Advances in Brief

Long-Term Survival of Dogs with Advanced Malignant Melanoma after DNA Vaccination with Xenogeneic Human Tyrosinase: A Phase I Trial[1]

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Abstract

Purpose: **Canine malignant melanoma (CMM) is a spontaneous, aggressive, and metastatic neoplasm. Preclinical mouse studies have shown that xenogeneic DNA vaccination with genes encoding tyrosinase family members can induce antibody and cytotoxic T-cell responses, resulting in tumor rejection. These studies provided the rationale for a trial of xenogeneic DNA vaccination in CMM using the human tyrosinase gene.**

Experimental Design: **Three cohorts of three dogs each with advanced (WHO stage II, III, or IV) CMM received four biweekly i.m. injections (dose levels 100, 500, or 1500 μg, respectively/vaccination) of human tyrosinase plasmid DNA i.m. via the Biojector2000 delivery device.**

Results: **Mild local reactions at injection sites were the only toxicities observed, with no signs of autoimmunity. One dog with stage IV disease had a complete clinical response in multiple lung metastases for 329 days. Two dogs with stage IV disease had long-term survivals (421 and 588+ days) in the face of significant bulky metastatic disease, and two other dogs with locally controlled stage II/III disease had long-term survivals (501 and 496 days) with no evidence of melanoma on necropsy. Four other dogs were euthanized because of progression of the primary tumor. The Kaplan-Meier median survival time for all nine dogs was 389 days.**

Conclusions: **The results of this trial demonstrate that xenogeneic DNA vaccination of dogs with advanced malignant melanoma is a safe and potentially therapeutic modality. On the basis of these results, additional evaluation of this novel therapeutic is warranted in locally controlled CMM and advanced human melanoma.**

Introduction

CMM[6] of the oral cavity, nail bed, foot pad, and mucocutaneous junction is a spontaneously occurring, highly aggressive, and frequently metastatic neoplasm (1–4). CMM is a relatively common diagnosis representing ~4% of all canine tumors, and it is the most common oral tumor in the dog (3, 5, 6). CMM and advanced HM are diseases that are initially treated with aggressive local therapies, including surgery and/or fractionated radiation therapy; however, systemic metastatic disease is a common sequela (1, 3, 7). Also, CMM and HM are chemoresistant neoplasms (8–10). On the basis of these similarities, CMM is a good clinical model for evaluating new treatments for advanced HM (11).

Canine patients with advanced disease (WHO stage II, III, or IV) have a reported median ST of <5 months with aggressive local excision (1–3). Human patients with deep American Joint Committee on Cancer stage II or stage III disease (locally advanced or regional lymph node involvement) have at least a 50% chance of recurrence after surgical resection; patients with stage IV melanoma (distant metastases) have a median survival of <10 months, and most of these patients eventually die of melanoma (9, 12). Standard systemic therapy is dacarbazine chemotherapy in HM and carboplatin chemotherapy in CMM (8, 10). Unfortunately, response rates to chemotherapy in humans or dogs with advanced melanoma range from 8 to 28% with little evidence that treatment improves survival (8–10). It is evident that new approaches to this disease are desperately needed.

Active immunotherapy in the form of vaccines represents one potential therapeutic strategy for melanoma. The advent of DNA vaccination circumvents some of the previously encoun-

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[6] The abbreviations used are: CMM, canine malignant melanoma; ST, survival time; HM, human melanoma; CR, complete response; PR, partial response; PD, progressive disease; ANA, antinuclear antibody; LDH, lactate dehydrogenase.

tered hurdles in vaccine development (13, 14). DNA is relatively inexpensive and simple to purify in large quantity. The antigen of interest is cloned into a bacterial expression plasmid with a constitutively active promoter. The plasmid is introduced into the skin or muscle with an intradermal or i.m. injection. Once in the skin or muscle, professional antigen-presenting cells, particularly dendritic cells, are able to present the transcribed and translated antigen in the proper context of MHC and costimulatory molecules. The bacterial and plasmid DNA itself contains immunostimulatory sequences that may act as a potent immunological adjuvant in the immune response (15, 16). In clinical trials for infectious disease, DNA immunization has been shown to be safe and effective in inducing immune responses to malaria and HIV (17, 18). Although DNA vaccines have induced immune responses to viral proteins, vaccinating against tissue-specific self-proteins on cancer cells is clearly a more difficult problem. One way to induce immunity against a tissue-specific differentiation antigen on cancer cells is to vaccinate with xenogeneic antigen or DNA that is homologous to the cancer antigen (19). It has been shown that vaccination of mice with DNA encoding cancer differentiation antigens is ineffective when self-DNA is used, but effective tumor immunity can be induced by orthologous DNA from another species (20).

