James L. LaBelle MD, PhD

Cancer Research Foundation Young Investigator Award -FINAL REPORT

Title*:* Defining the Mechanism and Therapeutic Targets of BIM BH3-Mediated Cell Death in Diffuse Large B-cell Lymphoma

Major Goals of the Project

The BCL-2 family of proteins comprises an essential network of proteins that govern the cell's decision to live or die. BIM, a pro-apoptotic BH3-only protein of the BCL-2 family is a master regulator of B cell homeostasis and its functional suppression is believed to be a key pathogenic factor in B cell lymphoma. The goal of this award is to investigate and modulate this critical, deregulated component of the apoptotic pathway in B cells and apply the mechanistic insights to advance a novel therapeutic strategy for reactivating cell death in treatment-refractory diffuse large B cell lymphoma (DLBCL).

DLBCL is the most common lymphoid malignancy, comprising over 40% of all lymphomas. Although treatment options for lymphoma have expanded in recent years, cases of DLBCL are refractory to current therapies in greater than 50% of patients. Thus, identifying new targeted therapies for this disease continues to be an urgent therapeutic goal. We hypothesize that the potency of BIM BH3 in triggering cell death reflects its capacity to engage a diversity of key protein targets and death pathways, and that pharmacologic replacement of BIM's "death domain" using a novel hydrocarbon-stapled peptide (BIM SAHB) will restore cell death for therapeutic benefit in DLBCL. To accomplish the aims of this project, we apply a multidisciplinary approach to (1) test the capacity of a hydrocarbon-stapled BIM BH3 helix to reactivate the death program in DLBCL driven by distinct mechanisms of apoptotic blockade, and (2) identify the explicit protein targets of BIM SAHB to link cellular activity to *in situ* mechanism of action. By intertwining chemistry, lymphoma biology, and developmental therapeutics, we aim to generate fresh mechanistic insight into the pro-apoptotic potency of the BH3-only protein BIM and determine how this unique BH3 death domain can be harnessed to reactivate cell death in diverse B cell lymphomas driven by distinct and clinically relevant chemoresistance mechanisms.

Results

Aim 1: We have extensively tested, and expanded upon a large panel of human DLBCL cell lines against BIM SAHB in addition to the leading small molecule BCL-2 therapeutics ABT-737, ABT-199, and Obatoclax. Unlike the ABT compounds, BIM SAHB induces cell death in all cell lines tested. Specificity of action has been confirmed using a point mutant control, BIM SAHB R153D, which shows no effects. Strikingly, BIM SAHB induces death in DLBCL expressing MCL-1, a BCL-2 anti-apoptotic protein that is resistant to ABT-737/199. While determining BIM SAHB's capacity to kill, we have uncovered two, non-mutually exclusive, mechanisms of BIM SAHB-medicated cell death in various DLBCL, suggesting a novel function for the BIM BH3 helix and a new network of proteins to therapeutically target. To determine if these effects are truly synergistic or simply additive, we plan on setting up combinatorial viability studies followed by CalcuSyn software analysis. Light microscopic images and confocal microscopy real-time movies have supported these results.

Aim 2: We continue to analyze the proteins responsible for controlling the molecular rheostat that directs BIM SAHB treatment towards cell death in susceptible DLBCL cell lines (Fig. 1). We have completed a comprehensive semi-quantitative western blot analysis of BCL-2 family members in the DLBCL lines and experiments are underway to explicitly characterize the BCL-2 protein targets of ABT-737 versus Obatoclax versus BIM SAHB through immunoprecipitation of treated cellular lysates.

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We have validated that the stapled and unstapled BIM BH3 peptides bind to BCL-2 anti-apoptotic family members in DLBCL lysates, a necessary validation as we move forward. We have now also successfully generated BIM expression vectors that will be used in tandem affinity purification (TAP)-TAG analysis. We are now concentrating on lentiviral expression variants of BIM that all include the BH3 death helix and have been shown to rapidly induce cell death in a Bcl-2 family-dependent manner. We are now expressing these constructs in select DLBCL cell lines to identify native BIM protein binding partners in these cells and compare them to the BIM SAHB protein targets. We plan on validating these targets in biochemical assays such as RNA interference (RNAi) and chemical blockade studies. We hope that these studies will be particularly enlightening as native BIM is thought to be the major inducer of ABT-737-mediated apoptosis in susceptible DLBCL.

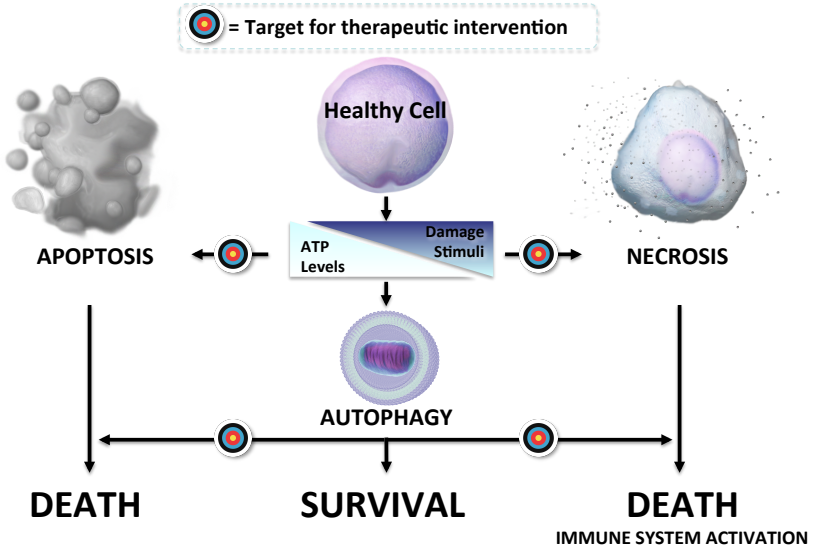

Fig 1: Death by BIM SAHB/BIM BH3 appears to toggle between apoptosis and necrosis in select DLBCL.

Research Development Planned Beyond CRF Funding

The validation of our BIM SAHB results using peptide amphiphiles has added further support towards our initial observations detailed above. Armed with this exciting new data, made possible by CRF funding, we plan on dovetailing these two approaches into a NIH grant submission by the end of the year. We remain very optimistic that we will uncover the mechanism behind the fundamental phenotypic observations we have measured using our lentiviral pull-down approach.

Publications (2014-2015)

1. Reynolds, C., Roderick, J., **LaBelle, J.L.,** Bird, G., Mathieu, R., Bodaar, K., Colon, D., Pyati, U., Stevenson, K., Qi, J., Harris, M., Silverman, L., Sallan, S., Bradner, J.E., Neuberg, D.S., Look, A.T., Walensky, L., Kelliher, M.A., Gutierrez, A. 2014. Repression of BIM mediates survival signaling by MYC and AKT in high-risk T-cell acute lymphoblastic leukemia. Leukemia, 28:1819-1827. PMID: 24552990. Reynolds, et. al. 2014
2. Darlington, W.S., Pinto, N., Hecktman, H., Cunningham, J.M., Cohn, S.L., **LaBelle, J.L.** 2015. Stem Cell Transplant-Associated Wernicke Encephalopathy in a 5-year-old with Stage 4, High-Risk Neuroblastoma. *Accepted.* Pediatric Blood and Cancer