RESEARCH PROGRESS REPORT SUMMARY

Grant 02317: The Role of Complex Translocations Associated with TP53 Somatic Mutations for Aiding Prognosis of Canine Diffuse Large B-Cell Lymphoma

Principal Investigator: Matthew Breen, PhD

Research Institution: North Carolina State University

Grant Amount: $177,327

Start Date: 1/1/2017 **End Date:** 6/30/2020

Progress Report: End-Year 3

Report Due: 12/31/2019 **Report Received:** 12/22/2019

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Original Project Description:

Lymphoma accounts for up to 24% of all cancers diagnosed in pet dogs. Among these cases diffuse large B-cell lymphoma (DLBCL) is the most common subtype. Despite continued advances in veterinary medicine, the response to treatment for canine lymphoma remains highly variable with no reliable means to predict response. Studies of lymphoma in people have identified characteristic genome changes that have both diagnostic and prognostic significance. In human DLBCL, mutations in the TP53 gene, and genome rearrangements involving the MYC, BCL2 and BCL6 genes have been shown to confer particularly poor prognosis in cases treated with standard of care multi-agent (CHOP-based) chemotherapy. The investigator's previous CHF-funded studies have shown that canine cancers, including lymphoma, exhibit genomic changes that are conserved with those observed in the corresponding human cancers, and have identified MYC and BCL2 rearrangements and a high frequency of TP53 mutation in canine DLBCL. This research will screen a well-defined collection of over 450 pre-treatment, canine DLBCL samples to determine accurate frequencies of these genome changes. The researchers will investigate the correlation of these target aberrations with duration of first remission, and identify key genomic signatures that may aid prognosis of prospective canine lymphoma cases. The data generated should assist owners and veterinarians with decisions regarding treatment with CHOP. Patients with signatures predictive of poor response to conventional CHOP chemotherapy may benefit from more aggressive treatment at the outset to improve outcome.

Publications: None at this time.

Presentations:

Katherine Kennedy, Tao Jiang, Rachael Thomas, Christina Williams, Alison Motsinger-Reif and Matthew Breen. A Comparative Assessment of Prognostic Genomic Signatures in Diffuse Large B-Cell Lymphoma (2018 Consortium for Canine Comparative Oncology, Durham, NC, 02/23/2018)

Katherine Kennedy, Tao Jiang, Rachael Thomas, Christina Williams, Alison Motsinger-Reif and Matthew Breen. A Comparative Assessment of Prognostic Genomic Signatures in Diffuse Large B-Cell Lymphoma (North Carolina State University PostDoctoral Research Symposium, Raleigh, NC, 05/25/2018)

Report to Grant Sponsor from Investigator:

This study involves the evaluation of a cohort of canine lymphoma specimens for the presence of tumor-associated abnormalities associated with four key cancer-associated genes (MYC, BCL6, BCL2 and TP53). The presence of these abnormalities, alone and in combination, has been shown to be predictive of the response to standard treatment modalities in human lymphoma patients, and provides powerful opportunities to predict prognosis in newly diagnosed patients. We hypothesize that the same may apply in dogs.

We have screened the full cohort of canine lymphoma cases for structural and numerical abnormalities involving MYC, BCL6, and BCL2. Overall the data suggest that rearrangement of the genome at the MYC and BCL6 loci is relatively rare within any given case, and occurs at a frequency similar to what is seen in human DLBCL (Li et al. 2018). While BCL2 rearrangement is highly infrequent in dogs (seen in only 2% of cases), and has a generally neutral copy number status, our initial analysis suggests an association of this event with disease-free interval. In an earlier study we showed that the incidence of BCL2 rearrangement and copy number imbalance is low in canine follicular lymphoma (Thomas et al. 2017). The rarity of this B-cell lymphoma subtype in the dog limited the ability to draw generalized comparisons with the human counterpart; however the present study suggests that these observations can be extended to other more common canine B-cell lymphomas. Analysis to date suggests that neither BCL6 nor MYC rearrangement is significantly associated with disease free interval. Assessment of the copy number status of both of these loci concur with previous studies (Thomas et al. 2011), with MYC demonstrating a trend toward copy number gain and BCL6 demonstrating largely neutral copy number status.

DNA sequencing analysis of the TP53 gene has revealed a diverse series of variants among those cases analyzed to date, the majority of which are clustered within a small genomic interval. Almost all variants are simple in structure but are predicted to have a deleterious effect on the function of the gene. We identified variants for which the equivalent alteration is highly recurrent in human tumors, including two key variants that have been reported previously in canine lymphomas, adding to their potential clinical significance. A subset of specimens yielded sequencing data that did not meet our quality control criteria. These specimens showed a high level of DNA degradation, which is likely a

consequence of prolonged exposure to formalin during the processing of the biopsy specimen for histologic analysis. Formalin exposure creates crosslinks between DNA and protein, which can confound downstream analyses, including DNA sequencing analysis. By modifying our DNA extraction protocol we were able to ameliorate this confounding factor for the majority of cases that originally failed to yield good quality TP53 sequence data; however a small number of cases remained intractable to this analysis. We were able to substitute these cases for alternates from our sample repository, and are now processing those specimens for genomic analysis. On completion we will integrate genomic data for each of the four genes studied for each case, and examine their status in context with patient outcome, to determine their potential as clinically predictive markers for canine lymphoma.