We have chosen to target defined melanoma differentiation antigens of the tyrosinase family. Tyrosinase is a melanosomal glycoprotein, essential in melanin synthesis. The full-length human tyrosinase gene was shown to consist of five exons and was localized to chromosome 11q14-q21 (21). Immunization with xenogeneic human DNA encoding tyrosinase family proteins induced antibodies and cytotoxic T cells against syngeneic B16 melanoma cells in C57BL/6 mice, but immunization with mouse tyrosinase-related DNA did not induce detectable immunity (22). In particular, xenogeneic DNA vaccination induced tumor protection from syngeneic melanoma challenge and autoimmune hypopigmentation (23, 24). Thus, xenogeneic DNA vaccination could break tolerance against a self-tumor differentiation antigen, inducing antibody, T-cell, and antitumor responses. Sequence identity between partially sequenced canine tyrosinase (284 bp) and human tyrosinase (1888 bp) is 91% (GI 6513592 and GI 37508, respectively).

In this study, we demonstrate that human tyrosinase DNA vaccination of dogs with advanced malignant melanoma is safe and potentially active, warranting additional xenogeneic DNA vaccine investigations as Phase II studies in CMM and Phase I studies for HM.

Materials and Methods

Patient Population. From April 2000 to December 2000, nine dogs with previously histologically confirmed spontaneous malignant melanoma were treated with xenogeneic human tyrosinase DNA vaccination. Pretrial evaluation included complete physical examination, a complete blood count and platelet count, serum chemistry profile, urinalysis, LDH, antinuclear antibody, and three-dimensional measurements of the primary tumor if present (or maximal tumor size from medical records if patient was treated before pretrial considerations). For evaluation of metastatic disease, 3-view radiographs of the thorax were obtained, and regional lymph nodes were evaluated with fine needle aspiration/cytology and/or biopsy/histopathology. All dogs were clinically staged according to the WHO staging system of stage II (tumors 2–4 cm diameter, negative nodes), stage III (tumor $>$ 4 cm and/or positive nodes) or stage IV (distant metastatic disease). The numbers of previous treatments with surgery, radiation, and/or chemotherapy were recorded. Dogs with WHO stage II (high grade), III, or IV histologically confirmed malignant melanoma were allowed entrance onto the study because of the lack of effective available systemic treatments. Additional entry criteria included an estimated life expectancy of $\geq$6 weeks, free of clinically detectable brain metastases, no previous therapy (surgery, radiation and/or chemotherapy) for at least 3 weeks, and no serious intercurrent medical illnesses. Written consent for entry onto this trial was obtained from each dog's owner before entry onto the study; this consent included request for necropsy upon death because of any reason. This study was performed under Animal Medical Center Institutional Review Board approval.

Table 1 Vaccine treatment and evaluation schedule

		Weeks				
	Pretreatment	0	2	4	6	8
Vaccine[a]		X	X	X	X	
Serology/PBMC's	X			X	X	X
Chest X-rays	X			X		X
Physical exam	X	X	X	X	X	X
CBC, Biochem Prof, LDH, anti-DNA antibody (ANA)	X			X		X

[a] 100, 500, and 1500 μg DNA dose levels.

Trial Design. Cohorts of three dogs each received increasing doses of xenogeneic plasmid DNA encoding human tyrosinase i.m. (100, 500, and 1500 μg/dose for each dose level) biweekly for a total of four vaccinations in the left semimembranosus/semitendinosus muscle (caudal thigh) region with the Biojector 2000 jet delivery device with no. 3 (i.m.) Bioject syringes. The Biojector2000 is a carbon dioxide-powered jet delivery device, which is Food and Drug Administration approved for administration of i.m. injections and has been used in DNA vaccine clinical investigations (25, 26). Subjective pain level responses and postvaccinal presence of a wheal or other reaction were assessed and recorded by the veterinarian administering the DNA vaccination. The dogs did not receive any concomitant treatments during the course of vaccination. Clinical safety patient rules were written such that the first dog in a new dose escalation group could not be entered until 2 weeks after the third dog from the previous dose group level received vaccination no. 1 without any subsequent evidence of serious toxicity. If any grade III or IV toxicities were noted, the size of the cohort of those dogs would be expanded for better delineation of the maximally tolerated dose. A schematic of the protocol is presented in Table 1.

