs were included. Thirty-seven dogs died of causes attributable to OMM;

12   median survival time (MST) was 455 days (95% CI, 313-597). Based on Kaplan-Meier survival analysis

13   with associated log-rank testing, age (<9 years; $p<0.001$), pigmentation (<50% pigmented cells; $p=0.02$)

14   and WHO stage ($p=0.03$) were statistically significant prognostic factors. In a multivariate model, only

15   age maintained significance ($p=0.01$). Eight of thirteen patients with macroscopic disease showed clinical

16   response.

17

18   **Clinical significance** – The patients treated with the melanoma vaccine in our study had MSTs similar to

19   dogs with OMM receiving the vaccine in the USA. Subpopulations of dogs with significantly varied

20   responses to the vaccine were identified. Response of patients with macroscopic disease was seen.

21

Introduction

Melanoma is the most common malignant tumour of the oral cavity in dogs (Ramos-Vara et al. 2000, Smith et al. 2002). It usually exhibits aggressive local behaviour and high, early metastatic potential, especially to regional lymph nodes and lungs (Bostock 1979, Modiano et al. 1999). However, individual tumour behaviour can vary, as a wide range of survival times has been described (Ottnod et al. 2013). Furthermore, a proportion of oral tumours are well-differentiated histologically and carry a good prognosis after surgery alone (Spangler & Kass 2006, Esplin 2008).

For untreated dogs with oral malignant melanoma (OMM), death due to progressive disease (PD) and/or metastases occurs after a median time of 2 months (Harvey et al. 1981). Following conventional treatments [surgery, radiation therapy (RT) and/or chemotherapy], median survival times (MST) range from 4·8 to 12 months (Kosovsky et al. 1991, Wallace et al. 1992, Bateman et al. 1994, Blackwood & Dobson 1996, Théon et al. 1997, Freeman et al. 2003, Proulx et al. 2003, Murphy et al. 2005, Boston et al. 2014). Surgery and RT are effective at achieving local tumour control, and curative-intent surgery has recently been associated with prolonged survival (Tuohy et al. 2014).

Nevertheless, metastatic disease is a common cause of death for patients with OMM (Blackwood & Dobson 1996, Théon et al. 1997, Murphy et al. 2005). Several studies evaluating the use of conventional chemotherapy in the adjuvant setting have failed to demonstrate a substantial survival benefit (Rassnick et al. 2001, Proulx et al. 2003, Murphy et al. 2005, Brockley et al. 2013, Dank et al. 2014).

Melanoma is a highly immunogenic tumour (Modiano et al. 1999). The Oncept vaccine (Merial, Duluth, GA, USA) contains plasmid DNA-targeting tyrosinase, a glycoprotein essential for melanin synthesis and demonstrated to be overexpressed in melanomas (Bergman & Wolchok 2008). Following promising phase I trial results (Bergman et al. 2003), the vaccine received conditional licensure from the United States Department of Agriculture in 2007 for treatment of stages II/III, locoregionally controlled canine OMMs. In a prospective clinical trial of 58 dogs with stage II/III locoregionally controlled OMM, the use of the vaccine significantly increased survival times compared to historical controls (Grosenbaugh et al. 2011). A retrospective study failed to demonstrate a survival advantage with the use of the vaccine in a similar population of dogs (Ottnod et al. 2013). Following USA licensure, the melanoma vaccine has been used in several UK referral centres to treat dogs with OMM. Recently, a retrospective study of 32 UK-based dogs with stages I to III OMM treated with the melanoma vaccine showed a MST of 355 days; no prognostic factors were identified (Treggiari et al. 2016). Nevertheless, the overall data reporting field use remain sparse. The aims of this retrospective study were to assess survival times of dogs with OMM treated with the

56   melanoma vaccine in UK and to identify possible prognostic factors that might influence survival, and to

57   describe the use of melanoma vaccine in patients with macroscopic disease.

58

59   **Materials and Methods**

60   Animals

61   Medical records for dogs with OMM that were presented to five UK referral centres from January 2009 to

62   December 2012 were reviewed (January 2009 is the date the melanoma vaccine became available in the UK).

63   Inclusion criteria were histological and/or immunohistochemical diagnosis of OMM and dogs that received

64   the melanoma vaccine as part of the treatment. Dogs diagnosed with well-differentiated oral melanoma or

65   melanoma involving the haired portion of the lip were excluded from the study because of the more

66   favourable prognosis associated with these types compared to those of the oral cavity or involving the

67   mucosal aspect of the lip (Esplin 2008, Smedley et al. 2011). Patients were also excluded if incompletely

68   staged.

