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## **RUNX1 and FPD/AML Translational Research**

**The Leukemia and Lymphoma Society / Babich Family Foundation Partnership**

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### **Background**

Inherited predisposition to hematological malignancies associated with germ line mutations has been reported in select families (Tawana and Fitzgibbon, 2016). Mutations involve *RUNX1*, *GATA2*, *ANKRD26*, or *ETV6*, which have been associated with hematological disorders manifesting as cytopenias or platelet dysfunction that can lead to acute myeloid leukemia (AML). In the case of *RUNX1* (known as acute leukemia 1 protein [AML1] or core-binding factor alpha-2 [CBFA2]), one copy of the gene is mutated. It is believed that this can create a pre-leukemic state that typically manifests as a familial platelet disorder (FPD) leading to AML upon acquisition of a second mutation, sometimes in the second *RUNX1* allele. Approximately 50% of patients with familial *RUNX1* mutations will develop myelodysplastic syndrome (MDS) or AML in their lifetime (Liew and Owen, 2011). Although most pedigrees have a propensity to develop MDS/AML, which is of myeloid lineage, a small fraction of family members develop T-cell acute lymphoblastic leukemia (T-ALL), which is clearly relevant since *RUNX1* also plays a role in T-ALL (Della Gatta et al., 2012).

Currently, it is poorly understood how *RUNX1* can mediate platelet defects or lead to AML. However, it is established that *RUNX1* encodes a subunit of a heterodimeric transcription factor that controls the expression of genes essential for hematopoiesis. Therefore, *RUNX1* mutations impair the protein, reduce the expression of *RUNX1*-targeted genes and ultimately impair differentiation to normal blood cells. When *RUNX1* is mutated in the presence of other mutations, progenitor cells do not differentiate and non-functional, leukemic cells can increase. Within the limited number of families with FPD/AML that have been examined, it has been found that mutations in *RUNX1* manifest as missense, nonsense, frameshifts, duplications, or deletion mutations scattered across the coding region for the protein (Nickels et al., 2013).

Although only approximately forty pedigrees have been reported in the United States in the scientific literature, it is important to note that *RUNX1* mutations are not limited to patients with FPD/AML. *RUNX1* mutations are found in approximately 15% of all AML patients (Papaemmanuil et al., 2016; Metzeler et

al., 2016). Within subtypes of AML, approximately 10% and 30% of patients with de novo and secondary AML, respectively, have RUNX1 mutations (Lindsley et al., 2015). Mutations in RUNX1 are also found in patients with MDS (Papaemmanuil et al., 2013). In patients with de novo or secondary AML, the RUNX1 mutation is typically found in patients who have mutations in other AML-promoting genes such as MLL (which may promote RUNX1 protein degradation; Zhao et al., 2014; MLL can also act as a co-factor of RUNX1; Huang et al., 2011) and IDH (which may promote gene repression through DNA methylation; Kernytsky et al., 2015). Both MLL and IDH mutations are associated with poor prognosis and response to chemotherapy (Gaidzik et al., 2011; Gaidzik et al., 2016). Overall, it has been suggested that RUNX1 may play a role in more than 50% of all AML patients, since the protein is mutated and / or works in conjunction with other transcriptional factors that mediate pre-leukemic states and AML (Huang et al., 2008; Ito et al. 2015; Lam et al., 2011; CGARN, 2013; Will et al., 2015). This suggests that unravelling the role of RUNX1 in leukemogenesis is likely to benefit patients with AML in general.

### **Key Areas of Investigation**

There are many important features of RUNX1 biology that need to be studied in order to improve treatment for pre- and post-leukemic FPD/AML patients. Research strategies to be funded include:

- A. Translational Research that includes, or can lead to, clinical trials
  - Discover and develop novel therapeutics that re-activate RUNX1 or downstream pathways or provide synthetic lethality via collateral pathways to restore impaired (mutated) RUNX1 function
  - Develop gene editing or gene therapy methods to correct RUNX1 mutations, thereby allowing allogeneic transplantations
  - Develop new prognostic assays to determine if or when to treat with such new therapeutics
- B. Laboratory research that supports Translational Research
  - Develop a deeper understanding of the interaction of RUNX1 with other transcription factor regulators or other relevant biology related to disease progression
  - Develop experimental systems either in vitro or in vivo that mimic FPD (or other RUNX1-mediated pre-leukemic conditions) that lead to AML.