Vaccine Information. Human tyrosinase cDNA was previously cloned and sequenced at Memorial Sloan-Kettering Cancer Center (21). The DNA has been inserted in the pING plasmid vector, which contains a cytomegalovirus promoter and kanamycin resistance selection marker. The vaccine was pro-

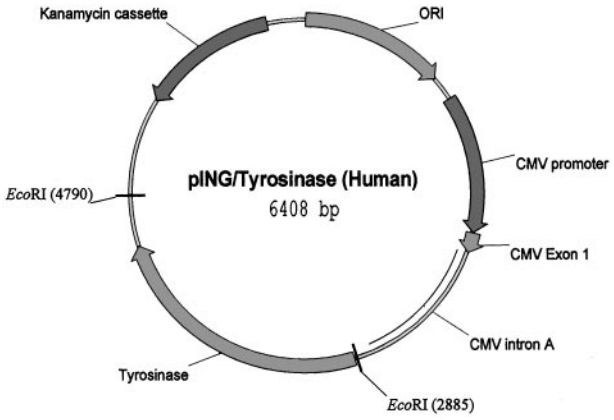

Fig. 1 Plasmid map of pING plasmid used for generation of human tyrosinase DNA vaccine given to nine dogs with advanced malignant melanoma.

duced and released from the Gene Transfer and Somatic Cell Engineering Facility at the Memorial Sloan-Kettering Cancer Center with permission from the United States Department of Agriculture. A schematic of the pING plasmid vector is presented in Fig. 1.

Clinical Response and Follow-Up Evaluations. The following criteria for antitumor effect were used: CR = disappearance of all clinical evidence of tumor on physical examination, radiographic examination, and biochemical evaluation for at least 1 month; PR = ≥50% decrease on physical and radiographic examination of the summed products of the perpendicular diameters of all measured lesions; stable disease = <50% decrease or <25% increase on physical and radiographic exams of the summed products of the perpendicular diameters of all measured lesions; or PD = ≥25% increase on physical and radiographic examination of the summed products of the perpendicular diameters of all measured lesions. After the fifth visit, 2 weeks after the fourth vaccination, serial rechecks with antitumor response evaluations continued to be performed as clinically indicated on an every 30–60-day interval. Historical abnormalities and problems found on physical examination were investigated as clinically indicated at each recheck visit. In addition, recheck evaluations continued as long as clinically necessary to determine ST for each dog. When requested by the dog's owner because of degradations in quality of life from advanced disease, euthanasia was performed with subsequent necropsy examination. A full necropsy was performed on all dogs that died or were euthanized with particular examination (gross and histopathological) of the primary tumor site, metastatic sites, left semimembranosus/semitendinosus vaccination site, and eyes.

Statistical Analysis. ST was defined as the time from receiving first xenogeneic human tyrosinase DNA vaccination until death. Median survival was calculated using the Kaplan-Meier product limit method using Statview statistical analysis software (27). Because of the limited number of cases evaluated in this Phase I clinical trial, appropriate nonparametric statistical testing (*e.g.*, Mann-Whitney *U* test, Spearman rank correlation, and so on) was used. All recorded variables (age, gender, neuter status, breed, weight, concurrent disease status, concurrent disease type, previous treatment, previous treatment type, number of previous treatments, and pain score) after each vaccination (none, mild, moderate, and severe), and toxicity from vaccination, ANA titer, change in ANA titer postvaccination, LDH level prevaccination, LDH level postvaccination, ST, survival censor, follow-up time, primary tumor location, WHO stage at diagnosis, WHO stage prevaccination, response at 2 week postvaccination, response at 6 months postvaccination, melanotic *versus* amelanotic malignant melanoma primary tumor, and DNA dose were evaluated when available for their effect on ST with Kaplan-Meier life table analysis and Cox proportional hazards analysis when appropriate. Dogs were censored if they were lost to follow-up or died because of disease other than malignant melanoma. In addition, all recorded variables were evaluated statistically for potential correlations and associations. To be evaluated for survival statistic purposes, dogs must have received at least three vaccinations; however, toxicity would be evaluated from dogs after entrance onto the trial independent of the number of vaccines received. A two-tailed P of <0.05 was considered statistically significant.

Results

Patient Demographics. The median age of the nine dogs receiving human tyrosinase DNA vaccination was 13 years (mean, 12 years; range, 9–14 years). The median weight was 13 kg (mean, 22 kg; range, 3.6–61 kg.). There were three spayed females, five castrated males, and one intact male dog. There were three mixed breed dogs, two Cocker Spaniels, and one each Siberian husky, Lhasa apso, Bichon frise, and German shepherd dog. All nine dogs received prior therapy before entrance onto the trial; seven dogs had a single previous surgery, two dogs had multiple previous surgeries, and three dogs received prior radiation therapy. Five dogs had primary malignant melanoma in the oral cavity, whereas three dogs had nail bed or footpad malignant melanoma, and one dog had an intraocular high-grade malignant melanoma treated with exenteration. Using a modified WHO staging scheme (3), four dogs had stage IV disease, two dogs had stage III disease, and three dogs had high-grade stage II disease. All nine dogs had a histological diagnosis of melanotic malignant melanoma verified by a veterinary pathologist. All nine dogs received all four biweekly vaccinations. Three dogs had concurrent nonmelanoma diseases but were still eligible for clinical trial; the concurrent diseases included histopathologically confirmed liver dysplasia, dermatopathy, and glaucoma. Table 2 presents an overview of the patient characteristics.