69

70   The following information were collected for each dog: signalment, date of diagnosis, tumour size and site

71   within the oral cavity, lymph node status, staging performed, completeness of -locoregional control achieved

72   immediately prior to vaccination [defined as no gross evidence at the excision site and, in cases of metastatic

73   regional lymph node(s), treatment of the lymph node(s) with surgery or RT prior to vaccination]. The initial

74   vaccination dates, number of vaccine doses, adverse effects, and other treatments apart from the vaccine

75   were also recorded. The following information was retrieved from the histopathology reports: percentage of

76   pigmented neoplastic cells, mitotic index (MI) and extent of surgical margins. Surgical margins were

77   considered complete if the narrowest histologic margin was >2 mm. Dogs were staged according to the

78   World Health Organisation tumour, node, metastases (TNM) guidelines (Table 1). For dogs with macroscopic

79   disease, response was retrospectively assessed according to the response evaluation criteria in solid tumours

80   (RECIST) (Nguyen et al. 2015). The vaccine was administered using the Vetjet transdermal device according

81   to the manufacturer's instructions as previously described (Grosenbaugh et al. 2011). The "induction course"

82   consisted of four doses 14 days apart; a booster vaccination was administered approximately every six

83   months. Minor variations in vaccination schedule occurred to meet clients' needs. Information regarding

84   cause of death and presence/absence of macroscopic or metastatic disease for patients alive at the end of

85   the study were assessed clinically and/or via diagnostic imaging by either the primary or referral clinician and

86   collected when available.

87

88   Data analysis

89   The primary outcome measures were overall survival (OS), defined as the time from date of surgery until

90 date of death or euthanasia, and disease-free interval (DFI), defined as the time from surgery to recurrence

91 or development of metastatic disease or death. An event was defined as death or euthanasia attributed to

92 OMM. Dogs were classified as censored if they died or were euthanased due to unrelated causes, were alive

93 at the end of the study or were lost to follow-up. Variables examined to determine their effect on OS

94 included MI [<4/10 high powered fields (hpfs) vs ≥4/10 hpfs], percentage of pigmentation by histopathology

95 (<50% pigmented neoplastic cells vs ≥50%), margins of excision (complete vs incomplete), WHO stage and

96 regional lymph node status (metastatic vs non-metastatic). Lymph nodes that were normal in size but not

97 sampled were considered non-metastatic in the statistical analysis. Kaplan-Meier product-limit estimation

98 was used to estimate OS plots for potential categorical risk factors, and log-rank testing was used to compare

99 survival impact of categorical variables. Values of P<0·05 were considered significant. Statistical analysis was

100 performed using commercial software (IBM SPSS Statistics version 20·0). Statistical analysis was performed

101 for patients with locoregional control prior to vaccination (stages I to III), while dogs with macroscopic

102 disease were considered separately. Due to the low number of patients in the latter group, Kaplan-Meier

103 product-limit estimation was used to estimate only OS plot.

104

105 **Results**

106 Sixty-nine dogs met the inclusion criteria; 56 patients had locoregional tumour control prior to vaccination

107 while 13 dogs had macroscopic disease. The median age for the entire population was 10·9 years (range 5·2

108 to 15·4 years). Patient signalment, clinical stage, treatments received and achievement of locoregional

109 tumour control prior to vaccine are shown in Table 2. Histopathology reports were available for review in 67

110 cases. Fifty-nine tumours (88·1%) had MI ≥4/10 hpfs (median 35·0/10 hpfs, range 4 to 150/10 hpfs) and 24

111 (35·8%) had <50% pigmented cells. Table 3 indicates details of the RT protocols and chemotherapy

112 treatments administered.

113 In 13 cases the melanoma vaccine was used in the presence of macroscopic disease. Eight dogs had tumour

114 recurrence after surgery (±RT), and concurrent regional lymph node involvement was found in three of

115 these. Three of these eight patients concurrently received non-steroidal anti-inflammatory drugs (NSAIDs)

116 and one received RT. Of the remaining dogs, the vaccine was used as palliative treatment for distant gross

117 metastatic disease (three cases) and for the primary tumour after incisional biopsy (one case). In one dog the

118 vaccine was initiated prior to surgery. Patients in the macroscopic group were staged as follows: four for

119 stage I and three each for stages II to IV.

120

121 Three hundred and seven doses of the melanoma vaccine were administered (mean 4·5, median 4)

122 beginning at a median of 42 days after diagnosis (range 4 to 409 days). Most dogs (76·8%) received four

123 doses of the vaccine; 16 (23·2%) dogs had one to five booster vaccinations (mean 1·9, median 1). All patients

124 completed the induction course of the vaccine. However, one dog in the macroscopic group had PD after

125 starting the vaccine, at which point surgery was performed. The vaccine was restarted after surgery and the

126 treatment was completed. Adverse effects suspected to be related to the vaccine were reported in 12 dogs

127 (17·4%) and included: pain at injection site (4), lethargy (2), local erythema (2), focal hair discolouration (2)
128 and one each of lethargy and anorexia, subcutaneous haemorrhage at injection site and restlessness. Most
129 of the adverse effects were temporary (<48 hours) and reported after the first or second vaccination; none
130 were described following boosters. Additionally, one dog developed a squamous cell carcinoma (SCC) at the
131 vaccination site.

