



## RESEARCH PROGRESS REPORT SUMMARY

**Grant 02309-T:** Targeting the Cancer Epigenome: The Effect of Specific Histone Lysine Methyltransferase Inhibition in Canine B-Cell Lymphoma

**Principal Investigator:** Angela McCleary-Wheeler, DVM, PhD

**Research Institution:** University of Missouri

**Grant Amount:** \$21,321

**Start Date:** 9/1/2018      **End Date:** 6/30/2021

**Progress Report:**

**Report Due:** 6/30/2021      **Report Received:** 4/28/2022

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### **Original Project Description:**

Canine lymphoma is one of the most common cancers in dogs. While some breeds appear more at risk than others, all can be affected. Although it is often treatable, canine lymphoma can rarely be cured. A continued understanding of the mechanisms causing lymphoma in dogs and identification of novel therapies are needed to improve survival in dogs with lymphoma. One area of research that has been actively explored and provided exciting breakthroughs for human lymphoma is epigenetics, or alterations in how genes are turned on and off independent of the DNA sequence. One way in which this occurs is due to modifications of the proteins that interact with DNA called histones. Various modifications to these histones can result in genes being turned on or off, leading to the development of cancer. One particular enzyme that modifies histones, EZH2, has been found to play a role in some human lymphomas. However, this has been unexplored in canine lymphoma. Given the striking similarities between human and canine lymphoma, the objective of this work is to characterize the function and role of EZH2 in canine lymphoma. The investigators will utilize an EZH2 inhibitor to study EZH2 in canine lymphoma cells. The information obtained from this study will help guide the future development of this targeted inhibitor for use as a novel therapy to treat canine lymphoma.

### **Publications:**

A manuscript showcasing this work is in preparation as the data is gathered and the aims of the project are achieved. An abstract was prepared for the annual conference of the Veterinary Cancer Society that was held in October 2017 and was published in the conference proceedings.



### **Presentations:**

The work was presented as a poster presentation at the Veterinary Cancer Society Annual Conference in October 2017. A portion of the work was included in a summary of the state of epigenetics in veterinary oncology presented by Dr. Jeffrey Bryan at the 2022 VCS Mid-Year conference with reference to the CHF for support of the work.

### **Report to Grant Sponsor from Investigator:**

Lymphoma, particularly the large, B-cell subtype, is one of the most common malignancies in dogs. Canine lymphoma can be treated, but it is rarely cured. Novel therapeutic strategies are necessary to improve outcomes in dogs diagnosed with lymphoma. Recently, advances in the understanding of human lymphomas have focused on the area of epigenetics. One area of this research involves understanding how genes are turned on or off based on different modifications to histone proteins, a specific group of proteins that interact with DNA. Specific enzymes that modify these histone proteins have altered activity that can lead to lymphoma development in human lymphomas. One of these enzymes is EZH2. Increased activity of EZH2 has been shown to play an important role in the development of some human lymphomas. Data from a Phase I study of an EZH2 inhibitor, tazemetostat, in relapsed or refractory human B-cell, non-Hodgkin lymphoma has shown to be a safe, oral therapy with potential clinical benefit. The role of EZH2, however, has not been evaluated in canine B-cell lymphomas to date. Given the similarity between human and canine B-cell lymphoma, we seek to investigate whether EZH2 activity plays a role in canine B-cell lymphoma. To do this, we use canine lymphoma cells and specific EZH2 inhibitors, including the tazemetostat used in early human studies, to evaluate the effect of EZH2 inhibition on cell growth and survival. Our data suggest that this inhibitor is highly potent and effective for inhibiting EZH2 effects on histone modification in canine lymphoma. This is important as this inhibitor is an orally bioavailable drug with a good toxicity profile in humans, making this inhibitor a candidate for clinical trials in dogs with lymphoma. Initial data suggests that EZH2 inhibition may not impede lymphoma cell proliferation or survival. However, we have confirmed that some genes that regulate the ability of canine lymphoma cells to replicate are altered with EZH2 inhibition. Specifically, one gene, CDKN1a, is turned back on when EZH2 is inhibited. The activation of CDKN1a is repeatable and profound. We will be continuing this work with a sequencing approach to further understand what genes are regulated by EZH2 in canine B-cell lymphoma cells. Additionally, evaluating the presence of some of these markers in banked tumor tissues will further our understanding of the role of this process in canine lymphoma. Our findings are suggesting an importance for EZH2 in canine lymphoma and for continued investigations into cell cycle regulators that may be abnormal. By understanding the genes regulated by EZH2, we can characterize the role this histone-modifying enzyme has in canine lymphoma. Moreover, we can assess how inhibition of EZH2 activity may be utilized clinically in dogs with lymphoma. We appreciate the generous support provided by this grant from the CHF and its supporting donors.