RESEARCH PROGRESS REPORT SUMMARY

Grant 01787: Clinical Advancement of a Cancer Vaccine in Dogs

Principal Investigator: Nicola Mason, BVetMed, PhD

Research Institution: University of Pennsylvania

Grant Amount: $96,660.00

Start Date: 1/1/2013 **End Date:** 12/31/2016

Progress Report: FINAL

Report Due: 12/31/2016 **Report Received:** 7/20/2018

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Original Project Description:

Canine lymphoma is the most common blood-based cancer in dogs with an estimated annual incidence of 30/100,000. Chemotherapy induces remission in 75-85% of patients; however, the majority of patients relapse with drug-resistant lymphoma within 8-10 months of diagnosis and most dogs die of their disease shortly thereafter. Cell-based vaccine strategies that stimulate anti-tumor immunity have shown promise in the treatment of many different cancer types including non-Hodgkin's lymphoma (NHL) in humans. In a previous study Dr. Mason developed a cell-based vaccine to induce anti-tumor immunity in dogs with NHL. Initial studies were hopeful as this early vaccine significantly prolonged second remission duration and overall survival, but ultimately the vaccine did not prevent relapse. These early findings suggest that while the lymphoma vaccine stimulated anti-tumor immunity it will require immunological boosting to achieve prolonged cancer-free survival. In the current study, Dr. Mason will optimize her cell-based vaccine approach to induce functional, long lasting tumor-specific immune responses that will prevent relapse and prolong survival in dogs with NHL.

Publications: None at this time.

Presentations:

a) IMMUNE THERAPY OF CANCER – AN UPDATE ON CURRENT CLINICAL TRIALS IN LYMPHOMA AND OSTEOSARCOMA AT UPENN – PENN Annual Conference March 2013

b) STIMULATING ANTI-TUMOR IMMUNITY IN THE CANINE CANCER PATIENT – Institute of Immunology, VetMedUni, Vienna July 2013

c) CLINICAL EVALUATION OF A CD40-ACTIVATED B CELL VACCINE TO PREVENT LYMPHOMA RELAPSE IN DOGS – Invited lecture at the ACVIM conference in Nashville Tennessee June 4-7, 2014

Report to Grant Sponsor from Investigator:

Our previous work has shown that white blood cells known as B cells found in the peripheral blood can be activated and grown outside of the body using special "feeder cells" that express a molecule known as CD40L. The stimulated B cells (known as CD40-B cells) can be loaded with genetic material (RNA) that has been extracted from the patient's tumor. When re-injected back into the patient, the CD40-B cells present the tumor material to the body's immune system and stimulate an anti-tumor immune response. We have shown in a phase I clinical trial that this approach has produced promising results with respect to prolonging overall survival in dogs with lymphoma. Since then we have been working to further improve this vaccine in 2 ways. Firstly, we aimed to generate a more robust system that induces greater B cell proliferation and produces B cells that have improved capacity to stimulate the patient's T cells against the cancer; and secondly to generate a more user-friendly system of B cell activation and expansion that would only require basic laboratory equipment to make these vaccines for canine patients. We saw this as an important step towards potential commercialization of the product enabling its use for many more dogs.

Current methods of generating the CD40-B cell vaccines from lymphoma patients are labor-intensive and require specialized laboratory equipment that is not available in most facilities. Therefore, we made second-generation feeder cells that stably express the human or canine form of CD40L (we previously used feeder cells that transiently express human CD40L). We found that our second-generation canine or human CD40L expressing feeder cells were simpler to maintain than the previously used transfected cells expressing human CD40L. We also performed several experiments to determine whether these cells could be irradiated prior to cryopreservation and then thawed to stimulate PBMCs. This would enable a master cell bank to be created. However, we found that B cell expansion using thawed, previously irradiated KTcCD40L feeder cells while possible is sub-optimal when compared to freshly irradiated feeder cells. We did experience technical difficulties in growing canine B cells with transduced feeder cells midway through our work and so we opted to use freshly irradiated KthuCD40L cells to generate CD40-B cell vaccines as previously described in our first pilot study for the current clinical trial.

The overall goal of the clinical trial was to determine whether repeat vaccinations with tumor RNA-loaded CD40-B cells administered to dogs with B cell lymphoma after successful induction chemotherapy would prolong remission time and overall survival. We recruited 20 dogs. 7 dogs failed screening and 3 dogs failed CHOP based chemotherapy. Ten dogs were in clinical remission at re-staging, and received their autologous CD40-B cell vaccines. The vaccine series consisted of an initial

series of three vaccines, given three weeks apart, followed by a maintenance phase of booster vaccines, given once every 2 months.

Of the 10 dogs that received CD40-B cell vaccines, 7 completed their initial series and 3 dogs relapsed with clinical disease prior to completing the initial series. Of the 7 dogs that completed the initial series, 5 received booster vaccines. The other 2 dogs relapsed at re-staging and were not eligible to receive further vaccinations. Of the 5 dogs that received booster vaccinations, 4 dogs received only one booster before relapse and 1 dog received 4 boosters before clinical relapse. All dogs participating in the clinical trial received rescue chemotherapy although different rescue protocols were used depending on clinician preference. We found a significant correlation between the number of vaccines a patient received and the time between first relapse and death. This finding supports the hypothesis generated from our original study that vaccine-induced anti-tumor immunity synergizes with rescue chemotherapy to prolong duration of second remission in dogs with B cell lymphoma. An alternative hypothesis is that long term survivors in this group had less aggressive disease which enabled prolonged time to progression and overall survival unrelated to vaccination. We are currently seeking to evaluate the immune response of these dogs and the genetics of the dogs' tumors to try to identify whether anti-tumor immunity or intrinsic differences in tumor behavior are responsible for these findings. We also found that the TTP and OS times of dogs in this study were considerably shorter than reported in our original publication on this topic. The reason for this difference is unknown although the small cohort sizes in each study are likely to contribute to the discrepancy.