Toxicity. A total of 36 vaccinations were given to the nine dogs on this trial. Three, 26, and 7 vaccinations were subjectively assessed to have moderate, mild, and zero pain responses, respectively (Table 3). Immediately after vaccination, the injection site had at most a minimal to mild wheal with no other local toxicity subsequently noted. No systemic toxicity as assessed by physical examination, hematopathology, or serum chemistry was noted throughout the trial and on subsequent serial examinations after vaccinations were completed. One patient had an abnormally elevated LDH before vaccination that returned to normal after completion of four vaccinations. One

Table 2 Patient characteristics

Dog no.	DNA dose (μg)	Breed	Weight (lbs.)	Gender	Age (yr)	WHO stage	Local tumor control before vaccine	Response at 2 week recheck	Response at last recheck or death	Survival time (days)	Cause of death
1	100	Mixed	8	Female neutered	14	II	No	PD	PD	57	1[a]
2	100	Siberian husky	67	Male neutered	12	IV	No	CR	PD	389	1b
3	100	Bichon frise	18	Male neutered	13	II	Yes	NED[b]	NED	496	2
4	500	Lhasa apso	21	Male neutered	13	II	No	PD	PD	96	1
5	500	German shepherd	130	Male neutered	9	IV	No	PD	PD	421	3
6	500	Cocker spaniel	27	Female neutered	13	III	No	PD	PD	54	1
7	1500	Mixed	59	Female neutered	13	III	Yes	NED	NED	501	4
8	1500	Cocker	29	Male neutered	12	IV	No	PD	PD	126	1
9	1500	Mixed	80	Male intact	9	IV	No	SD	PD	588+	Alive

[a] Cause of death: 1, progressive local disease; b, concomitant sepsis; 2, hyperadrenocorticism complications; 3, progressive metastatic disease; 4, hepatocellular carcinoma.
[b] Response: NED, No evidence of disease; SD, stable disease.

Table 3 Toxicities

DNA dose/vaccination	Pain score = 0[a]	Pain score = 1	Pain score = 2	Local toxicity	Systemic toxicity
100 μg	0	11	1	None	None
500 μg	3	7	2	None	None
1500 μg	0	8	4	None	None

[a] Pain score 0 = none; pain score 1 = mild; pain score 2 = moderate.

patient had an abnormally elevated LDH after completion of the four vaccinations; this patient was diagnosed with liver dysplasia before entrance into the study and died with a ST of 501 days of hepatocellular carcinoma. For patients entering the trial with normal ANA titers ($n = 5$), there were no subsequent abnormal results. For those patients entering the trial with baseline abnormally elevated ANA titers ($n = 4$), there was no subsequent increase in ANA titers during or after the vaccination protocol. All dogs that died or were euthanized had complete necropsies performed with particular attention and examination (gross and histopathological) of the primary tumor site, metastatic sites, previously vaccinated region, and the eyes. No toxicity was noted, except for two dogs having a mild pleocellular inflammatory infiltrate in the s.c. and i.m. regions of the vaccination site.

Response. Response data are shown in Table 2. At the latest evaluation, there was one stage IV dog alive (588+ days) with stable disease on this trial. This dog has an unchanged, solitary 4-cm pulmonary lesion that was confirmed to be melanoma via ultrasound-guided fine needle aspirate and cytological examination before entrance on the trial. One stage IV dog had a long-term stabilization of disease for 196 days with significant bulky pulmonary metastases. After experiencing PD, this dog went on to receive additional xenogeneic DNA vaccines (mouse tyrosinase-related protein 1/gp75 and then later murine tyrosinase) in subsequent clinical trials and was euthanatized at 421 days (28).

One stage III dog was alive for 496 days and was euthanatized because of complications from pituitary-dependent hyperadrenocorticism and was found to be free of any gross and histopathological evidence of melanoma on necropsy. An additional stage III dog was alive for 501 days and was euthanized because of complications from a subsequent hepatocellular adenocarcinoma (dog with histopathologically confirmed liver dysplasia before entrance onto study) and was found to be free of any gross or histopathological evidence of melanoma on necropsy. Both of these dogs were censored on Kaplan-Meier survival statistics at 496 and 501 days, respectively.