132 Outcomes

133 At the time of analysis, 50% (28/56) of dogs with stages I to III OMM had died or were euthanased for causes
134 attributable to OMM: 10 (17·9%) because of local recurrence at the surgical or RT site (five had concurrent
135 involvement of the regional lymph node) and 16 (28·6%) due to metastasis (including regional lymph nodes,
136 lungs, liver, brain, tonsil and skin), and two of unknown causes (but assumed to be melanoma-related by
137 clinical determination). The MST for dogs staged I to III was 455 days (95% CI: 324 to 586 days; Fig 1) and the
138 median DFI was 222 days (95% CI: 175 to 269 days; Fig 2). The MST from start of vaccination was 422 days
139 (95% CI: 255 to 589 days).

140 Eight dogs (14·3%) staged I to III survived less than 6 months; 62·5% of these had <50% pigmented neoplastic
141 cells. Eleven dogs (19·6%) experienced long-term survival (615 to 1070 days), six of which were still alive at
142 the end of the study. Of these 11 patients, eight had a melanotic tumour and three amelanotic. In eight dogs
143 complete margins were achieved. Three of the eleven long-term survivors had stage I disease, five stage II
144 and three stage III at initial presentation; six had surgery before the vaccine, four had surgery + RT (three
145 before and one after the induction course of the vaccine) and one surgery + chemotherapy.

146 In the censored group, 22 dogs (39·3%) were alive and 18 were free of detectable disease (11 cases based on
147 clinical examination; for the other seven patients, diagnostic imaging confirmed remission). Of the remaining
148 four dogs, three had documented local recurrence (in two patients there was also regional lymph node
149 involvement) and one dog had suspected pulmonary metastases by CT. Of the 18 dogs considered disease-
150 free, nine staged I, four staged II and five staged III at initial presentation. Median follow-up time for
151 censored dogs was 300 days (range 72 to 1070 days). One dog was lost to follow-up 615 days after initial
152 diagnosis and considered free of detectable disease based on diagnostic imaging findings. Six dogs died or
153 were euthanased due to causes other than OMM (10·7%), including second malignancy (multi-centric
154 lymphoma, osteosarcoma, haemangiosarcoma), gastric dilation/volvulus, pulmonary-thromboembolism and
155 hyperadrenocorticism.

156 A Kaplan-Meier survival plot and associated log-rank test suggested that MI (P=0·47), degree of
157 pigmentation (P=0·09), margins (P=0·27), WHO stage (P=0·19) and lymph node status (P=0·68) were not
158 statistically-significant prognostic factors.

159 Of the 13 patients with macroscopic disease at the time of first vaccination, three dogs achieved complete
160 response (CR) of the primary tumour during the vaccine induction course. Two of these patients did not
161 receive any other treatment and developed local recurrence 125 days and 139 days after the first
162 vaccination; the third dog received firocoxib and had no evidence of local recurrence based on clinical
163 examination 232 days after the first vaccination. One patient with local disease and involvement of the
164 regional lymph node prior to vaccine had a partial response (PR) during the induction course but CR was
165 described at the time of the first booster vaccination; this dog was receiving meloxicam and was alive and
166 considered disease-free based on clinical examination 232 days after the first vaccination. A PR was seen in
167 another dog though the tumour was also treated with RT at the same time. Of three dogs with stable disease

168   (SD), one was receiving meloxicam and had PD after 164 days; the other two were treated with the vaccine
169   only and the disease considered stable at last follow-up. The rest of the patients, including all stage IV
170   melanomas, had PD. The MST for patients in the macroscopic group was 179 days from diagnosis (95% CI: 95
171   to 263 days; Fig 3). The three patients with stage IV melanoma survived 171, 178 and 288 days from
172   diagnosis, and 129, 130 and 241 days from the vaccination. None of these patients received other type of
173   treatments, apart from surgery and melanoma vaccine.

174   **Discussion**

175   The purpose of this study was to retrospectively evaluate the survival times of dogs with OMM in the UK
176   treated with the melanoma vaccine and to possibly identify associated prognostic factors for response. An
177   additional aim was to describe the response of patients with macroscopic disease to the vaccine.

178   The Oncept vaccine has been used to treat canine melanoma in UK referral centres after acquiring
179   conditional FDA licensing in 2007 but publications evaluating effectiveness are limited. Furthermore, the two
180   largest studies assessing the vaccine efficacy reported contrasting results (Grosenbaugh et al. 2011, Ottnod
181   et al. 2013). The overall MST described in our study (455 days) for patients with stages I to III disease, was
182   similar to that described by Ottnod et al. (2013) in which MST for vaccinated dogs was 477 vs 491 days for
183   historical "controls". However, our study included 14 patients (25%) with stage I disease which may have
184   positively impacted survival compared to studies in which the vaccine was used for stages II and III OMMs
185   only. Conversely, if we compare our data to those from a recent UK study including stages I to III (Treggiari et
186   al. 2016), improved survival was achieved in this study.

