



RESEARCH PROGRESS REPORT SUMMARY

Grant 01787: Clinical Advancement of a Cancer Vaccine in Dogs

Principal Investigator: Dr. Nicola J Mason, BVetMed, PhD

Research Institution: University of Pennsylvania

Grant Amount: \$96,660.00

Start Date: 1/1/2013 **End Date:** 12/31/2014

Progress Report: End-Year 1

Report Due: 12/31/2013 **Report Received:** 2/2/2014

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Canine lymphoma is the most common hematopoietic cancer in dogs with an estimated annual incidence of 30/100,000. Chemotherapy induces remission in 75-85% of patients; however, the majority 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. We have used a cell-based vaccine to induce anti-tumor immunity in dogs with NHL. This vaccine given three times after successful induction chemotherapy significantly prolonged overall survival. However, in the majority of dogs the vaccine did not prevent relapse but significantly prolonged second remission duration induced by rescue chemotherapy when compared to unvaccinated controls. These findings suggest that the lymphoma vaccine stimulated anti-tumor immunity but that this was insufficient to prevent relapse and only upon immunological boosting (through a poorly defined but previously recognized chemotherapy effect) could prolonged cancer free survival be realized. Here we aim to optimize our cell-based vaccine approach to induce functional, long lasting tumor-specific immune responses that aim to prevent relapse and prolong survival in dogs with NHL. This cellular vaccine will be generated in the presence of a potent immune stimulant and will be given every 2 months to dogs with NHL. The effects on tumor specific immunity will be evaluated. The goal is to optimize our vaccine/protocol to stimulate more effective anti-tumor immunity that will prevent relapse and prolong overall survival in dogs with NHL.



Grant Objectives:

To optimize our cell-based vaccine approach to induce functional, long lasting tumor-specific immune responses that aim to prevent relapse and prolong survival in dogs with non-Hodgkin's lymphoma.

Publications:

None at this time.

Report to Grant Sponsor from Investigator:

The goal of this proposal is to build on our previous work developing a cell-based vaccine that aims to stimulate potent tumor-specific immune responses that will kill lymphoma cancer cells. 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 an important 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 are able to 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. We have now improved the generation of this vaccine by generating canine specific feeder cells that are moderately more efficient at inducing canine B cells to grow from PBMCs. We have started to evaluate ways to improve the immune stimulatory function of these vaccine cells but have not yet identified an immune stimulant that can significantly augment the CD40-B cell capacity to stimulate canine T cells in vitro beyond that already achieved. Our studies in this area continue.

Our current methods of generating the CD40-B vaccine from lymphoma patients are labor-intensive and require specialized laboratory equipment that is not available in most facilities. Therefore, we have made second-generation feeder cells that stably express the canine form of CD40L (we previously used the human CD40L molecule in our feeder cells) and have evaluated a non cell-based technique for canine B cell culture. We found that our second generation canine CD40L expressing feeder cells work well in our culture system and are much simpler to maintain in the laboratory than the previously used transfected cells expressing human CD40L. We also performed several experiments to evaluate whether these second-generation feeder cells can be irradiated, frozen and then thawed prior to their effective use in B cell generation. This would enable these cells to be distributed to other centers that do not have ready access to an irradiator and enable those centers to generate CD40-B cell vaccines on site. We have found that canine B cell expansion using thawed, previously irradiated KTcCD40L feeder cells is possible however it is sub-optimal when compared to freshly irradiated feeder cells. Unfortunately, we found that a soluble form of



CD40L was not effective at activating and expanding canine B cells from peripheral blood lymphocytes in culture. Therefore, we will continue to generate CD40-B cell vaccines using freshly irradiated feeder cells.

We have also tested the hypothesis that the ability to stimulate anti-tumor immunity can be improved through the addition of a potent immune adjuvant (CpG DNA) to our CD40-B cell cultures. Our preliminary results indicate that while the addition of CpG may induce a mild increase in the number of CD40-B cells generated we have not identified any significant increase in expression of B cell surface molecules over and above a negative GpC control. These experiments are currently being repeated.

Regulatory approval for our second clinical trial using our improved CD40-B cell technology is being sought and we expect to begin recruitment for the second phase of this proposal in the near future.