Update on AKC CHF Grant 01113:

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

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Report to Grant Sponsor from Investigator:

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

Non-Hodgkin lymphoma (NHL) represents a heterogeneous group of tumors divided into more than 25 subtypes based on cell lineage and histological morphology. Histological classification and clinical stage are used to predict patient outcomes; however, disease progression is variable even within the same NHL subtype, and therefore additional means of establishing prognosis and outcomes are needed. The growth of small blood vessels into tumors (angiogenesis) has been correlated with the extent of disease and progression of lymphoid tumors in dogs and humans, but the prognostic value of mature endothelial cells (cells which form the lining of blood vessels/ECs) and endothelial progenitor cells (immature cells which may proliferate to form additional endothelial cells/EPCs) as surrogate markers for angiogenesis in canine NHL is unclear. Furthermore, the significance of lymphoid progenitor cells (immature cells which may proliferate to form lymphocytes/LPCs) in this disease has not been addressed. Here, we evaluated the predictive value of ECs, EPCs and LPCs in dogs with naturally occurring NHL.

We used flow cytometry to identify cells that expressed markers that identify EPCs; or hat expressed markers that identify LPCs, as well as cells that expressed markers ECs, in blood and lymph nodes from dogs with NHL treated using standard of care multi-agent chemotherapy. The normalized frequency of ECs, EPCs and LPCs was compared with patient outcomes. ECs, EPCs, and LPCs were routinely present in all lymph node samples. No correlation was observed between these cells types and patient outcomes. Significantly, our investigations also showed that LPCs occur in all canine lymphomas at levels far above those in normal lymph nodes. By establishing the identity of the LPCs within canine NHL we have accomplished the critical first step in understanding this basic aspect of lymphoma. Further understanding of the biology of LPCs in canine NHL may inform the development of more effective treatments for this devastating cancer.

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 know 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 assays and are now doing the 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 method by which we proposed to evaluate the phenotype of the lymphoma stem cells was 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 continuing to prepare cell samples from lymphomas as we acquire them and separating the putative stem cell populations from the rest of the tumor cells. These activities are ongoing and no data has as yet been acquired.