Student: David Bobrowski
PI: Dr. Shiela Singh

Progress Report

Over the duration of the summer, I had the privilege to work at the McMaster Stem Cell and Cancer Research Institute (SCC-RI) in Hamilton under the supervision of Dr. Sheila Singh. In applying a stem cell biology framework to the study of brain tumours, the Singh laboratory has identified and purified a subpopulation of cells, termed brain tumour initiating cells (BTICs), from human glioblastoma (GBM) tissue samples which exhibit stem cell properties, namely self-renewal, in both culture and animal models. BTICs are thought to be responsible for cancer initiation, progression, recurrence, and drug resistance. The BTIC hypothesis has gained a great deal of attention over the past several years because these cellular changes raise the potential for the development of novel therapies. Treatment interventions can take advantage of a perceived Achilles' heel by targeting cell-surface molecular markers or various intracellular signaling pathways involved in regulating these special properties of the BTIC population likely responsible for poor patient outcomes. This approach represents a paradigm shift from treating the manifestations of cancer to addressing the cause.

The Eph project focused on EphA2 and EphA3 co-expression as a marker for a highly tumorigenic cell population in recurrent GBM that is enriched in BTIC marker expression. The Singh laboratory and collaborators devised a new therapeutic strategy by engineering a novel bispecific antibody (BsAb) that co-targets EphA2 and EphA3. Treatment with this BsAb reduces the tumorigenic potential of recurrent GBM by down regulating Akt and MAPK signaling pathways and increasing differentiation. The strategic co-targeting of both EphA2 and EphA3 presents a novel immunotherapeutic approach for recurrent GBM.

During my time in the Singh laboratory, I had the opportunity to work on the Eph project and assist Maleeha Qazi, a PhD student and lead investigator. I was initially tasked with writing a literature review on previous publications that explored the role of EphA2 or EphA3 in GBM and other cancers. I performed a Pubmed and Google Scholar search of the English language literature published on this topic and summarized eight articles. In the laboratory, I was taught how to process brain tumour tissue and culture brain tumor cells, applying culture conditions to enrich for BTICs, and characterize patient-derived tumour samples using flow cytometry. Specifically, I characterized BT428 to determine its CD133, CD15, Sox2, and Bmi1 expression levels. As well, I helped conduct *in vitro* experiments. I read several sphere formation and proliferation assays using PrestoBlue cell viability reagent, and counted sphere-formation in limiting dilution assays to assess self-renewal capacity, often following knockdown of EphA2 and/or EphA3. Moreover, I learned about and aided in running western blot and polymerase chain reaction experiments. In addition, animal studies were performed using recurrent GBM cells sorted based on expression of EphA2 and EphA3 and intracranially injected into the right frontal lobes of NOD-SCID mice. In association, intracranial treatment with BsAb or control IgG was also conducted. I sectioned these xenografts using a brain-slicing matrix, and sent them for paraffin-embedding and H&E staining. Subsequently, I scanned and analyzed these stained brain sections for total tumour area to

evaluate engraftment and treatment effect. I received authorship for my efforts on a manuscript submitted to Cancer Cell.

I am excited by the work that I have been doing, and I know my experiences in the Singh laboratory will serve me well in my future pursuits. After graduating from the Bachelor of Health Sciences Program at McMaster University, I was admitted to the University of Toronto Faculty of Medicine. I believe the road between bench and bedside is a two-way street and it is my intention to travel it in both directions. The insights provided by biomedical research, including the work being done to understand the molecular pathways that underpin cancer, can lead to profound changes in the way physicians approach treating disease. In this way, the medical field is constantly evolving and this progression is directed by research findings that are produced by teams like Dr. Singh's laboratory. My education at the University of Toronto will allow me to make a more fulsome contribution to the medical sciences and I hope to maintain my association with the Singh laboratory team because I consider this affiliation to be a privilege that I continue to value.