One stage IV dog had a CR 2 weeks after the fourth DNA vaccination that was durable for 329 days. This dog continued to have PD (increasing size of pulmonary metastases on thoracic radiographs) while being vaccinated; however, thoracic radiographs at the time of the fourth vaccination showed mild-moderate scalloping of the pulmonary metastases. Two weeks after the fourth vaccination, the pulmonary metastases disappeared with a subsequent long-term CR (Fig. 2). In clinical trials, cancer vaccines can take ~4–8 weeks or more to induce cellular and humoral immune responses and months for clinical responses (29, 30). The one dog experiencing a long-term CR fits into this temporal model of response. This dog eventually was euthanized because of complications from acute sepsis and was found to have a recurrent 2-cm malignant melanoma in the caudal pharynx ~3-cm caudal to the site of the original oral primary malignant melanoma. No evidence of the previously radiographically documented pulmonary metastases was found on gross and histopathological necropsy examination. Because of the presence of recurrent oral malignant melanoma, this dog was not censored and considered to be dead possibly because of melanoma, although there was no clinical evidence linking systemic sepsis to the local melanoma recurrence.

The Kaplan-Meier median ST for all nine dogs is 389 days (Fig. 3). No dogs were lost to follow-up, and the median follow-up time is 389 days, suggesting adequate data maturation. Two dogs were euthanatized at the 2-week recheck after the fourth vaccination because of progressive local disease. All four dogs with STs < 130 days were euthanized because of

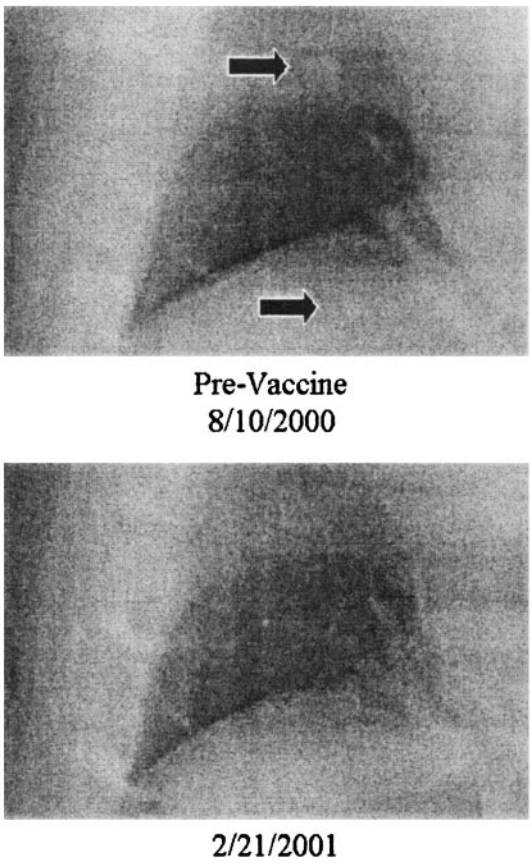

Fig. 2 Detail photographs of lateral chest radiographs from dog no. 2 (12-year-old neutered male Siberian husky; stage IV disease) placed on trial. *Top panel:* lateral chest radiograph before DNA vaccination documenting evidence of pulmonary metastases (note *arrows*). *Bottom panel:* lateral chest radiograph from same dog in *top panel* showing complete resolution of pulmonary metastases after four 100-μg human tyrosinase DNA vaccinations. The dog's complete response lasted for 329 days.

local tumor progression and not because of quality of life implications from metastatic disease. All four of these dogs did not have primary tumor control before entrance onto the study.

None of the recorded variables had a statistically significant association or effect on ST, likely because of the small number of patients. For association evaluation of continuous variables, a positive correlation was found for age and LDH level postvaccination (Kendall rank correlation; $P = 0.0333$), and a negative correlation was found for age and weight (Spearman rank correlation; $P = 0.0113$). For association evaluation of continuous and nominal variables, a positive association was found between increased LDH postvaccination and female gender (Mann-Whitney U test; $P = 0.0201$) as well as increased LDH prevaccination and no previous radiation therapy (Mann-Whitney U test; $P = 0.0389$). Statistically significant positive associations were found for the presence of a perivaccinal pain response between vaccination nos. 2 and 3, vaccination nos. 2 and 4, and vaccinations nos. 3 and 4 (χ^2; $P = 0.0111$, $P = 0.0111$, $P = 0.0027$, respectively).

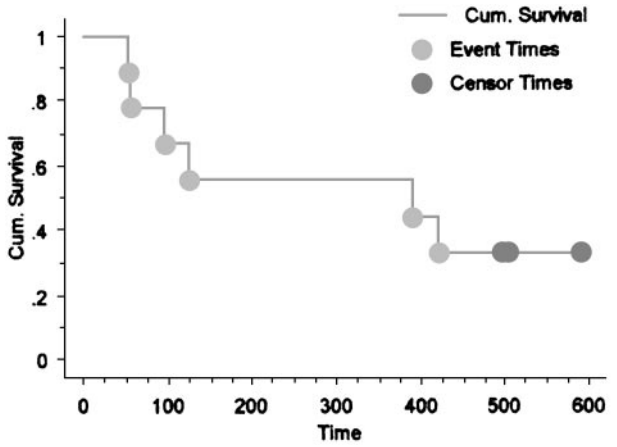

Fig. 3 Kaplan-Meier survival curve for nine dogs with advanced malignant melanoma treated with four biweekly xenogeneic human tyrosinase vaccinations. Kaplan-Meier median survival time is 389 days. Time as shown on X-axis is in days, and three dogs are censored at 496, 501, and 588 days.