187   The degree of pigmentation of canine melanoma has been associated with survival in some studies (Esplin
188   2008, Bergin et al. 2011); however, others have failed to demonstrate any correlation (Harvey et al. 1981,
189   Hahn et al. 1994, Ramos-Vara et al. 2000). Although not statistically significant in our study population the
190   MST for tumours with ≥50% pigmented neoplastic cells was 508 days compared to 310 days for tumours
191   with <50% pigmented neoplastic cells. Our results support the idea that less pigmented tumours might be
192   clinically more aggressive but this notion requires further formal testing in larger case series.

193   In our population of dogs with stages I to III OMM, complete surgical margins were achieved in 13 patients
194   (23·2%). Only 19 dogs (44·1%) with incomplete margins received adjuvant radiation therapy. Completeness
195   of excision was not a statistically significant prognostic factor in our study. This is similar to results described
196   by Grosenbaugh et al. (2011) and Ottnod et al. (2013), in which margins of excision did not correlate with
197   survival. Nevertheless, for our patients with complete margins the MST was not reached and the mean
198   survival was 628 days (95% CI: 388 to 869 days) compared to a MST of 417 days [95% CI: 266 to 568 days;
199   mean 477 days (95% CI: 378 to 575 days)] for patients with incomplete margins. The lack of significance seen
200   in this study could again be due to type II error or to the lack of histopathology review for confirmation of
201   the margins of excision.

202   In our study, WHO stage was not statistically significantly associated with survival (P=0·19). However, for
203   patients with stage I disease the MST was not reached while the mean survival was 687 days (95% CI: 462 to
204   912 days). Interestingly, dogs with stage II disease had a shorter MST compared to patients with stage III
205   disease [269 days (95% CI: 118 to 421 days) and 342 days (95% CI: 214 to 470 days), respectively]. Several
206   factors could have contributed to this. For example, 10/22 dogs with stage II disease had amelanotic
207   tumours (45·4%) vs 5/20 (25%) patients with stage III disease. Furthermore, complete margins were
208   achieved in 20% of staged III patients vs 9% of the dogs with staged II tumours. In regards to other treatment

209   modalities (e.g. RT and chemotherapy) and time of delayed vaccination, these were similar for both groups.
210   If we compare our results to the recent UK study on the melanoma vaccine (Treggiari et al. 2016), our stage
211   II population experienced shorter survival while dogs with stages I and III disease had increased survival.
212   Both studies are retrospective in nature and several confounding factors may have contributed to these
213   results. Additionally, the dogs in this study received a combination of treatment modalities including surgery,
214   RT, chemotherapy and NSAIDs. Based on the small number of patients for each subgroup, no statistical
215   analysis was performed to assess the influence of different treatments on survival. However, it is not
216   possible to exclude the potential role of chemotherapy and NSAIDs on tumour control. COX-2 expression has
217   in fact been demonstrated in canine melanoma tissue, particularly in aggressive OMMs (Pires et al. 2010).
218   Nevertheless, two previous studies (Boria et al. 2004, Murphy et al. 2005) showed that the use of NSAIDs for
219   canine melanomas did not confer a biological response or survival advantage.

220   Interestingly, in our study, 50% of patients with stages I to III disease died due to local recurrence (with or
221   without regional lymph node involvement; 17·9%) or due to metastatic disease (29·6%); lymph nodes, lungs,
222   liver, brain, tonsil and skin, were the described metastatic sites. This suggests that the melanoma vaccine
223   may not be effective in all the patients treated or that its effect may be temporary.

224   Eleven dogs (19·6%) staged I to III experienced long-term survival (615 to 1070 days), six of which were still
225   alive at the end of the study. Several factors may have contributed to this result, including the presence of
226   pigmented tumours in eight patients as well the achievement of complete margins for most of them (72·7%).

227   As previously described by Hahn et al. (1994) and Proulx et al. (2003), regional lymph node metastasis did
228   not appear to impact survival in our study population. However, seven patients with palpably normal lymph
229   nodes did not have lymph node sampling performed at initial staging. This may have underestimated the
230   number of cases with regional lymph node involvement, as up to 40% of normal lymph nodes are metastatic
231   (Williams & Packer 2003). Nevertheless, none of the patients developed detectable lymph node metastases
232   during the study period.

