RESEARCH PROGRESS REPORT SUMMARY

Grant 01918-G: Discovery of Biomarkers to Detect Lymphoma Risk, Classify For Treatment, and Predict Outcome in Golden Retrievers

Principal Investigator: Dr. Jeffery N. Bryan, DVM, PhD

Research Institution: University of Missouri, Columbia

Grant Amount: $404,813.00

Start Date: 7/1/2013 **End Date:** 6/30/2017

Progress Report: End-Year 3

Report Due: 12/31/2016 **Report Received:** 1/6/2017

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 strikes 1 in 8 Golden Retrievers, approximately one-third of the cases being B cell. While T cell classifications currently inform therapy choices for dogs, B cell classifications have been investigated little in Golden Retrievers. Epigenetic DNA methylation changes clearly underlie lymphomagenesis in humans, but have been evaluated minimally in dogs. Cancers con-tain tumor initiating cell (TIC) populations that resist therapy by expressing efflux pump and pro-survival genes that have not been identified clinically in lymphoma of dogs. We propose to improve diagnostic, classification, and prognostic ability using flow cytometry paired with biopsy to characterize the B cell lymphomas of Golden Retrievers. With these same samples, we will identify DNA methylation changes in lymphoma cells not present in normal cells to develop bi-omarkers of each class of lymphoma and identify new therapy targets for affected Goldens. More significantly, because DNA methylation changes occur so early in the process of cancer formation, we hypothesize that they could serve as biomarkers of risk, allowing medicine or diet to prevent lymphoma in Goldens before it develops. Finally, we propose to identify TICs in lymphoma biopsies to characterize stem-like cells by surface markers and DNA methylation changes. Identifying these cells will aid therapeutic strategy development. Each project advanc-es a current frontier of research. By performing them in parallel, the markers from each can be combined, correlated, and translated into biomarkers of

risk, diagnosis, and prognosis to advance the prevention and management of lymphoma in Golden Retrievers.

Grant Objectives:

1. Characterize the types of B cell lymphoma in Golden Retrievers by flow cytometry.

2. Define the methylomes of B cell lymphomas in Golden Retrievers.

3. Identify and characterize subpopulations of cells within types of B cell lymphoma in Golden Retrievers with TIC phenotype.

Publications:

None at this time.

Report to Grant Sponsor from Investigator:

Progress continues at all 3 institutions. The proposed immunohistochemical evaluations and flow cytometry techniques have identified that the population of B cell lymphomas appears to be a monomorphic group of diffuse large B cell variety (DLBCL) similar to the aggressive form in humans. An immunohistochemistry panel is now functional to identify these and flow cytometry has being optimized, but does not clearly distinguish among them. Gene expression analyses are underway that we expect to further characterize these samples and better our understanding of the etiopathogenesis of the disease. Completed sequencing experiments have identified differentially hypermethylated genes in B cell lymphomas of Golden Retrievers that is similar to those in human lymphoma. A diagnostic PCR panel is in progress for further screening of affected and unaffected Golden Retrievers for lymphoma methylation marks. We are beginning to add whole genome, exome, and transcriptome sequencing in a subset of cases to understand how mutation and DNA methylation interact. TAMU has successfully generated tumor initiating cell populations from cultured lymphocytes and has optimized the procedures to be performed on fine-needle aspirates of lymphoma nodes. It appears that multiple aspirates will be necessary to get all the material needed for characterization. Sufficient TIC cells can be generated for sequencing with the protocol in place at MU. Because the lymphoma samples are so similar across the board, we have changed Aim 1 to evaluate gene expression in these tumors as it relates to the methylation profile.