Discussion

This clinical trial was designed to determine the safety and efficacy of xenogeneic DNA vaccination in advanced CMM. From the results of this study, we can conclude that xenogeneic human tyrosinase DNA vaccination of dogs is: (*a*) safe, based on minimal local toxicity and a lack of systemic toxicity; and (*b*) potentially efficacious, based on clinical antitumor responses and remarkably prolonged median STs. Historical Kaplan-Meier median STs for dogs with WHO stage II malignant melanoma treated with surgery is ≤5 months, and dogs with WHO stage III or IV have median STs of ≤2–3 months (2, 3, 31). The median ST of dogs treated with xenogeneic human tyrosinase DNA vaccination found in this study is 389 days, supporting clinical efficacy. These data warrant additional clinical evaluations in both CMM and HM.

Vaccine strategies to date in CMM have either used autologous tumor cell vaccines (with or without transfection with immunostimulatory cytokines), allogeneic tumor cell vaccines transfected with interleukin 2, or a bacterial super-antigen approach with granulocyte macrophage colony-stimulating factor or interleukin 2 as immune adjuvants (31–35). Although these approaches have produced clinical antitumor responses, the methodology for the generation of these vaccines is time consuming, sometimes dependent on patient tumor samples being established into cell lines, and fraught with the difficulties of consistency, reproducibility, and other quality control issues. Xenogeneic DNA vaccines are relatively easy to produce, inexpensive, break tolerance, and induce antitumor responses in mouse systems. In addition, DNA vaccines can induce both cellular and humoral immunity, and this combined immunity may be more effective than either arm alone. Importantly, the results of this study show that the xenogeneic model can be extrapolated to CMM as we believe this is the first report of xenogeneic DNA vaccination in spontaneous cancer. This study emphasizes how spontaneous canine cancers serve as an important bridge between preclinical studies in mouse model systems

and clinical trials in humans with cancer and additionally supports the synergy of collaborations between veterinary and human cancer centers.

It is believed that immunotherapy holds the most promise for cancer patients with minimal residual disease (24, 29, 36). The results of this trial support this long-held premise as cases of CMM without local tumor control before vaccination had STs ranging from 54 to 126 days ($n = 4$), whereas those dogs with good local tumor control via radical surgery and/or coarse fractionation radiation therapy (7) and no evidence of metastasis at the start of vaccination ($n = 2$) had STs of 496 and 501 days with both cases having no gross or histopathological evidence of melanoma at death. These data in addition to the aforementioned prolongation of median ST when compared with historical controls strongly argue for Phase II xenogeneic DNA vaccine investigations in locally controlled but not grossly metastatic CMM and HM settings.

Carboplatin is presently the therapy of choice for late-stage CMM; however, response rates are poor and predominately consist of short-term PRs with a median PR time of only 156 days (10). The results of this trial using xenogeneic human tyrosinase DNA vaccination in CMM appear to be substantially superior to carboplatin based on the lack of toxicity and increased median STs noted in this trial. In summary, we propose that xenogeneic tyrosinase DNA vaccination may be an effective treatment for stage II–IV CMM after locoregional control of primary tumor growth, presumably through inhibiting progression of metastases (and perhaps through therapeutic effects on established metastases in occasional patients).

CMM of the oral cavity, digit/footpad, and mucocutaneous junction appears clinically similar to aggressive HM because both diseases are chemoresistant, radioresistant, and share similar metastatic phenotypes and site selectivity. Importantly, CMM is a spontaneous syngeneic cancer occurring in outbred, immune-competent large mammals that live in the same environment that humans do. For these reasons, we believe CMM is a more clinically faithful therapeutic model for HM when compared with the more traditional mouse systems, and further use of CMM as a therapeutic model for HM is strongly encouraged.

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References

1. Bostock, D. E. Prognosis after surgical excision of canine melanomas. Vet. Pathol., *16:* 32–40, 1979.

2. Harvey, H. J., MacEwen, G., Braun, D., Patnaik, A. K., Withrow, S. J., and Jongeward, S. Prognostic criteria for dogs with oral melanoma. J. Am. Vet. Med. Assoc., *178:* 580–582, 1981.

3. MacEwen, E. G., Patnaik, A. K., Harvey, H. J., Hayes, A. A., and Matus, R. Canine oral melanoma: comparison of surgery *versus* surgery plus Corynebacterium parvum. Cancer Investig., *4:* 397–402, 1986.

