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

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Progress Report: FINAL

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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 (Consortium for Canine Comparative Oncology 2018 Meeting & North Carolina State University Post-Doctoral Research Symposium)

Report to Grant Sponsor from Investigator:

This study involved the evaluation of canine lymphoma biopsy specimens for the presence of tumor-associated abnormalities associated with four key cancer-associated genes (*MYC, BCL6, BCL2*, and *TP53*). In a subset of human lymphoma patients, cancer cells show structural rearrangements of *MYC, BCL2*, and/or *BCL6*, meaning that the normal organization of the gene has become disrupted in the tumor. Human lymphomas also frequently show DNA sequence mutations in the *TP53* tumor suppressor gene. The presence of these abnormalities, alone and particular in combination, has been shown to be predictive of a poor response to standard treatment modalities in human lymphoma patients, and provides powerful opportunities to predict prognosis in newly diagnosed patients.

We hypothesized that the same may apply in dogs. We tested this hypothesis using a cohort of canine lymphoma cases that were all treated using standard of care chemotherapy protocols, for which the disease-free interval (DFI) and outcome is known. The aims of the study were to establish a) whether canine lymphomas exhibit abnormalities in these genes and b) to assess whether the presence of one or more of these abnormalities in a tumor specimen is associated with the clinical course of the disease for that case.

We screened the 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. The frequency of MYC rearrangement in dog lymphomas (19% of cases) was similar to that seen in human cases (10-15%), while *BCL6* rearrangements were less commonly observed in dog versus human cases (18% and 30%, respectively, Li et al. 2018). Furthermore, 50% of dog cases showed an increase in the number of copies of the MYC gene in tumor cells, compared to normal controls.

In contrast, while *BCL2* rearrangements are found in 20-30% of human lymphomas, this abnormality was detected in only 2% of dog tumors. Interestingly, however, dog cases harboring this rearrangement were among the poorest responders to chemotherapy, with a DFI of 38-42 days, compared to a mean of 250 days across the cohort. Furthermore, one case with *BCL2* rearrangement was the only case found to also exhibit rearrangements of both *MYC* and *BCL6*. In human lymphomas, this combination of events is termed a 'triple hit', and is associated with a particularly poor prognosis.

DNA sequence mutations of the *TP53* gene were also infrequent among dog cases. As in human patients, the majority were located in exons 7 and 8 of the *TP53* gene. Of the mutations observed, 87% were predicted to have a deleterious impact on the function of the gene, suggesting that they play a role in tumor pathogenesis rather than being coincidental events. Adding further support, four of the mutations have been identified in prior studies of dog lymphoma. Additionally, the dog counterpart of the three most frequently affected sites within human *TP53* were represented as mutations in our canine lymphomas. Interestingly, no *TP53* mutations were found within the subset of dogs whose tumors responded well to chemotherapy, and *MYC* was the only one of the four abnormalities to be found in good responders.

Statistical analysis of these findings showed that cases with mutation within *TP53* exon 5-8 mutation were more at greater risk of responding poorly to standard of care chemotherapy compared to those cases in which no mutation was detected. Similarly, cases with *BCL6* rearrangement were at greater risk of responding poorly compared to cases in which this alteration was not detected. No significant correlation was evident when considering *MYC* alterations, while the incidence of *BCL2* rearrangement within the lymphoma cohort was too low to permit statistical evaluation. Interestingly, combinatorial assessment showed that there was no compounding effect associated with the presence of both *TP53* exon 5-8 mutation and *BCL6* rearrangement. Instead, the treatment response of dogs with tumors bearing both these alterations was comparable to that of those with *TP53* exon 5-8 mutation only, although the significance of this observation was lower due to the small number of cases with both alterations. Analysis of additional cases will aid a more complete understanding of the clinical relevance of cases with alterations of both genes. Further investigation will also be required to determine whether *TP53* mutation and *BCL6* rearrangement are predictive of therapeutic response for other treatment regimens, and to develop these findings into a tool for application to newly diagnosed cases in a clinical setting. Continued investigation will also be necessary to establish whether *TP53* mutation and *BCL6* rearrangement are predictive of therapeutic response for other treatment protocols used for canine lymphoma, and to develop these findings into a tool for application to newly diagnosed cases in a clinical setting.