John M. Cunningham, MD - 2010 Fletcher Scholar

"Characterization of the nuclear architecture of leukemia stem cells"
2012 Final Report

I want to thank the Foundation for its support of my program and the broader tAML program. It has facilitated our studies of the epigenetic changes that induce the induction of leukemogenesis and its maintenance.

During the period of the award, we have focused on two complementary areas. Our first goal was to advance our understanding of the defects in the histone code that characterize leukemia stem cells. We sought to compare these events with those in normal blood stem cells and in other stem cell populations. The rationale for these studies lies in the observations by ourselves, and others, that the factors modifying this code are not only critical proteins necessary for leukemic transformation and maintenance of the malignant phenotype, but also represent potential therapeutic targets that can be exploited to develop novel and successful curative strategies.

Central to the success of our approach was the development of two assays, ChIP-Seq and FAIRE. These assays are usually effective only in the context of large numbers of cells (>1 million cells/reaction). Modifying the approaches of others, we have now being able to perform these assays on less than 50,000-100,000 cells, allowing us to evaluate samples derived either from patient material directly, or from newly established animal models of therapy-related acute myeloid leukemia, or acute lymphoblastic leukemia.

Working with a team of talented biochemists, animal modelers and bioinformaticists within my laboratory, as well as faculty colleagues within the BSD, we have used these assays to identify key networks activated by components of the PRC1 and PRC2 multiprotein complexes and by histone methyltransferases (PRDM16) and demethylases (ARID5B). These studies are ongoing but we expect to publish our observations in the next 12 months.

In a second area of investigation, we are exploring the divergent roles of distinct sub-nuclear compartments in leukemic transformation and maintenance. As reported previously, this has been a highly demanding initiative, especially given the small number of cells available to study. By focusing on amplifying the target cell population and refining the e4C assay, a technique that measures interactions between different chromosomes, we have been able to demonstrate novel interactions between regulatory regions that may explain leukemic transformation. We are now in the process of a) validating these observations, and b) confirming their functional significance in our model systems. We expect that these defects may explain a significant component of the leukemogenic transformation associated with the chromosomal gains and losses observed during leukemic transformation.

In summary, the funding of the Fletcher award has been instrumental in developing a highly informative, novel, and challenging program in my laboratory. I expect that at least two manuscripts will result from these studies in the next eighteen months. In addition, we anticipate approaching both the NCI and the American Cancer Society for funding in the next year.