ALEX'S LEMONADE STAND FOUNDATION

IMPACT REPORT

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ALSF Charity Partner

Targeting TGFb Pathway Dependencies in Group 3 Medulloblastoma

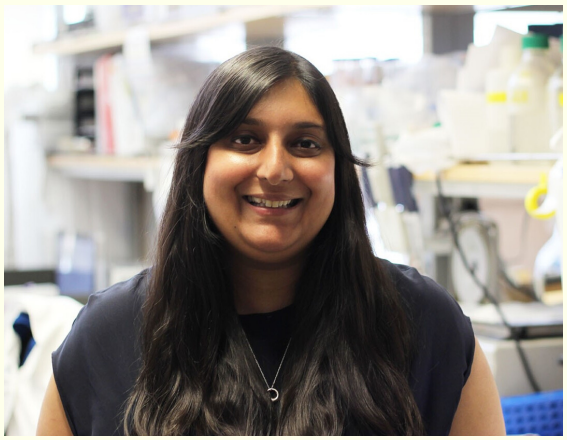
Zulekha Qadeer, PhD
University of California, San Francisco
2019 Young Investigator Grant

Updates on Dr. Qadeer's project will be available after receipt and review of her first progress report in Summer 2020.

Project Update

Recent work has identified alterations in distinct developmental pathways that regulate gene expression in several pediatric cancers, notably medulloblastoma. One such pathway, TGFβ, was recently found to be overexpressed in aggressive Group 3 medulloblastoma that has overall poor prognosis. The functional significance of this pathway and how it cooperates with other cancer driving events, such as MYC amplification, is currently unclear.

My studies have focused on using an novel humanized model for medulloblastoma utilizing human induced pluripotent stem cells (iPSC) differentiated to neuroepithelial stem cells (NES), the presumptive cell of origin for medulloblastoma. These cells have been genetically engineered to express MYC and/or TGFβ pathway effectors that were identified to be altered in Group 3 medulloblastoma patient samples. Excitingly, I have validated that the combination of MYC and TGFβ pathway effectors accelerated tumorigenesis when injected into the brain of immuno-compromised mice with some TGFβ pathway effectors causing tumor growth alone. Further, I have utilized these cells as a platform to test out clinically relevant TGFβR1 inhibitors and found reduced growth in TGFβ expressing cells that was ablated upon MYC overexpression. This supports that MYC may be promoting drug resistance in these cells. I plan to test out other TGFBR1 inhibitors that are in clinical



trials for other cancers to validate this phenotype. Further, I am currently analyzing gene expression data from NES lines driven by MYC and TGFB pathway effectors to identify new targetable genes/pathways that can overcome intrinsic resistance of Group 3 medulloblastoma.

"The Swifty Foundation has been essential to conduct these innovative studies and I am grateful for their investment and dedication to childhood cancer research. They have provided me with exceptional support to achieve our mutual goals of improving patient outcomes for this devastating disease."

Group 3 medulloblastoma remains a challenging disease to treat and this is due, in part, to the difficulty in modeling this disease. Using our unique humanized models, I hope to generate critical information about Group 3 medullo-blastoma that could have important therapeutic implications.

Targeting symmetric division in pediatric cancers

Rosalind Segal, MD/PhD
Dana-Farber Cancer Institute
2019 Innovation Grant

Project Update

A protein known as “Eya1” has a critical role in the development and progression of Sonic Hedgehog (SHH)-subtype medulloblastoma, a common brain tumor in children. We already know that Eya1 is an enzyme that removes phosphates from particular proteins. We are using proteomic approaches to identify the specific phosphoproteins that are altered by Eya1, and find out how these proteins work in tumor cells. We have initial compounds that target Eya1, and we are working to understand how they target Eya1 function, so that better drugs may be developed. This same protein may also play a role in the growth of other pediatric cancers. Therefore, we propose that targeting Eya1 may be a new way of treating pediatric brain cancers.

There is a drug that is known to target a related protein in the same family. We worked with that earlier drug and we have developed 60 new derivatives to find ones that work on Eya1. We have three different tests we are using to find the best potential drug among these compounds. Several of the compounds show better efficacy than current drugs in preventing the growth of medulloblastoma. After determining the important structural features of these compounds and narrowing down to the compounds that show the best results in all three preliminary tests, we will move forward to testing the top compounds in mice. Overall, our preliminary tests show promising results in providing new therapeutics for pediatric cancers.

"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 and Alex's Lemonade Stand Foundation. The support has allowed us to fully delve into the project without reserve to find a potential therapy for these devastating cancers."

Updates on Dr. Segal's project will be available after receipt and review of her first progress report in Fall 2020.

De-escalation of Radiotherapy for Medulloblastoma by a Novel DNA Damage Checkpoint Inhibitor

Marc Symons, PhD
The Feinstein Institute for Medical Research
2017 Innovation Grant

Dr. Symons was approved for a no-cost extension of his research grant, with a final report to be completed in Summer 2020.

