Grant: 01418: *PET 2.0: Providing Engineered T-cells (PET): New Genetic and Immunotherapy Targeting Canines with Spontaneous B-cell Lymphoma*

Principal Investigator: Dr. Heather M. Wilson, DVM
Research Institution: Texas A&M Research Foundation
Grant Amount: $150,000.00
Start Date: 1/1/2011 **End Date:** 12/31/2013
Progress Report: End-Year 2
Report Due: 12/31/2012 **Report Received:** 2/25/2013
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:

Lymphoma is the most common malignancy of dogs representing up to 25% of diagnosed cancers. Dogs often develop an aggressive form of lymphoma that is rarely curable, with most unfortunately succumbing to disease within 12 months of diagnosis despite best-available chemotherapies. We seek to develop a new treatment to re-train the dog's own immune system to attack the most common type of canine lymphoma, B-cell lymphoma. We plan to obtain a small number of circulating white blood cells, called T cells, from the blood of affected dogs and insert a gene that will cause the T cell to express a receptor which recognizes the tumor "fingerprint". After docking with the lymphoma, the T cell will be triggered to mount an immune response against the tumor cells with the specific fingerprint. This therapy could be used alone or in combination with chemotherapy. Our preliminary data demonstrate that we can indeed express a desired immunoreceptor in genetically modified T cells and we show that it is possible to harvest and grow T cells in the laboratory and return them safely to the dog. Furthermore, the infused cells can be found in the blood and tumor weeks after infusion, showing that it is possible for these cells to survive in the dog. These data support our plans to develop first-in-dog T-cell therapy targeting a tumor that is basically untreatable.

Grant Objectives:

Hypothesis: This grant seeks to test whether ex vivo propagated autologous T cells can be (i) rendered specific for canine B-lineage NHL, (ii) feasibly manufactured as clinical-grade material, (iii) safely infused, and (iv) exert an anti-tumor effect.
Objective 1: To develop a panel of three canine CD20-specific CARs for fully-competent T-cell activation.
Objective 2: To determine the safety and feasibility of infusing autologous canine CD20specific CAR+ T cells into canine patients with CD20+ NHL.
Objective 3: To determine the persistence and anti-tumor effect of CAR+ T-cell infusions.

Publications:

O'Connor CM, Sheppard S, Hartline CA, Huls H, Johnson M, Palla SL, Maiti S, Ma W, Davis RE, Craig S, Lee DA, Champlin R, Wilson H, Cooper LJ. Adoptive T-cell therapy improves treatment of canine non-Hodgkin lymphoma post chemotherapy. Sci Rep. 2012;2:249. doi: 10.1038/srep00249. Epub 2012 Feb 13.

Report to Grant Sponsor from Investigator: The purpose of this grant is to develop a treatment for canine B-lineage NHL using autologous canine T cells which have been non-virally genetically modified using the Sleeping Beauty (SB) transposase/transposon gene transfer system (Figure 6) to express a canine chimeric antigen receptor (cCAR) that recognizes and targets the canine B-cell marker, CD20.

The goal of specific aim 1 is to develop a panel of three canine CD20-specific cCARS for fully-competent T-cell activation. The goals described in specific aim 1 include: designing three cCARS with endodomain

combinations, gene transfer into canine T cells, expanding cCAR+ T cells, and recording descriptive data (cytotoxicity, phenotypes, cytokine secretion, and T-cell expansion kinetics).

This end-of-the-year progress report describes the advancements we have made, as well as, the need for further technique optimization. In order to achieve clinically meaningful numbers and satisfy P.E.T. 2.0 release criteria set forth by our human CAR trials, a majority of T cells must be cCAR+ post transfection and expansion. First, this requires the development of efficient electroporation protocols which include various parameters not yet identified for canine T cells. We have focused on increasing the cCAR expression and T-cell viability post transfection. This has taken several months of re-optimization studies with GFP and the cCAR plasmids. We are also optimizing the type of transposon used in the transfection. We have added selection markers, as well as, imaging genes to the cCAR plasmids to aid in the in vivo detection by Positron Emission Tomography and in vitro propagation of the T cells. As an enhanced safety method, we inserted a suicide gene which is linked to the cCAR expression. In case of a serious adverse event attributed directly to the cCAR+ T cells, the genetically modified T cells can be killed immediately using a certain drug. During this year, we were able to successfully transfect the canine T cells with SB cCAR. Our efforts are now focused on expanding cCAR+ T cells to clinically sufficient numbers. Secondly, now that we can electroporate the canine T cells with DNA, we are trying to establish the appropriate cCAR+ Tcell expansion culture conditions. These cells require a specific number, order and type of signal to grow in vitro. Therefore, cytokine concentrations, selection marker concentrations, and the type and number of artificial antigen presenting cells must be combined in a certain way to promote cCAR+ T cell growth. Based on initial experiments, this seems to vary from the human CAR+ T cell conditions. We have developed several variations of the artificial antigen presenting cells to test for optimal T cell expansion. Additionally, the right proportion of helper to killer T cells is also important. Not all T cells are created equal. Some are responsible for directing the immune systems response, others actually kill the tumor cells and some provide memory so that if the tumor returns the infused T cells can target the malignant cell and increase the potency of the anti-tumor immunity. Finally, we are currently working with the USDA for approval on the use of the SB system to treat pet dogs with B-cell lymphoma. The P.E.T 2.0 study is progressing nicely at this point. We are extremely happy with the progression of the project in light of the challenges associated with transfecting and growing canine T cells.

