A charity partner of ALSF, the Swifty Foundation has made a huge impact through co-funding high impact medulloblastoma research projects, bringing us closer to a day where no child has to suffer from medulloblastoma. Since 2017, the Swifty Foundation has co-funded 6 cutting-edge research projects across the U.S. The Swifty Foundation was founded by Michael Gustafson before passing away from brain cancer in 2013 at the age of 15. Michael’s “Master Plan” was to donate his tumor tissue to science so a cure might be found for other children. Since then, the Swifty Foundation has started a national initiative to promote post-mortem tissue donation, focuses on funding medulloblastoma research, and promotes collaboration within the childhood cancer community.
Targeting TGFβ Pathway Dependencies in Group 3 Medulloblastoma

Zulekha Qadeer, PhD
University of California, San Francisco
Young Investigator Grant

Project Update

Currently, Group 3 medulloblastoma (MB) have poorly understood biology, insufficient representation in mouse models, and as a result, few actionable targets for therapies. Amplification of MYC and TGFβ signaling is common in aggressive and therapy resistant Group 3 MB and we have established the first humanized model for Group 3 MB from NES cells and demonstrated that TGFβ effectors drive tumor formation in vivo alone and in combination with MYC. However, the functional significance of TGFβ pathway being overexpressed in Group 3 MB and how it cooperates with MYC amplification remains poorly understood.

I hypothesized that the TGFβ pathway represents a targetable driving event in Group 3 and I have preliminarily characterized the transcriptional pathways in TGFβ driven Group 3 MB utilizing a panel of NES-derived cells transduced with MYC alone, TGFβR1 and TGFβ1 alone, and MYC in combination with these TGFβ pathway effectors. I have also observed that the introduction of MYC to NES cells expressing TGFβR1 and TGFβ1 leads to resistance to inhibitors targeting TGFβR1. To decipher the mechanism of resistance, we have identified a few candidate genes via our RNA-seq analysis, notably HES5, that could be new genes and pathways upregulated upon MYC and TGFβ pathway cooperation. I anticipate that my studies will provide insight on the mechanism(s) of how TGFβ promote tumor progression and new therapies to overcome resistance to improve patient outcomes.

"The Swifty Foundation has provided me with exceptional support to achieve our mutual goals of improving patient outcomes for this devastating disease."

Future Plans

In Year 2, our priority is to further decipher the mechanism(s) of drug resistance mediated by MYC and the TGFβ pathway through CRISPRi and/or RNAi knockdown of candidate targetable genes of interest in NES cells treated with TGFβR1 inhibitors. We will assess if these genes affect proliferation of the NES cells in the presence of TGFβR1 inhibitors and whether they can potentially reverse the growth advantage that MYC confers on these cells. We will also validate our in vitro drug resistance data by generating MYC and TGFβ-driven tumors in vivo and then treating the mice with TGFβR1 inhibitors to determine if there are any changes in tumor growth. In parallel, our other main focus will be to perform single-cell RNA seq on these tumors to further understand how MYC and TGFβ are contributing to tumor heterogeneity and resistance. Collectively, we hope to overcome the inherent resistance of Group 3 MB and potentially identify novel combination treatments.

Dr. Qadeer plans to present her findings at the AACR meeting in Boston, MA on Brain Cancer in October 2021. Dr. Qadeer’s final report summary will be available in March 2022.
Single cell analyses have shown insight in potential avenues of developing targeted therapies for cancers like medulloblastoma. It has previously been found that the haloacid dehalogenase (HAD) phosphatase Eya1 is highly expressed in SHH-medulloblastomas and single cell sequencing indicates that Eya1 can be detected in every individual cancer cell. In addition, reduced levels of Eya1 expression has decreased mortality rates in mouse SHH-medulloblastoma models. Previously, we have found that Eya1 is critically involved in normal development, promoting symmetric division of cerebellar granule cell precursors (GCPs), the cells of origin for SHH-subtype medulloblastoma.