233   The time between diagnosis and vaccination was extremely variable in our study (4 to 409 days). This was
234   due to the fact that the melanoma vaccine was not available in the UK before 2009. The median time of first
235   vaccination was 43 days after surgery (range 4 to 364 days) for patients with stages I to III disease, and 33
236   days (range 11 to 409 days) for dogs in the macroscopic group. Most of the dogs with stages I to III tumours
237   (71·4%) received the first dose of the melanoma vaccine within 2 months from diagnosis. Although there are
238   no clear guidelines about when to start the vaccine, an early vaccination is recommended in order to give
239   time to mount an immune response. Therefore, the delayed vaccination may have negatively influenced the
240   outcome of some of our patients. Nevertheless, patients that received the vaccine at later stage may have
241   also had less aggressive tumours. No statistical analysis was performed as to whether delayed vaccination
242   was a significant prognostic factor in our population; however, the MSTs of stages I to III patients from
243   diagnosis and vaccination were similar (455 days and 422 days, respectively).

244   The melanoma vaccine was well-tolerated. Minimal adverse effects were described by owners, most being
245   mild and self-limiting; the frequency of adverse reactions declined throughout the vaccination course. One
246   dog developed SCC in the area of the vaccination site nine months after the first vaccine diagnosed using
247   cytology. It is unclear whether this was related to the vaccine.

248

249 The effect of the vaccine on macroscopic disease was described for the first time in this paper. Overall, eight
250 of 13 patients showed clinical response. However, four of these patients had received other forms of
251 treatment concurrent with the vaccine, including RT (one) and NSAIDs (three dogs). The overall response
252 rate of the nine patients with macroscopic disease treated with the vaccine only was 44·4%. Of the four
253 responders, two had CR and two had SD. It is interesting that in three dogs, response was documented
254 during the induction course of the vaccine. The early response seen in this study was unexpected as a
255 humoral response is reportedly detected within three to nine months after completion of the induction
256 course of the melanoma vaccine (Liao et al. 2006). In our population it seems that some patients were able
257 to develop a much more rapid response. Nevertheless, in most cases, the response was short-lasting (<4·5
258 months).

259 Three patients with stage IV disease treated with surgery and vaccine only survived between 5·6 and 9·5
260 months from the initial diagnosis. Although these patients developed eventually -progressive disease, it is
261 not possible to exclude that the vaccine had a potential role in slowing disease progression. Based on this
262 preliminary data and the tolerability of the treatment, the melanoma vaccine could be considered as
263 palliative treatment in patients with macroscopic disease, when surgery or radiotherapy is not an option, or
264 for patients with advanced disease (i.e. stage IV). However, the response seen was extremely variable and
265 further studies are required to investigate the role of the vaccine in the macroscopic setting.

266 Due to its retrospective nature, there are several limitations in this study. Necropsy was not performed in
267 any dogs; the attributed cause of death was often based on clinical examination by the referring or referral
268 veterinarian and standard diagnostic procedures. Histopathology records were not available for two dogs
269 and tumour samples were not reviewed, so variation between pathologists regarding histologic features of
270 malignancy might exist. There was no control group, which limited the possibility of assessing the effect of
271 the vaccine on survival. Additionally, the limited number of dogs in some subcategories might have
272 introduced bias. Finally, cases were managed by multiple investigators and treatment type and time of
273 vaccine initiation varied.

274 In conclusion, our study dogs with OMM treated with the Oncept melanoma vaccine showed similar MSTs to
275 those reported by Ottnod et al. (2013). In addition, our stage III patients had improved survival compared to
276 a recent UK study on dogs treated with the vaccine (Treggiari et al. 2016). However, considering the
277 limitations of this study, the lack of a control group and the different treatment modalities used for each
278 patient, it is not clear whether the vaccine resulted in a survival advantage in our population. None of the
279 prognostic factors analysed in the present study was statistically significant. Although this could be a genuine
280 result, it is not possible to exclude type II error, based on the low number of patients in each subcategory
281 and on the limitations of a retrospective study.

282 Nevertheless, this is the first study that describes the use of the vaccine in dogs with macroscopic disease.
283 Patients with macroscopic disease had a 44·4% response rate to the vaccine and the MST for dogs with stage
284 IV disease was 178 days. Therefore, given its very good tolerability, the melanoma vaccine may be
285 considered as palliative treatment for patients with advanced disease (e.g. stage IV) or for dogs with
286 macroscopic tumours when other treatment modalities are not an option.

287

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401 *Association* **222**, 1234-1236
402

403   **Table 1.** World Health Organisation staging system for dogs with OMM

Clinical stage
I. Tumour < 2 cm diameter II. Tumour 2-4 cm diameter III. Tumour > 4 cm diameter and/or evidence of regional lymph node involvement IV. Any tumour size, any lymph node status, evidence of distant metastasis

404

405   **Table 2.** Characteristics of the 69 dogs with OMM treated with the melanoma vaccine