4. Ramos-Vara, J. A., Beissenherz, M. E., Miller, M. A., Johnson, G. C., Pace, L. W., Fard, A., and Kottler, S. J. Retrospective study of 338 canine oral melanomas with clinical, histologic, and immunohistochemical review of 129 cases. Vet. Pathol., *37:* 597–608, 2000.

5. Schwarz, P. D., Withrow, S. J., Curtis, C. R., Powers, B. E., and Straw, R. C. Mandibular resection as a treatment for oral cancer in 81 dogs. J. Am. Anim. Hosp. Assoc., *27:* 601–610, 1991.

6. Schwarz, P. D., Withrow, S. J., Curtis, C. R., Powers, B. E., and Straw, R. C. Partial maxillary resection as a treatment for oral cancer in 61 dogs. J. Am. Anim. Hosp. Assoc., *27:* 617–624, 1991.

7. Bateman, K. E., Catton, P. A., Pennock, P. W., and Kruth, S. A. 0-7-21 Radiation therapy for the treatment of canine oral melanoma. J. Vet. Intern. Med., *8:* 267–272, 1994.

8. Chapman, P. B., Einhorn, L. H., Meyers, M. L., Saxman, S., Destro, A. N., Panageas, K. S., Begg, C. B., Agarwala, S. S., Schuchter, L. M., Ernstoff, M. S., Houghton, A. N., and Kirkwood, J. M. Phase III multicenter randomized trial of the Dartmouth regimen *versus* dacarbazine in patients with metastatic melanoma. J. Clin. Oncol., *17:* 2745–2751, 1999.

9. Houghton, A. N., Meyers, M. L., and Chapman, P. B. Medical treatment of metastatic melanoma. Surg. Clin. N. Am., *76:* 1343–1354, 1996.

10. Rassnick, K. M., Ruslander, D. M., Cotter, S. M., Al-Sarraf, R., Bruyette, D. S., Gamblin, R. M., Meleo, K. A., and Moore, A. S. Use of carboplatin for treatment of dogs with malignant melanoma: 27 cases (1989–2000). J. Am. Vet. Med. Assoc., *218:* 1444–1448, 2001.

11. MacEwen, E. G. Spontaneous tumors in dogs and cats: models for the study of cancer biology and treatment. Cancer Metastasis Rev., *9:* 125–136, 1990.

12. Sirott, M. N., Bajorin, D. F., Wong, G. Y., Tao, Y., Chapman, P. B., Templeton, M. A., and Houghton, A. N. Prognostic factors in patients with metastatic malignant melanoma. A multivariate analysis. Cancer (Phila.), *72:* 3091–3098, 1993.

13. Pardoll, D. M. Cancer vaccines. Nat. Med., *4:* 525–531, 1998.

14. Moelling, K. DNA for genetic vaccination and therapy. Cytokines Cell. Mol. Ther., *3:* 127–135, 1997.

15. Chu, R. S., Targoni, O. S., Krieg, A. M., Lehmann, P. V., and Harding, C. V. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. J. Exp. Med., *186:* 1623–1631, 1997.

16. Roman, M., Martin-Orozco, E., Goodman, J. S., Nguyen, M. D., Sato, Y., Ronaghy, A., Kornbluth, R. S., Richman, D. D., Carson, D. A., and Raz, E. Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants. Nat. Med., *3:* 849–854, 1997.

17. Wang, R., Doolan, D. L., Le, T. P., Hedstrom, R. C., Coonan, K. M., Charoenvit, Y., Jones, T. R., Hobart, P., Margalith, M., Ng, J., Weiss, W. R., Sedegah, M., de Taisne, C., Norman, J. A., and Hoffman, S. L. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. Science (Wash. DC), *282:* 476–480, 1998.

18. Edgeworth, R. L., San, J. H., Rosenzweig, J. A., Nguyen, N. L., Boyer, J. D., and Ugen, K. E. Vaccine development against HIV-1: current perspectives and future directions. Immunol. Res., *25:* 53–74, 2002.

19. Perales, M. A., Blachere, N. E., Engelhorn, M. E., Ferrone, C. R., Gold, J. S., Gregor, P. D., Noffz, G., Wolchok, J. D., and Houghton, A. N. Strategies to overcome immune ignorance and tolerance. Semin. Cancer Biol., *12:* 63–71, 2002.

20. Weber, L. W., Bowne, W. B., Wolchok, J. D., Srinivasan, R., Qin, J., Moroi, Y., Clynes, R., Song, P., Lewis, J. J., and Houghton, A. N. Tumor immunity and autoimmunity induced by immunization with homologous DNA. J. Clin. Investig., *102:* 1258–1264, 1998.

21. Bouchard, B., Fuller, B. B., Vijayasaradhi, S., and Houghton, A. N. Induction of pigmentation in mouse fibroblasts by expression of human tyrosinase cDNA. J. Exp. Med., *169:* 2029–2042, 1989.

