St. Baldrick's FOUNDATION
Conquer Childhood Cancers

The Ty Louis Campbell Foundation St. Baldrick's Fellow Stewardship Report

St. Baldrick's Fellow: **Anya Levinson, MD**
Title: Clinical Instructor

Title of Research Project: *Intrinsic and acquired glucocorticoid resistance in T-cell ALL*

Grant Period: 7/1/20-4/15/21
Institution: University of California San Francisco Mentor: Dr. Kevin Shannon

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Please summarize your work in everyday language.

Leukemia is the most common form of childhood cancer. While most children with leukemia are curable, patients whose leukemia comes back after an initial response to therapy often die of their disease. I study a classes of medicines used to treat leukemia called "glucocorticoids." Though glucocorticoids are generally very good at killing leukemia cells, some patients have been found to be "resistant" to them, making their disease far more difficult to treat. I try to understand how and why resistance develops in certain patients in an effort to learn how to overcome such resistance and increase overall survival in T-ALL.

Please describe any advance in childhood cancer diagnosis or treatment furthered by your research under this St. Baldrick's Fellow Award.

I hope to identify patients who will not respond well to glucocorticoids, which are a standard component of therapy that confers many adverse side effects. These patients could either be spared high but ineffective doses of such drugs, or (ideally) benefit from treatment with additional medications that could overcome their steroid resistance.

Describe the value of this data to future research and why your research is something donors would be excited about.

This research has therapeutic implications for patients with leukemia, one of the most common childcare cancers. It is particularly important to prevent relapse in T-ALL (vs. B-ALL) as options for treatment of relapsed disease are incredibly limited.

Why was it important for the St. Baldrick's Foundation to fund your St. Baldrick's Fellow Award?

The SBF grant has been absolutely instrumental in my ability to continue this work since the completion of my fellowship in pediatric hematology oncology. It has fully supported my salary and enabled me to remain at UCSF as a young faculty member.

If you could thank our donors and volunteers for making St. Baldrick's funding possible, what would you say?

Thank you very much for your generosity and confidence in my research. I could not be continuing my work if it weren't for your support. I am humbled and tremendously grateful for the opportunity.

Mentor's Comments:

Dr. Anya Levinson is a young pediatric oncologist who is studying why some acute lymphoblastic leukemia (ALL) cells respond poorly to glucocorticoid drugs (also called steroids). Steroid treatment has played a key role in the remarkable advances in pediatric ALL therapy and resistance to these drugs is a major cause of relapse. Thus, Dr. Levinson's work may identify ways to improve ALL treatment by identifying children at high risk of relapse and suggesting better ways of treating them.

Despite substantial disruptions in our research activities due to the COVID pandemic, Anya has made exemplary progress on her SBF-funded project. Anya is a very careful and rigorous scientist who has rapidly mastered a number of complex techniques including CRISPR/Cas9-mediated gene editing and BH3 profiling and used them to advance the goals of her project. She has the potential to becoming a leading researcher in pediatric leukemia as her career progresses.

--Dr. Kevin Shannon

Part A: Report of Results:

1. **Specific Results:** Using no more than two pages, please describe the overall goals for the project for the current reporting period. List the Specific Aims (SA), using the titles from the original application. For each SA provide description of the results achieved within this reporting period. If no progress has been made on a SA state this and provide an explanation/justification.

Aim 1. To investigate Jdp2 overexpression as a mechanism of DEX resistance and to test strategies to overcome it.