Category	Variable	n (%)
Breed	Purebreed	51 (68.9%)
	Golden retriever	16 (21.6%)
	Labrador retriever	6 (8.1%)
	Flat-coated retriever	1 (1.4%)
	Mixed breed	23 (31.1%)
Gender	Male	53 (71.6%)
	Neutered	34 (46%)
	Entire	19 (25.7%)
	Female	21 (28.4%)
	Spayed	18 (24.3%)
	Entire	3 (4%)
Location	Gingival	
	Maxillary	24 (32.4%)
	Mandibular	19 (25.7%)
	Labial (mucosal aspect)	21 (28.4%)
	Lingual	5 (6.8%)
	Hard palatal	3 (4%)
	Oropharyngeal	2 (2.7%)
Investigations performed	Physical examination	74 (100%)
	Blood tests	63 (85.1%)
	Thoracic radiography	55 (74.3%)
	CT scan	
	Thorax	34 (45.9%)
	Abdomen	16 (21.6%)
	Abdominal ultrasonography	18 (24.3%)
	Regional lymph node assessment	62 (83.8%)
	Cytology	55 (88.7%)
	Histopathology	13 (21%)
Clinical stage	I II	18 (24.3%)
	III	24 (32.4%)
	IV	23 (31.1%)
	Unknown	3 (4%)
		6 (8.1%)

| Treatment modality | Surgery | 74 (100%) |
	Complete margins	18 (24.3%)
	Incomplete margins	56 (75.7%)
	Radiation therapy	24 (32.4%)
	Primary site	6 (8.1%)
	Primary site and local lymph nodes	18 (24.3%)
	Systemic treatment	28 (37.8%)
	NSAIDs	18 (24.3%)
	Chemotherapy	11 (14.9%)
	Toceranib phosphate	2 (2.7%)
Local tumour control prior to vaccine	Yes	61 (82.4%)
	No	13 (17.6%)
	Residual primary mass	7 (9.5%)
	Regional lymph nodes	6 (8.1%)
	Lungs	3 (4.1%)

415

416

417 **Table 3.** Radiation therapy protocols and chemotherapy treatments for the 69 patients with OMM

| Radiation therapy	n (%)	Chemotherapy/TKI treatment	n (%)
Patients treated	24 (32.4%)	Patients treated	13 (17.6%)
Type of treatment		Type of treatment	
Adjuvant to surgery	20 (27.0%)	Adjuvant to surgery and/or RT	6 (8.1%)
Main treatment	2 (2.7%)	At local recurrence	7 (9.5%)
At local recurrence	2 (2.7%)		
		In relation to melanoma vaccine	
In relation to melanoma vaccine		Pre-vaccine	6 (8.1%)
Pre-vaccine	12 (16.2%)	Concurrent to vaccine	4 (5.4%)
Concurrent to vaccine	10 (13.5%)	Post-vaccine	11 (14.9%)
Post-vaccine	2 (2.7%)		
		Protocol	
Protocol		Carboplatin	5 (6.8%)
4 fractions x 8 Gy	18 (24.3%)	Carboplatin and mitoxantrone	1 (1.4%)
4 fractions x 8.5 Gy	1 (1.4%)	Metronomic chlorambucil/NSAIDs	3 (4.0%)
4 fractions x 9 Gy	5 (6.8%)	Metronomic CXP/NSAIDs	4 (5.4%)
		Toceranib phosphate	2 (2.7%)
Treatment of regional lymph node	18 (24.3%)		
Prophylactic	13 (17.6%)		
Metastatic lymph node/s	5 (6.8%)		