Symmetric division of precursor cells has been implicated in the progression of pediatric cancers in general. Finding and verifying Eya1 substrates will give critical insight on the development of tumor biology and open the possibilities for other targets for therapy. In addition, our results show promise in the value of inhibiting the growth of SHH-medulloblastoma via targeting Eya1 phosphatase activity. Potent Eya1 inhibitors derived from benzarone may provide a new effective therapeutic approach to not only SHH-medulloblastoma but also other pediatric cancers.

Targeting Symmetric Division in Pediatric Cancers
Rosalind Segal, MD/PhD
Dana-Farber Cancer Institute
Innovation Grant

“The scientific progress to finding a novel therapy for pediatric brain cancers through Eya1 inhibition would not have been possible without the valuable support from the Swifty Foundation.”

Future Plans
To identify novel EYA1 substrates in a medulloblastoma based cell model, we will use our CRISPR KO EYA1 MB cells to carry out a phosphoproteomics screen coupled with mass spectrometry. We will validate identified phosphorylation shifts by (1) rescuing with WT EYA1 and (2) expressing a phosphatase dead EYA1 in our CRISPR KO MB cells. Further validation will be conducted using our in vitro phosphatase assay.

Among these compounds, we have narrowed down to the top optimal derivatives with the greatest efficiency in all preliminary tests. We are currently in the midst of directly assessing these compounds effect on the proliferation and cell death through flow cytometry. These compounds will be tested in patient-derived xenograft (PDX) medulloblastoma models that we will have available this year.

Dr. Segal’s final report summary will be available in April 2022.
Effective treatment of brain tumors is strongly limited by the presence of the blood-brain barrier, a feature of the brain vasculature that protects the brain against soluble insults, but also prevents access of most drugs to these tumors. In collaboration with Dr. Robert Mitchell (University of Louisville, KY), we have identified a blood-brain barrier permeable drug that makes glioblastoma tumors more sensitive to temozolomide chemotherapy. Current efforts are geared toward translating these findings into clinical benefit.

The ultimate goal of this study was to reduce the toxicity caused by radiotherapy in medulloblastoma patients. To examine the therapeutic effects of minocycline, we implemented an immuno-competent model of Group 3 medulloblastoma. We first performed a number of pilot experiments to evaluate the time to morbidity of the model in our hands. These Myc/DNp53 medulloblastoma cells do not grow in vitro, and are expanded by passaging as orthotopic implants in vivo. We observed a highly variable survival time, ranging from 20 to 40 days. We extracted the GFP-labeled tumor cells from the mouse cerebella under a fluorescence microscope. This approach shortened the survival time to a median of approximately 20 days. To optimize therapeutic dosing of minocycline to target TAMs, we used quantification of in vivo invasion. Because the behavior of TAMs is largely conserved over distinct cancers, we decided to use minocycline at the MTD.

To examine the radiosensitization potential of minocycline, we performed pilot studies and observed a dose-dependent increase in survival, with 2 Gy of radiation resulting in a 5-6 day increase in survival. Next, we examined whether minocycline can sensitize a single dose of radiation therapy. Surprisingly, combining radiation with minocycline did not result in any survival increase, and minocycline monotherapy also did not improve survival over control.

Key Findings

Interestingly, in preliminary studies, the direct inhibitory effects of 4-IPP on glioblastoma cell survival appears to be most potent for patient-derived glioblastoma cells that are cultured in stem cell conditions. This is a remarkable finding, because stem cells, including glioblastoma stem cells, are known to be largely resistant to chemo- and radio-therapy. Notably, stem cells, in glioblastoma as well as in most other cancers, are thought to be largely responsible for tumor propagation and therapeutic resistance. Based on these in vitro results, we expect that 4-IPP would show increased effectiveness in glioblastoma stem cell-based animal models as GL261 cultures contain very few stem cells. Such results would further underline the promise of 4-IPP in a clinical setting, as most patient’s tumors, upon diagnosis, contain a sizable fraction of stem cells.

A manuscript describing the results presented in this summary is currently in preparation.