



## GRANT PROGRESS REPORT

**Grant:** 01113: *Canine Non-Hodgkin Lymphoma: Characterization and Prognostic Value of Cancer Stem Cells*

**Principal Investigator:** Dr. Timothy D. O'Brien, DVM PhD

**Research Institution:** University of Minnesota

**Grant Amount:** \$150,071.40

**Start Date:** 1/1/2009      **End Date:** 12/31/2010

**Progress Report:** 6 month

**Report Due:** 6/30/2009      **Report Received:** 6/26/2009

**Recommended for Approval:** Approved

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### Original Project Description:

Stem cells are cells which, in general, have the ability to give rise to multiple different (differentiated) cell types while at the same time maintaining their own population of undifferentiated stem cells. Embryonic stem cells are the quintessential stem cell and have the ability to form any tissue of the embryo, fetus, and adult. However, in the adult animal, most tissues or organs also have a stem cell population (adult stem cells) with a more limited repertoire, which can give rise to any of the mature cell types of that organ or tissue (eg. skin, brain, liver, or blood and lymphocyte stem cells). Recently, cells have also been identified in several human and animal cancers that have the essential features of stem cells (cancer stem cells) and which are thought to be responsible for the growth and spread of the tumor. We have identified cells within dog lymphomas that have features highly suggestive of a cancer stem cell. We have also found evidence that increased numbers of these cells tend to correlate with worsening prognosis. In this study we propose 1) to evaluate the numbers of these suspected cancer stem cells in various subtypes of lymphoma to firmly establish whether increasing numbers of these cells do correlate with worsening prognosis across all forms of canine lymphoma, and 2) to obtain key information regarding which genes are characteristically expressed in the cancer stem cells in contrast to those expressed in the remainder of the tumor cells. Thus, this study will potentially give us a new tool to diagnose canine lymphoma and assess prognosis, and secondly, give us a detailed look into the biology of the cancer stem cells, revealing much about their origin and functions and possibly indicating new methods to eliminate these common and lethal cancers.

### Original Grant Objectives:

Objective 1: Enumerate cancer stem cells and endothelial precursor cells in archived and freshly isolated samples of canine non-Hodgkin lymphoma.

Objective 2: Define the phenotype of putative cancer stem cells in non-Hodgkin lymphoma.

### **Publications:**

#### **Report to Grant Sponsor from Investigator:** (Lay Update allowed to be reproduced)

Specific Aim 1: Enumerate cancer stem cells and endothelial precursor cells in archived and freshly isolated samples of canine non-Hodgkin lymphoma.

Toward completing this aim, we have obtained 34 new canine lymphoma samples, including 24 B-cell lymphomas, 9 T-cell lymphomas, and 1 non-T/B cell lymphoma. These were obtained through our collaborations with the veterinary oncology services at the University of Minnesota and Tufts University, as well as through local veterinarians and breeders. We analyzed these samples for the expression of cancer stem cell markers and have confirmed that all of these samples contained cell populations corresponding to the putative cancer stem cells that we detected in our preliminary studies. These cells were found to be present in all tumors in various percentages, generally between 0.1 to 3.0%. Parallel evaluations are also being completed for the endothelial precursor cells. We are now following the outcome (length of survival) of each of these dogs to expand our preliminary data which had suggested that there was a significant (negative) prognostic value associated with the numbers of our putative lymphoma stem cells in a tumor.

Specific Aim 2: Define the phenotype of putative cancer stem cells in non-Hodgkin lymphoma.

Stem cells, such as the well-characterized embryonic stem cells, have been shown to have characteristic patterns of gene expression. Similar patterns of "stem cell marker" genes have also been shown to occur in cancer stem cells. Therefore, one approach to evaluating the putative cancer stem cells that we have identified in dog lymphomas is to use the RT-PCR (reverse transcriptase-polymerase chain reaction) technique to look for expression of specific genes that are known to occur in various types of normal stem cells. To make sure that these are not just genes expressed in all of the tumor cells we will compare the levels of these genes expressed in our putative lymphoma stem cells to the non-stem cell populations of tumor cells. Toward this goal we have developed the initial tools to accomplish this which consist of DNA PCR primers specific for each of the canine genes of interest. We have developed these primers for Oct4, Sox2, Nanog, c-Myc, (all expressed in embryonic stem cells) and CD34 (expressed in hematopoietic stem cells), along with a primer to GAPDH (a "house keeping" gene which is expressed at similar levels in most cells) to be used to normalize comparisons between different cell samples. In a preliminary trial, using these primers on lymphoma stem cell enriched samples from 2 B-cell lymphomas we detected Oct4 and c-Myc in both tumors, Sox2 in one tumor, and both were negative for Nanog. We are continuing to develop these assays and are now preparing to do quantitative RT-PCR on the samples that we have collected to fully evaluate the gene expression profiles of the lymphoma stem cells and compare them to the rest of the tumor cells.

The second means that we proposed to evaluate the phenotype of the lymphoma stem cells was to do DNA microarray analysis which allows the simultaneous assessment of expression of tens

of thousands of genes. This assay is less sensitive than PCR but is an extremely powerful way to assess overall features of gene expression in cell populations. Toward achieving this goal we are preparing cell samples from lymphomas as we acquire them and separating the putative stem cell populations from the rest of the tumor cells and then saving (freezing) the cell samples for later study. These activities are ongoing and no data has as yet been acquired.