22. Wang, S., Bartido, S., Yang, G., Qin, J., Moroi, Y., Panageas, K. S., Lewis, J. J., and Houghton, A. N. A role for a melanosome transport signal in accessing the MHC class II presentation pathway and in eliciting CD4+ T cell responses. J. Immunol., *163:* 5820–5826, 1999.

23. Bowne, W. B., Srinivasan, R., Wolchok, J. D., Hawkins, W. G., Blachere, N. E., Dyall, R., Lewis, J. J., and Houghton, A. N. Coupling and uncoupling of tumor immunity and autoimmunity. J. Exp. Med., *190:* 1717–1722, 1999.

24. Wolchok, J. D., and Livingston, P. O. Vaccines for melanoma: translating basic immunology into new therapies. Lancet Oncol., *2:* 205–211, 2001.

25. Aguiar, J. C., Hedstrom, R. C., Rogers, W. O., Charoenvit, Y., Sacci, J. B., Jr., Lanar, D. E., Majam, V. F., Stout, R. R., and Hoffman, S. L. Enhancement of the immune response in rabbits to a malaria DNA vaccine by immunization with a needle-free jet device. Vaccine, *20:* 275–280, 2001.

26. Williams, J., Fox-Leyva, L., Christensen, C., Fisher, D., Schlicting, E., Snowball, M., Negus, S., Mayers, J., Koller, R., and Stout, R. Hepatitis A vaccine administration: comparison between jet-injector and needle injection. Vaccine, *18:* 1939–1943, 2000.

27. Fleming, T. R., and Lin, D. Y. Survival analysis in clinical trials: past developments and future directions. Biometrics, *56:* 971–983, 2000.

28. Vijayasaradhi, S., Bouchard, B., and Houghton, A. N. The melanoma antigen gp75 is the human homologue of the mouse b (brown) locus gene product. J. Exp. Med., *171:* 1375–1380, 1990.

29. Greten, T. F., and Jaffee, E. M. Cancer vaccines. J. Clin. Oncol., *17:* 1047–1060, 1999.

30. Chan, A. D., and Morton, D. L. Active immunotherapy with allogeneic tumor cell vaccines: present status. Semin. Oncol., *25:* 611–622, 1998.

31. MacEwen, E. G., Kurzman, I. D., Vail, D. M., Dubielzig, R. R., Everlith, K., Madewell, B. R., Rodriguez, C. O. J., Phillips, B., Zwahlen, C. H., Obradovich, J., Rosenthal, R. C., Fox, L. E., Rosenberg, M., Henry, C., and Fidel, J. Adjuvant therapy for melanoma in dogs: results of randomized clinical trials using surgery, liposome-encapsulated muramyl tripeptide, and granulocyte macrophage colony-stimulating factor. Clin. Cancer Res., *5:* 4249–4258, 1999.

32. Hogge, G. S., Burkholder, J. K., Culp, J., Albertini, M. R., Dubielzig, R. R., Yang, N. S., and MacEwen, E. G. Preclinical development of human granulocyte-macrophage colony-stimulating factor-transfected melanoma cell vaccine using established canine cell lines and normal dogs. Cancer Gene Ther., *6:* 26–36, 1999.

33. Hogge, G. S., Burkholder, J. K., Culp, J., Albertini, M. R., Dubielzig, R. R., Keller, E. T., Yang, N. S., and MacEwen, E. G. Development of human granulocyte-macrophage colony-stimulating factor-transfected tumor cell vaccines for the treatment of spontaneous canine cancer. Hum. Gene Ther., *9:* 1851–1861, 1998.

34. Quintin-Colonna, F., Devauchelle, P., Fradelizi, D., Mourot, B., Faure, T., Kourilsky, P., Roth, C., and Mehtali, M. Gene therapy of spontaneous canine melanoma and feline fibrosarcoma by intratumoral administration of histoincompatible cells expressing human interleukin-2. Gene Ther., *3:* 1104–1112, 1996.

35. Dow, S. W., Elmslie, R. E., Willson, A. P., Roche, L., Gorman, C., and Potter, T. A. *In vivo* tumor transfection with superantigen plus cytokine genes induces tumor regression and prolongs survival in dogs with malignant melanoma. J. Clin. Investig., *101:* 2406–2414, 1998.

36. Fernandez, N., Duffour, M. T., Perricaudet, M., Lotze, M. T., Tursz, T., and Zitvogel, L. Active specific T-cell-based immunotherapy for cancer: nucleic acids, peptides, whole native proteins, recombinant viruses, with dendritic cell adjuvants or whole tumor cell-based vaccines. Principles and future prospects. Cytokines Cell. Mol. Ther., *4:* 53–65, 1998.