### **LLS Proposed Plan**

The overarching goal for The Babich Family Foundation and LLS is to build a team of researchers focused on RUNX1 biology with the hope that novel diagnostics and therapeutic agents can be developed. The Babich Family Foundation has created the RUNX1 Research Program ([www.runx1.com](http://www.runx1.com)) in order to fund research into RUNX1 biology and to help educate patients with FPD/AML. A research program focused on FPD/AML has already been initiated with The Babich Family Foundation and Alex's Lemonade Stand Foundation (ALSF) in April 2016. This work will develop knowledge that will provide a better understanding of the disease through retrospective or prospective analysis of FPD/AML family members and identify ways to block the transition of a preleukemic state to leukemia in patients with familial RUNX1

disorders. The partnership between The Babich Family Foundation and LLS is intended to further enhance this effort and focus on translational research that can bring laboratory findings to the clinic.

The understanding of RUNX1 biology, and in fact most transcription factors, is at an early stage. Moreover, therapeutic manipulation of transcription factors, which typically involve protein-protein or protein-DNA interaction are typically difficult drug targets (in contrast to kinases, which are enzymes). The development of new model systems in the laboratory will be required to achieve an understanding of how the germ line RUNX1 mutation progresses to AML and to test prospective therapeutics. Therefore, LLS and The Babich Family Foundation will build a broad-based team of researchers to address the major issues stated above. The team will likely include members with the following expertise: leukemia (with special interest in FPD and/or AML), transcription factor structure and function, animal models of blood cancer, transplantation, genetics, drug development and computational biology. Two key features of the plan are the sharing of data among researchers in the field and an annual conference to present and discuss research results.

### **Implementation of the Plan**

The proposed strategy to fund this work will be by the Translational Research Program (TRP) by LLS. The TRP program provides \$600,000 to each grantee over a 3 year period. It is designed to fund laboratory research that could translate to experimental clinical programs. The plan calls for funding up to three TRPs related to RUNX1 biology.

In September 2016, LLS re-opened the Translational Research Program (TRP). A Request for Proposal (RFP) outlines the intent of the RUNX1-related TRP program (see <https://www.lls.org/research/translational-research-program>). The announcement of the RFP for this specific program has occurred simultaneously with a broad RFP request for the entire LLS TRP program (covering leukemia, myeloma, and lymphoma). Applications for the RUNX1 program will be permitted after the letter of intent is reviewed to ensure they meet the intent of the RFP. This is followed by full application. Evaluation of the full application for the RUNX1-related grants will be done by primary and secondary reviewers selected by LLS and The Babich Family Foundation. Scoring of the grant applications (using the NIH system) will be done by the entire TRP leukemia review panel, although further consideration of RUNX1-related TRP applications will be done separately from the general leukemia TRP grant applications. Decisions for funding will be made in June 2017 and the grants activated October 1, 2017.

LLS and The Babich Family Foundation believe that it is highly likely that TRP grant applications of exceptional quality will be identified during this process since two applications related to RUNX1 biology were identified in the last round of TRP applications in 2015 to LLS (Lucy Godley, University of Chicago in conjunction with Nancy Speck of U Pennsylvania and Paul Gadue/Deborah French/Mortimer Poncz of CHOP and an undisclosed investigator). During this same time period, one of the canonical downstream targets of RUNX1, the transcription factor PU.1, has been shown to be critically involved in the formation of pre-leukemic stem cells and their progression to AML (Will et al., 2015). There is also a report on a small molecule inhibitor of the transcription factor fusion, core factor –beta –smooth muscle myosin, which can reactivate RUNX1 (Illendula et al., 2015). Beyond this, the role of cohesion mutations in the faulty regulation of RUNX1 and other transcription factors leading to AML has received considerable attention in the published literature (Mazumdar et al., 2015) as well as in emerging work examining the utility of epigenetic regulators controlling cohesion-mediated progression of hematopoietic stem cell to MDS and

AML. It is also important to note that, in general, the TRP grant program is highly competitive with approximately 10% of all grants applications being funded in 2016.

In sum, the goal of this program is to activate up to three TRPs on October 1, 2017. Awardees will be brought together to discuss their findings, along with other current LLS grantees and members of the ALSF / The Babich Family Foundation partnership, as well as experts in RUNX1-related biology to review progress on an annual basis. The total cost for the program is up to \$1.8 M for grants, which is shared equally between LLS and The Babich Family Foundation. LLS will administer the grant program.

## **Conclusion**

This grant program aims to fund cutting-edge research with the potential to transform therapeutic options for pre- and post-leukemic FPD/AML patients as well as to contribute to current science's limited understanding of the role RUNX1 plays in leukemogenesis. LLS and The Babich Family Foundation believe an opportunity exists to apply other technological advances in the study of cancers and inherited diseases to the study of AML, where there has been a very limited advancement in therapies over the last several decades. We believe this opportunity will have a direct and significant clinical impact for patients, and we encourage applications to this endpoint.

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