418

419

420   **Figure 1.** Kaplan-Meier plot showing the median survival time of the 56 patients with locoregional control
421   prior to vaccination (stages I to III). The overall median survival time was 455 days (95% CI, 324 to 586 days).
422   Crosses represent censored observations.
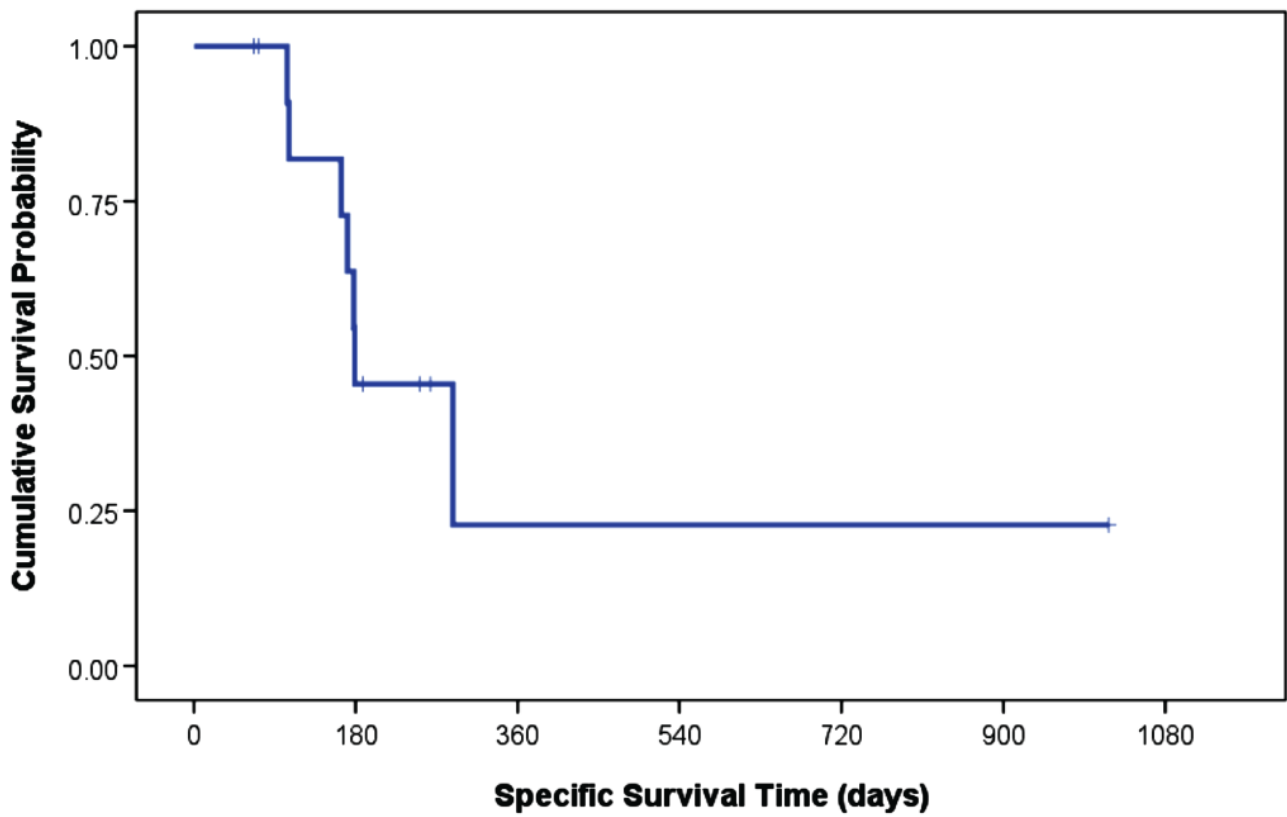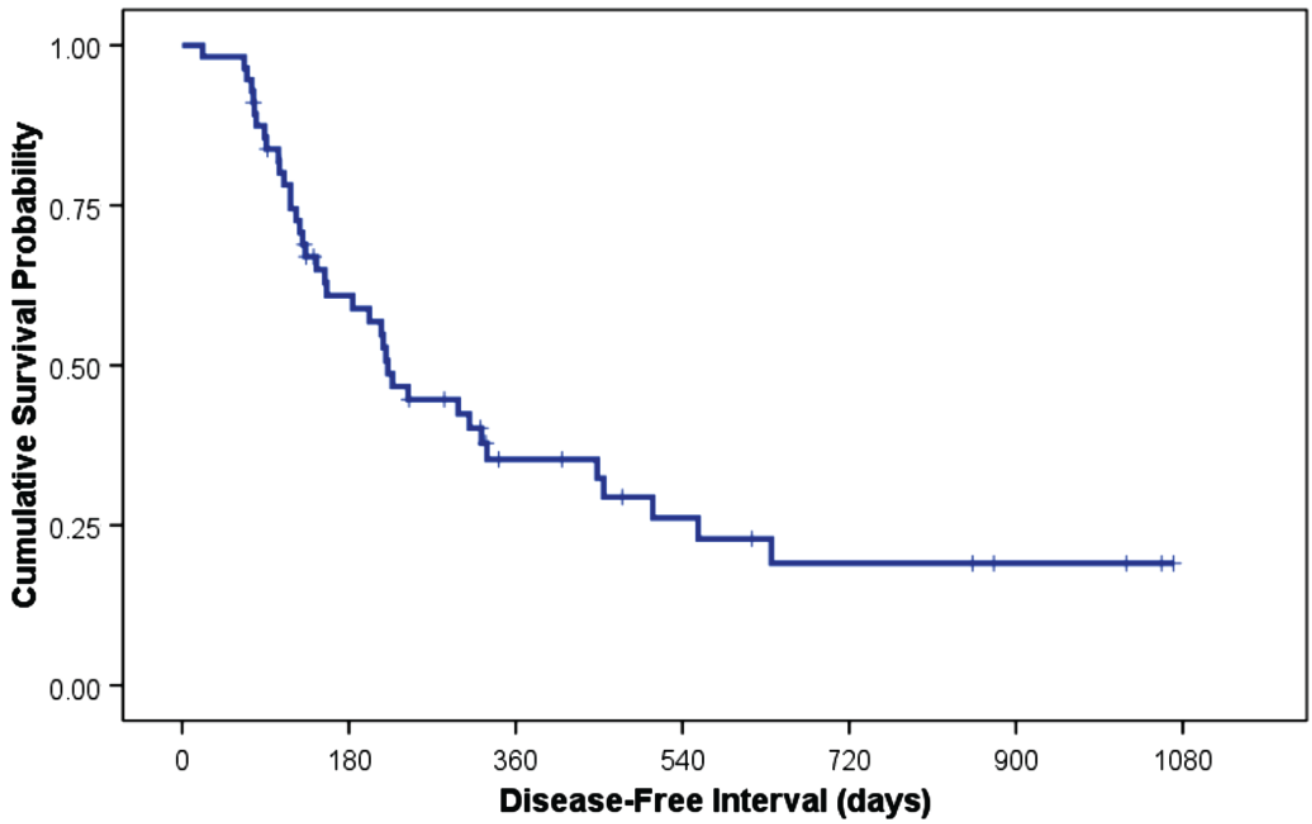

