I Care I Cure Proposal - Bushweller

Project Title: Small Molecule Inhibitors of ERG for Pediatric Leukemia and Sarcoma

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Institution: University of Virginia, School of Medicine

Type of Grant: Innovation

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Type of Cancer: Leukemia\AML

Scientific Abstract

ERG is transcription factor with a functional role in normal hematopoietic stem cell function whose expression is altered in a number of pediatric cancers. ERG is over-expressed in and is a definitive driver of T-ALL (1). ERG over-expression is an unfavorable prognostic marker for survival of pediatric AML patients (2). ERG is the target of a chromosomal translocation with EWS in Ewing's sarcoma (3). All of this identifies ERG as a useful potential therapeutic target for pediatric cancer. Transcription factors such as ERG have traditionally been considered "undruggable" due to the challenges of targeting either the protein-DNA or protein-protein interactions on which their activity depends. However, many transcription factors are auto-inhibited, i.e. other regions of the protein fold back on the DNA binding domain to inhibit its activity. Such auto-inhibition presents a unique opportunity to inhibit these proteins, namely small molecule stabilization of auto-inhibition will decrease DNA binding and therefore transcription factor activity. As these auto-inhibitory modules differ among members of families of transcription factors, there is also the potential to achieve extraordinary selectivity of action within a family. We have identified fragments which are selective and act via modulation of auto-inhibition of ERG. Based on an initial fragment with an IC_{50} of >2 mM, we have developed inhibitors with IC_{50} values of 81 and 187 μM. We are proposing to further optimize this class of compounds to develop a potent and selective inhibitor of ERG for testing in appropriate models of pediatric cancer.

Impact Statement

Current treatment for childhood AML, T-ALL, and Ewing's sarcoma is limited in efficacy and has profound long- term side-effects due to the use of traditional cytotoxic agents rather than targeted drugs inhibiting specific drivers of the diseases. A targeted agent which inhibits ERG, clearly a driver of these diseases, has the potential to improve both survival and quality of life for children with AML, T-ALL, and Ewing's sarcoma. It is likely that such an ERG inhibitor can be used in combination with existing approaches to enhance their efficacy and reduce the dose or duration of exposure to cytotoxic agents, thereby improving outcome as well as quality of life.

Specific Aims

Specific Aim 1: Development of a potent selective small molecule inhibitor of ERG- DNA binding. The central hypothesis of this grant is that by identifying and optimizing compounds which bind and stabilize the auto- inhibitory domain of ERG, we can achieve specific, potent inhibition of its DNA binding activity. The DNA binding activity of many transcription factors is regulated by auto- inhibition, namely regions of the protein outside the DNA binding domain fold back onto the DNA binding domain to decrease its affinity for DNA (4). Such auto-inhibition has been observed for numerous transcription factors including p53, HSF, C/EBPb, and RUNX1 as well as numerous members of the Ets family (5) including ERG (6). In the case of the Ets family, the structural basis for auto-inhibition appears to differ among family members as no homology is seen for the auto- inhibitory regions in the proteins. Unlike the DNA binding interface on transcription factors such as ERG, the auto-inhibitory modules present "normal" binding surfaces with appropriate electrostatics and curvature for drug-like small molecules to bind. The Ets family of transcription factors has clear relevance to a variety of cancers and its members are regulated by auto- inhibition. We have chosen to focus on ERG, based on its clear relevance to pediatric cancer, for testing this hypothesis. It is important to note that the regions mediating auto-inhibition in these proteins differ among family members, so unlike targeting the highly conserved Ets domain which mediates DNA binding in the Ets family, targeting the auto-inhibitory modules likely provides a route to achieve a very high degree of selectivity among family members. As pan inhibition of Ets family members is likely to be toxic, such exquisite selectivity is an important component of this proposed approach. This is a potentially powerful approach to the allosteric regulation of ERG as well as transcription factors in general.

We have screened a fragment library to identify compounds which act via the auto-inhibitory domain of ERG, resulting in the identification of several hits. We have made analogs with increased potency. We are proposing to optimize these to improve the potency as well as selectivity for ERG with the goal of developing a potent and selective inhibitor of ERG which can be used for in vivo studies.

Specific Aim 2: Demonstrate effects of an ERG inhibitor on leukemia and Ewing's sarcoma cell lines as well as patient samples. The second hypothesis of this grant is that small molecule inhibition of ERG will be of utility in the treatment of pediatric cancers AML, T-ALL, and Ewing's sarcoma. To establish the utility of the inhibitors we develop, we will explore their effects on AML and Ewing's sarcoma cell lines to demonstrate effects on proliferation, apoptosis, gene expression, and ERG occupancy on target genes. Subsequently, we will test effects on patient derived AML and T-ALL cells.