Investigating *Kdm6a* as a Candidate Cooperating Mutation. Building upon my previous work showing that Jdp2 over-expression reduces the sensitivity of a CCRF-CEM (human T-ALL) cells to dexamethasone (DEX), I hypothesized that loss of *Kdm6a*, another epigenetic modifier, might cooperate with *Jdp2* over-expression to drive further steroid resistance. I successfully performed CRISPR-Cas9 editing to knock out *Kdm6a* in CCRF-CEM sub-clones S1 and S19 (**Figure 1a**) and generated independent sub-clones of each characterized by *Jdp2* over-expression. I then investigated the following four genotypes in each sub-clone: (1) empty vector (EV), (2) *Jdp2* over-expression only, (3) *Kdm6a* knock-out only or, (4) combined *Jdp2* over-expression and *Kdm6a* knock-out. I unexpectedly discovered that *Kdm6a* inactivation *increases* the sensitivity of CCRF-CEM cells to DEX as assessed by Hoechst live/dead staining (**Figure 1b, c**) or a cleaved-caspase 3 assay (data not shown). *Jdp2* over-expression rescued these cells from their augmented DEX sensitivity, driving resistance that overcomes the effect of *Kdm6a* loss. As shown (**Figure 1c**), in one CCRF-CEM sub-clone (S19), I independently generated two replicates for S19_EV_KDM6A and two replicates for S19_JDP2_KDM6A and results were consistent between replicates. I generated similar genotypes in a different CCRF-CEM subclone (S1, **Figure 1b**) and the results were generally consistent in both sub-clones. These findings support the hypothesis that *Kdm6a* inactivation in T-ALL 8633 predisposed this leukemia to somatic *Jdp2* retroviral integrations in response to *in vivo* DEX treatment, which resulted in Jdp2 over-expression and acquired resistance.

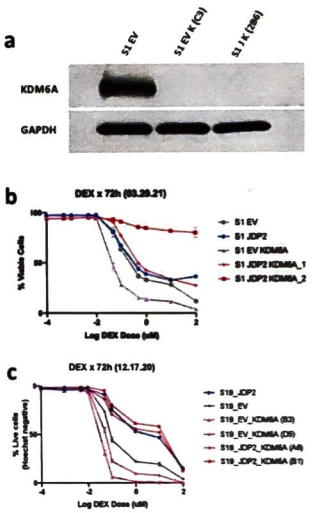

***Figure 1. A)** Western blot depicting Kdm6a protein levels in three related cell lines. **B)** DEX dose-response assay in the S1 genotypes. **C)** DEX dose-response assay in the S19 genotypes.*

Jdp2 Integrations and Expression in T-ALL 8633. This the last stage of this Aim, which I have recently begun to work on. So far, I have designed primers to amplify several of the integration sites near Jdp2 in the relapsed 8633 samples. Based on the functional data presented above, I hypothesize that the integrations present in relapsed (DEX-treated) leukemias will not be present in the parental leukemia, supporting the idea that they were induced independently under selective pressure from DEX treatment.

Effects of *Jdp2* Over-expression on Apoptotic Priming. In the S19 CCRF-CEM sub-clone, I found that loss of *Kdm6a* results in increased expression of glucocorticoid receptor (GR) target genes after treatment with DEX, including *Nr3c1* (encoding the GR) and *BIM* and *FKBP5,* which encode downstream effectors of GR (**Figure 2a-c**). Furthermore, *Jdp2* over-expression in the context of *Kdm6a* inactivation reduced the expression of these key GR target genes (**Figure 2a-c**). These data support the hypothesis that *Kdm6a* and *Jdp2* exert their effect on DEX sensitivity by modulating GR pathway activation in response to DEX.

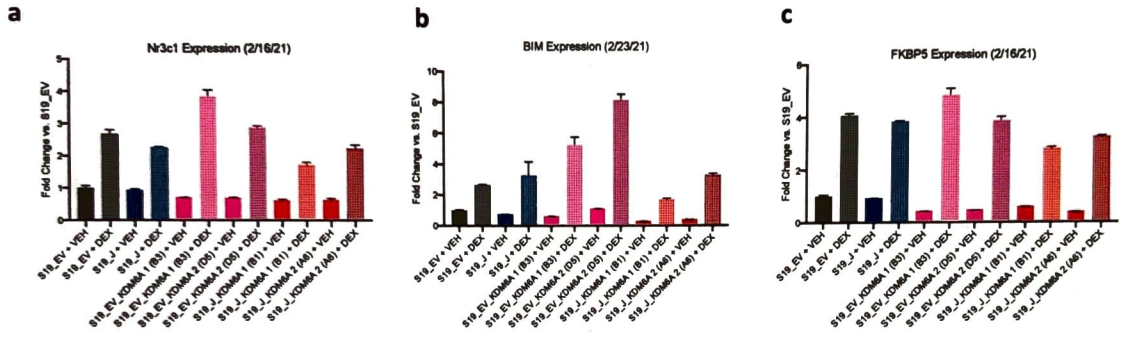

Figure 2. *Expression levels measured by qPCR of three glucocorticoid receptor-pathway components.* ***A)*** *Nr3c1.* ***B)*** *BIM (BCL2L11).* ***C)*** *FKBP5.*

Lastly, using a technique called BH3 profiling that measures apoptotic potential upon exposure to the pro-apoptotic protein BIM, I showed that T-ALL cells with loss of *Kdm6a* are more primed to undergo apoptosis in their basal state than those with intact *Kdm6a.* This apoptotic priming is reduced by *Jdp2* over-expression.

