Title: **Identification of Key Signalling Pathways in Chronic Myeloid Leukemia Stem Cells**

MicroRNA182 (miRNA182) upregulation has been reported in several solid tumors and hematological malignancies but the mechanistic role is currently poorly understood. A particular question of both biological and clinical significance is the role of miRNA182 in contributing to the late stage of chronic myeloid leukemia (CML) progression in the absence of TK (tyrosine kinase) gene mutations. Following an unbiased screen for potential microRNAs in the context of TKI (tyrosine kinase inhibitor) resistance, increased expression of miRNA182 was detected in Bcr-Abl-inhibited K562 cells. We further verified the increased expression of miRNA182 in other CML cell lines and in a panel of primary CML cells. Additionally, erythroid differentiation was found positively correlated with TKI resistance and ectopically expressed miRNA182 respectively. We undertook a strategy involving transient inhibition and complete knockout of MIR182 locus. CRISPR/Cas9 mediated MIR182 knockout successfully deleted the MIR182 locus in K562 cells. Transient inhibition of miRNA182 and Δ182 cells revealed biased and complete myeloid phenotypes respectively. The phenotype was rescued by ectopic expression of miRNA182-5p in Δ182 cells. Additionally, our experimental analysis of the role of Hes1, which is linked to miRNA182 through indirect evidence, further substantiates a role for the miRNA182- Hes1 axis in regulating the erythroid to myeloid switch. A conserved binding site at 3’UTR of Hes1 mRNA and rescue of Hes1 expression upon miRNA182 inhibition are suggestive of Hes1 as a putative target of miRNA182. Further, Hes1 overexpression increased myeloid differentiation and Hes1 downregulation increased erythroid differentiation. In conclusion, we reveal a key role for miRNA182 in regulating the differentiation of leukemic cells. We propose that the Δ182 cell line will be valuable in designing experiments for generating next generation pharmacological interventions.

I undertook my thesis work in collaboration with a senior hematologist Cecil Ross of St. John’s Medical College. The program evolved out of a joint hematology centric teaching program. The program was centered around hematology genomics and our approach was to develop platforms and in addition ask questions that addressed basic biological mechanisms in the context of therapies. Overall, the hematology program in our group has had three major overlapping themes-

i) development of tools to build HLA registries Gowda, M., et al. (2016). Comparative analyses of low, medium and high-resolution HLA typing technologies for human populations. Journal of Clinical and Cellular Immunology, 7, 399-406