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425

426   **Figure 2.** Kaplan-Meier plot showing the disease-free interval of the 56 patients with locoregional control
427   prior to vaccination (stages I to III). The overall disease-free interval was 222 days (95% CI, 175 to 269 days).
428   Crosses represent censored observations.
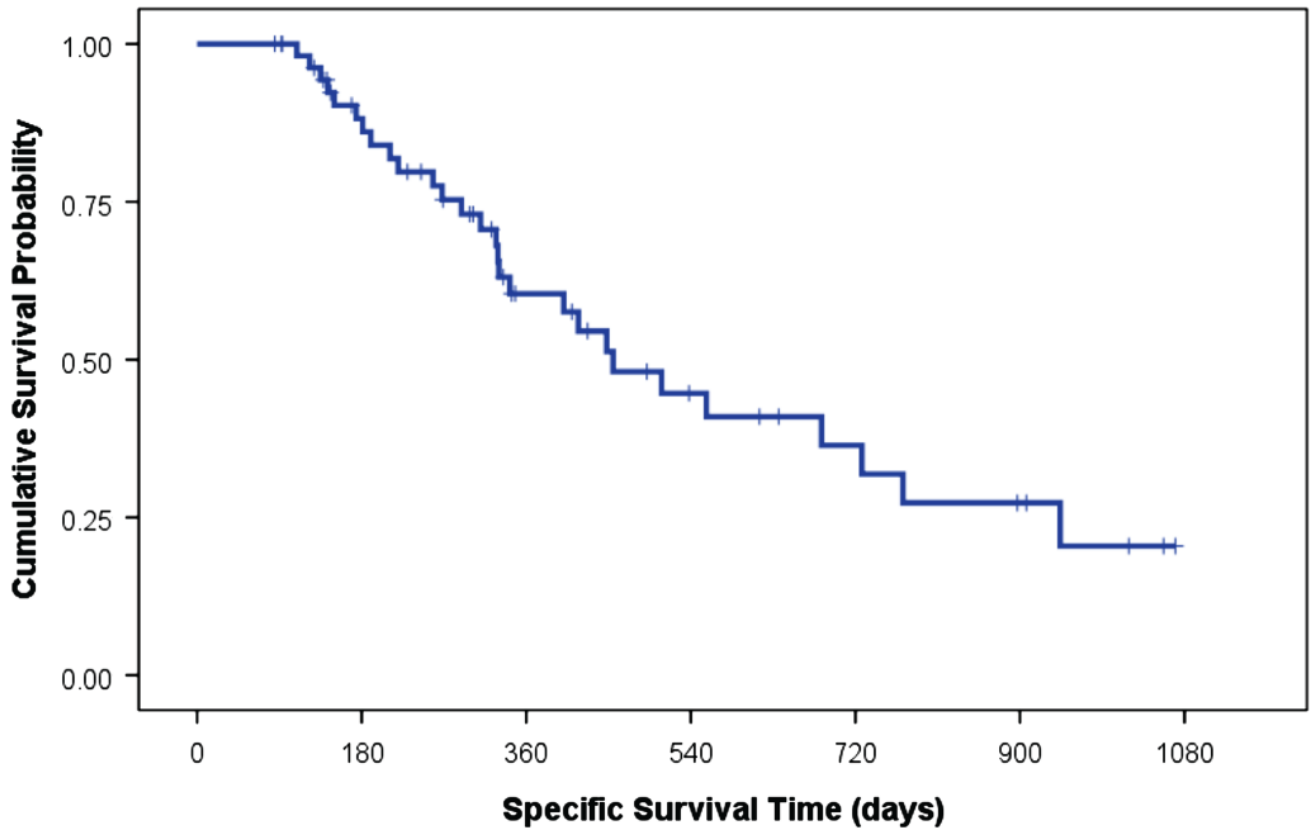
429

430

431 **Figure 3.** Kaplan-Meier plot showing the median survival time of the 13 patients with macroscopic disease
432 prior to vaccination. The overall median survival time was 178 days (95% CI, 95 to 263 days). Crosses
433 represent censored observations.

434