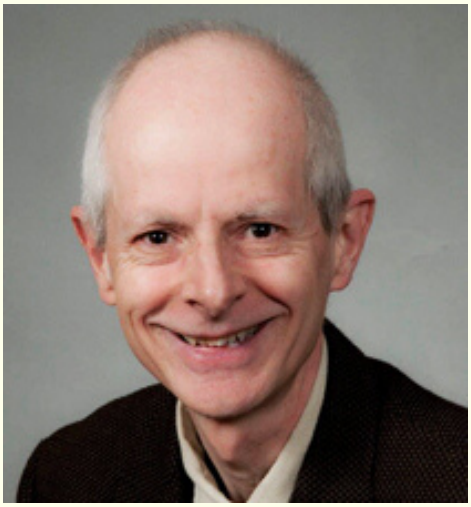
Project Update

The recent results that eliminating macrophages and microglia has an adverse outcome in a mouse model of SHH medullobastoma, strongly limits the therapeutic potential of targeting TAMs to radiosensitize these tumors to radiation therapy. Our findings using a group 3 medulloblastoma model underline this conclusion. Our results that targeting TAMs using a small molecule inhibitor of MIF strongly sensitizes glioblastoma tumors to chemo-therapy, indicate that targeting MIF is a novel therapeutic approach for the treatment of glioblastoma. Thus, further investigation of the role of MIF in the malignant behavior of glioblastoma is warranted.

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 from the University of Louisville in Kentucky, 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.

Future Plans

There are a number of mechanistic questions that remain to be addressed to complete our initial characterization of the therapeutic



effects of 4-IPP in the context of glioblastoma. One important question is whether 4-IPP directly reprograms TAMs in tumors. To examine this, we recently have established a panel of 15 PCR-based markers to determine the activation profile of TAMs in vivo. In addition, we also would like to understand the molecular basis for the observed toxicity of 4-IPP in combination with radiotherapy. To this end, noting that MIF has been implicated in the regulation of angiogenesis, we plan to analyze the tumor vasculature in the presence and absence of radiotherapy and 4-IPP. We should be able to accomplish both goals in the 4 months of the requested no-cost extension of the grant.

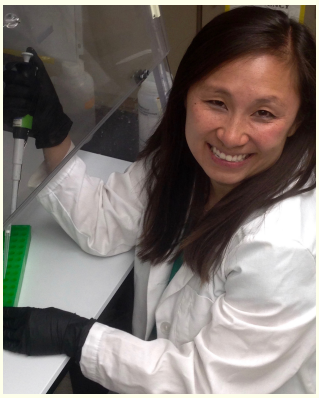
A manuscript on Dr. Symons' findings with regard to the preclinical results on 4-IPP in the context of glioblastoma will be prepared once they complete the characterization of the effects of 4-IPP on the activation profile of TAMs in vivo. These results will likely be published in an open access journal.

Targeting Eya2 to Inhibit c-Myc driven Medulloblastoma Tumor Progression

Melanie Vincent, PhD
University of Colorado, Denver
2016 Young Investigator Grant

Dr. Vincent left the University of Colorado to accept a position at Vigeo Therapeutics in Cambridge, Massachusetts where she is continuing to develop novel cancer therapies. Her former mentor, Dr. Heide Ford, intends to publish project findings once lab operations resume.

Final Project Update



With Group3 medullo-blastoma being the most malignant and lethal of all medullo-blastomas, there is an urgent need to identify novel potential targetable pathways. Our data suggest that Eya2, a developmental protein which is highly expressed in Group3 medullo-blastoma patients, regulates c-Myc at both the transcriptional level and regulates c-Myc stability at the post-transcriptional level.

From our results, we were able to conclude that loss of Eya2 leads to a decrease in c-Myc at the protein level and a subsequent decrease in c-Myc driven growth in Group3 medulloblastoma in both in vitro and in vivo models of two Group3 medullo-blastoma cell lines. These results suggest that Eya2 is a novel target for c-Myc driven Group3 medulloblastoma patients.

We were also able to conclde that loss of Eya2 caused an increase in PT58 levels, suggesting that Eya2 can also regulate c-Myc post-transcriptionally in the context of Group3 medulloblastoma. Taken together, we have shown that Eya2 contributes to Group3 medulloblastoma by transcriptionally regulating and stabilizing c-Myc, thus driving c-Myc driven growth in Group3 medulloblastoma in multiple cell line models.

Future Plans

We have shown a role for Eya2 in regulating c-Myc mRNA and protein levels and in enhancing the growth and progression of Group 3 medulloblastoma both in vitro and in vivo (for growth). We plan to publish a manuscript around this topic, in which we will dissect the individual roles of the different activities of Eya2 in Group3 medulloblastoma progression. Our lab will generate new Thr phosphatase dead mutants, to determine if the Tyr and/or Thr phosphatase activity of Eya2 is critical for stabilizing c-Myc. With this additional Eya2 Thr phosphatase dead cell line, our lab will repeat all of the experiments outlined above in the dox-inducible Eya2 overexpression Eya2 KO D425 cell line.

Dr. Vincent presented her findings at the Pediatric Hematology and Oncology Symposium in Aspen, Colorado as well as a Pharmacology Retreat in Keystone, Colorado.