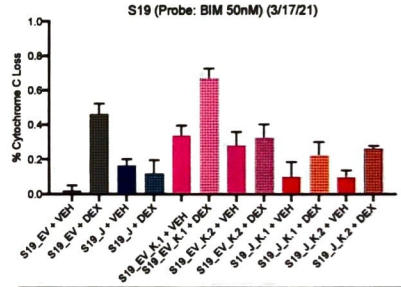

Figure 3. *BH3 profiling assay. Cells are treated with DEX or VEH, exposed to BIM, and then cytochrome C loss is measured.*

Aim 1 Summary.

I have confirmed that *Jdp2* confers modest glucocorticoid resistance to CCRF-CEM cells, and that effect is amplified in cells with baseline loss of *Kdm6a*, which I unexpectedly demonstrated renders them particularly sensitive to DEX. I have shown that over-expression of *Jdp2* can fully overcome the sensitization of cells to DEX induced by loss of *Kdm6a.* These findings support the hypothesis that murine leukemia 8633 likely acquired recurrent retroviral integrations near *Jdp2* to escape DEX sensitivity induced by its loss of *Kdm6a.*

Aim 2. To characterize the role of STAT5B as a driver of intrinsic DEX resistance and to test strategies to overcome it.

I am optimizing two assays in the BaF3 leukemia cell line: (1) DEX sensitivity assays, and 2) interleukin 3 (IL3)-independence assays. My studies to date indicate that BaF3 cells are sufficiently sensitive to DEX to serve as useful model to answer our research questions regarding whether the *STAT5A* and *STAT5B* mutations seen in human T-ALL cause glucocorticoid resistance and for investigating functional differences between these STAT5 isoforms.

2. **Future plans:** Please describe your plans, if any, for continuation of this research. If a SA has been achieved, please state the value of this data to future research.

   Aim 1. I will begin preparing a manuscript in the next few months. Outstanding items include repeating some of the functional assays in the S1 sub-clone, completing integration sequencing, delving deeper into the pronounced resistance observed in the S1_JDP2_K.2 sub-clone, and mining the COG AALL0434 database to determine whether *Kdm6a* mutations affect response to induction therapy and/or expression of Jdp2.

   Aim 2. Now that I have nearly completed assay optimization, I will mutagenize and clone several mutant STAT5B and STAT5A vectors into BaF3 cells and perform IL3-independence and DEX sensitivity assays. Depending on what I find, I will consider transitioning to a more representative cell line such as CCRF-CEM (which, as opposed to BaF3s, is a human T-ALL cell line) to further investigate the relationship between STAT5 mutations and glucocorticoid resistance. Should I discover that STAT5B mutants do confer glucocorticoid resistance, I will test ruxolitinib and venetoclax to see if these agents overcome the resistance (I would hypothesize that venetoclax would, and ruxolitininb would not, based on their locations in the JAK/STAT pathway).

3. **Data sharing: efforts and evidence of use.** Describe steps taken to share data generated from your research. Please clearly address each sub question below:

   a. Please include details as to the type of data and the repository for the deposit of your data.

   The Shannon Laboratory provides any reagent generated including vectors, strains of mice, and cryopreserved primary leukemia cells to any academic investigator at the time they are reported in a peer-reviewed research publication. The University of California may require a Materials Transfer Agreement in some circumstances. We provide these reagents with no restrictions, and never require that we be included as collaborating authors on published work. We make all primary sequencing and gene expression data publicly available in raw and normalized forms.

   b. Please include details about the availability of this data to other researchers.

   Our laboratory provides any reagent that we generate including vectors and strains of mice to any academic investigator at the time they are reported in a peer-reviewed research publication. We make all primary sequencing and gene expression data publicly available in raw and normalized forms. Gene expression data is deposited in the NCBI Gene Expression Omnibus (GEO). We are also open to providing unpublished reagents to collaborating investigators on a case-by-case basis. There is no time limit on this commitment to share all reagents and data with the scientific community.