While we are optimizing cCAR+ T cell growth and expression, we have developed the CARneg T cells as a successful treatment to significantly extend the survival of NHL+ CHOP-treated dogs (P.E.T. 1.0). Unfortunately, while survival time was increased with CARneg T-cell infusions post CHOP chemotherapy, the majority of dogs eventually relapsed. However, we currently have 2 in complete remission from the original 8 treated. Thus, the need for genetically modified with redirected specificity to provide long lasting and permanent remission without the need for chemotherapy is still necessary. Regulatory permission was granted for the P.E.T. 1.0 trial by the USDA. The infusion of CARneg T cells into dogs with B-cell NHL has allowed us to develop new biomarkers and tracking techniques which can be directly applied to the P.E.T. 2.0 study. We were able to show, post CARneg T-cell infusion, rapid killer T-cell reconstitution within a chemotherapy lympho-depleted immune system, CARneg T-cell homing to LN and tumor, biomarker predicting the prognosis of the patient as well as success of the T cell infusion, and the significant increase in tumor-free and overall survival (almost 5 times longer compared to the CHOP only control group) in our patients who received T cell infusions after CHOP chemotherapy. These data are currently in press with the Nature Publishing Group journal, Scientific Reports. The data from this project and Scientific Reports paper was well received by various media outlets and garnered attention from the national media, such as ABC World News with Diane Sawyer, FoxNews Health Online, and the local NBC Houston news station.

Although P.E.T. 2.0 AIM #1 is taking longer than initially expected, we feel that there will be no problems recruiting/infusing patients, expanding cCAR+ T cells, and achieving regulatory permission for the trial in

the next year due in part to the P.E.T. 1.0 data and our diligence with cCAR+ T cell manufacturing. Thanks to your support and we are looking forward to infusing our first patients in the coming months.

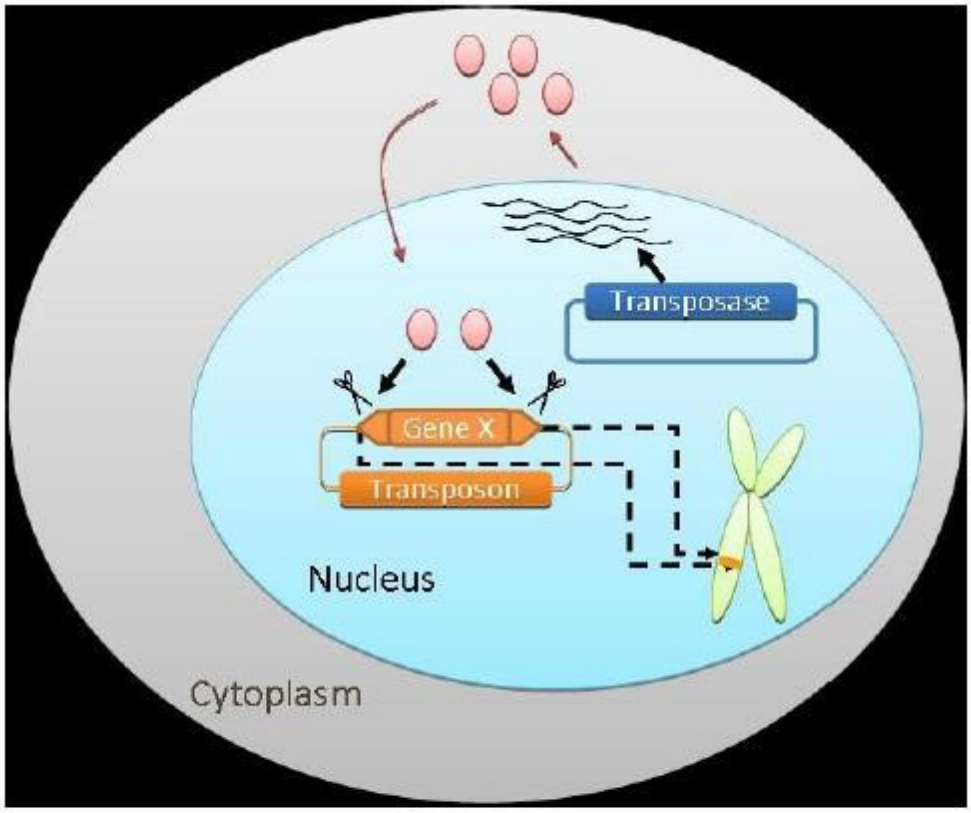

Figure 6: *SB* non-viral DNA integration system. The transposon contains the gene of interest (e.g. CAR), which is electroporated along with the transposase. The transposase cuts the gene of interest out of the transposon and inserts it into the host genome.