STRONG CHILDREN'S RESEARCH CENTER

Summer 2014 Research Scholar

Name: Allison Eberhardt

School: University of Rochester

Mentor: Jason Mendler, M.D., Ph.D.

ABSTRACT

Title: Treatment of RUNX1-Mutated Acute Myeloid Leukemia with Focal Adhesion Kinase Inhibitors

Background: Acute myeloid leukemia (AML) is an aggressive form of blood cancer that arises from precursor cells of granulocytes and monocytes and results in their uncontrolled proliferation. AML is both molecularly and cytogenetically heterogeneous, and while a variety of mutations are associated with a good prognosis, others confer a much poorer prognosis. Runt-related transcription factor 1 (*RUNX1*)-mutated AML, with a prevalence of about 10-20%, is a negative prognostic indicator, with many patients failing to achieve complete remission. The *RUNX1* mutation is a loss-of-function mutation, making the locus difficult to effectively target. Upregulation of focal adhesion kinase (FAK) has been shown to be correlated with the *RUNX1* mutation. Drugs have been developed to specifically target FAK, and treatment of AML with FAK inhibitors might aid in the killing of cells that are normally unaffected by standard chemotherapy. Our hypothesis is that FAK may be a more realistic therapeutic target for AML patients, and if these FAK-inhibiting drugs are capable of preventing phosphorylation of FAK, then they could help make these cells more susceptible to chemotherapy and result in a better prognosis for patients with *RUNX1*-mutated AML.

Objective: The goal of my project was to identify a *RUNX1*-mutated and FAK-expressing primary AML sample to create a model for this type of leukemia. The cells were then treated *in vitro* with FAK inhibitors to assess whether or not the drugs were capable of specifically targeting phospho-FAK. The cells were also injected into mice to observe whether or not they were able to engraft.

Results: Sample 121813, a sample that highly expresses FAK and is *RUNX1*-mutated, is viable in culture and engrafts in mice in 4 weeks. Drug concentrations greater than 10 μM were toxic to both the FAK-expressing and non-expressing cell lines at 24 hours, as well as sample 121813. Lower concentrations (100 nM, 1 μM) of Compound 14 and PF-562271 had little effect on the ability of CG-SH and U937 cells to form colonies. Drug PF-562271 had a mild effect on FAK phosphorylation at 10 μM and 100 μM at 2 and 4 hours.

Conclusion: Primary sample 121813 provides a great model for a FAK-expressing, RUNX1-mutated AML. It is viable in cell culture and capable of engrafting in mice. Because 121813 expresses phospho-FAK so highly, it is a good sample on which to test the efficacy of FAK inhibitors both *in vitro* and *in vivo*.

Low doses of the FAK inhibitors Compound 14 and PF-562271 had little effect on the colonizing ability of both FAK expressing and non-expressing cells. Although doses higher than 10 mM were toxic to both FAK expressers and non-expressers in culture, we believe that the lower concentrations are capable of effectively inhibiting FAK; the process of lysing he cells has yet to be optimized.