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OF MICE & MEN (AND DOGS!): XENOGENEIC DNA VACCINES FOR THE TREATMENT OF

INTRODUCTION - Canine malignant melanoma (CMM) of the oral cavity, nail bed, foot pad and mucocutaneous junction is a spontaneously occurring, highly aggressive and frequently metastatic neoplasm. CMM is a relatively common diagnosis representing ~ 4% of all canine tumors and it is the most common oral tumor in the dog. CMM and advanced human melanoma (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. Based on these similarities, CMM appears to be a good clinical model for evaluating new treatments for advanced HM. Canine patients with advanced disease (WHO stage II, III or IV) have a reported median survival time of 1-5 months with standardized therapies. A combination of hypo-fractionated radiation therapy and chemotherapy have a reported median survival time of one year in stage I oral CMM. Human patients with deep AJCC 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 less than ten months and most of these patients eventually die of melanoma. Standard systemic therapy is dacarbazine chemotherapy in HM, and carboplatin chemotherapy in CMM. Unfortunately, response rates to chemotherapy in humans or dogs with advanced melanoma range from 8-28% with little evidence that treatment improves survival. It is easily evident that new approaches to this disease are desperately needed and multiple methodologies have been reported to date.

Active immunotherapy in the form of vaccines represents one potential therapeutic strategy for melanoma. The advent of DNA vaccination circumvents some of the previously encountered hurdles in vaccine development. 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 intramuscular 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 major histocompatibility complex and costimulatory molecules. The bacterial and plasmid DNA itself contains immunostimulatory sequences that may act as a potent immunological adjuvant in the immune response. In clinical trials for infectious disease, DNA immunization has been shown to be safe and effective in inducing immune responses to malaria and human immunodeficiency virus. 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. It has been shown that vaccination of mice with DNA encoding cancer differentiation antigens is ineffective when self-DNA is used, but tumor immunity can be induced by orthologous DNA from another species.

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. 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. In particular, xenogeneic DNA vaccination induced tumor protection from syngeneic melanoma challenge and autoimmune hypopigmentation. Thus, xenogeneic DNA vaccination could break tolerance against a self tumor differentiation antigen, inducing antibody, T-cell and anti-tumor responses.

RESULTS – The signalment of dogs have been similar to those in previously reported CMM studies. No toxicity was seen in any dogs receiving the aforementioned vaccines with the exception of minimal to mild pain responses at vaccination, one muGP75 dog experienced mild aural depigmentation, and one muTyr dog has experienced moderate foot pad vitiligo. Dogs with stage II-III loco-regionally controlled CMM across the xenogeneic vaccine studies have a Kaplan-Meier (KM) median survival time (MST) of > 2 years (median not yet reached). The KM MST for all stage II-IV dogs treated with huTyr, muGP75 and muTyr are 389, 153 and 224 days, respectively. The KM MST for stage II-IV dogs treated with 50mcg MuTyr, 100/400/800mcg HuGM-CSF or combination

MuTyr/HuGM-CSF are 242, 148 and > 900 (median not reached, 6/9 dogs still alive) days, respectively. For dogs on the Phase Ib MuTyr/HuGM-CSF/Combination trial, significant differences in MST were noted across pre-vaccination stage (stage IV MST = 99 days, stage III = 553 days and stage II > 401 days, P < .001). The results from dogs vaccinated with huTyr were published in 2003 (Bergman et al, Clin Cancer Res 2003).

DEVELOPMENT OF SPECIFIC ANTI-TYROSINASE HUMORAL IMMUNE RESPONSES - We have investigated the humoral responses of dogs receiving HuTyr as a potential explanation for the long-term survivals seen in some of the dogs on this study. Utilizing standard ELISA with mammalian expressed purified human tyrosinase protein as the target of interest (kind gift of C Andreoni & JC Audonnet, Merial, Inc.), we have found 3/9 dogs with 2-5 fold post-vaccinal humoral responses compared to pre-immune sera. We have confirmed these findings utilizing a flow-cytometric-based assay of pre- and post-vaccinal sera in permeabilized human SK-MEL melanoma cells expressing endogenous human tyrosinase. Interestingly, the three dogs with post-vaccinal anti-HuTyr humoral responses are dogs with unexpected long-term tumor control (Liao et al, Cancer Immunity, 2006). Co-Investigators have also determined that normal dogs receiving the HuTyr-based melanoma vaccine develop Ag-specific IFN-γ T cells (Goubier et al, Vaccine, 2008).

CONCLUSIONS - The results of these trials demonstrate that xenogeneic DNA vaccination in CMM is: 1) safe, 2) develops specific anti-tyrosinase humoral and cell-mediated immune responses, 3) potentially therapeutic with particularly exciting results in stage II/III local-regional controlled disease and dogs receiving MuTyr/HuGM-CSF combination, and 4) an attractive candidate for further evaluation in an adjuvant, minimal residual disease Phase II setting for CMM. A safety and efficacy USDA licensure multi-institutional trial investigating HuTyr in dogs with locally controlled stage II/III oral melanoma was initiated in April, 2006. Human trials of xenogeneic tyrosinase DNA vaccination have recently initiated. In March 2007 and December 2009, we received conditional followed by full licensure (respectively) from the USDA for the canine melanoma vaccine. This represents the first US-government approved vaccine for the treatment of cancer across species.

In summary, CMM is a more clinically faithful therapeutic model for HM when compared to more traditional mouse systems as both human and canine diseases are chemoresistant, radioresistant, share similar metastatic phenotypes/site selectivity, and occur spontaneously in an outbred, immuno-competent scenario. In addition, this work also shows that veterinary cancer centers and human cancer centers can work productively together to benefit veterinary and human patients afflicted with cancer.

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