# I Care I Cure Proposal – Faber

**Project Title: Pharmacogenomics and Drug Screening Lead to a Novel Targeted Therapy with Potent and Specific Activity Against Mouse Models of MYCN-amplified Neuroblastoma**

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**Institution: Virginia Commonwealth University**

**Type of Grant: Innovation**

**Year Awarded: 2015**

**Type of Cancer: Neuroblastoma**

## Scientific Abstract

Neuroblastoma is the second leading cause of cancer-related death in the pediatric population. This is attributable to high-risk neuroblastoma, of which amplification of the oncogenic transcription factor MYCN is a hallmark. We have a significant interest and history of developing rational targeted therapies to bring into clinical trials, particularly in neuroblastoma. To this end, using a combination of pharmacogenomics, drug screening and gene expression data mining, we have developed a novel targeted therapy combination that is specific for *MYCN*-amplified neuroblastoma. Parsing data from a large-scale drug screen of roughly 500 cancer cell lines, we found *MYCN*-amplified neuroblastomas were exquisitely sensitive to the in-clinic BCL-2/BCL-XL inhibitor ABT-263. In addition, we found *MYCN*-amplified neuroblastomas express high levels of the pro- apoptotic PUMA through MYCN-directed upregulation, which helps explain the observed sensitivity to ABT-263. We also found *MYCN*-amplified neuroblastomas had markedly low BCL-XL levels, resulting in preserved sensitivity to the select and less toxic BCL-2 inhibitor, ABT-199. Using a small molecule “anchor" drug screen to find sensitizers to ABT-199 in *MYCN*-amplified neuroblastomas, we uncovered the Aurora A inhibitor MLN8237 as a drug that combines with ABT-199 to induce dramatic apoptosis, specifically in *MYCN*- amplified neuroblastoma. Strikingly, this combination was specifically synergistic in human xenograft mouse models of *MYCN*-amplified neuroblastoma to induce regressions and even cures. In a unique model of a patient- derived xenograft (PDX) of *MYCN*-amplified neuroblastoma established in our laboratory, the combination shrank all tumors tested. Importantly, this combination has demonstrated no signs of toxicity in mice and is effective at the optimal dosing schedule of MLN8237 as established from early phase trials at The Children’s Hospital of Philadelphia (CHOP). Therefore, we propose to further define this unique sensitivity of the combination and the role of amplified *MYCN* in sensitivity, the precise molecular mechanism(s) of combination efficacy, and further expand our PDX studies to bolster the preclinical evidence of efficacy and safety to quickly bring this novel therapy into the clinic at CHOP.

## Impact Statement

Less than half of children with high-risk neuroblastoma survive despite recent therapeutic advances. Amongst the victims of this terrible disease was Alex Scott. The goal of this grant is to further develop a combination targeted therapy to treat high-risk neuroblastoma. We have leveraged data from a high-throughput drug screen in combination with analyses of neuroblastoma expression data to uncover a novel and potent therapy for high- risk, *MYCN*-amplified neuroblastoma. This therapy combines a new in-trial BCL-2 inhibitor (ABT-199) with the in-trial Aurora A inhibitor MLN8237. This therapy has potent activity to kill *MYCN*-amplified neuroblastoma cells with no overt toxicity in traditional human xenograft models of *MYCN*-amplified neuroblastoma and in unique patient-derived xenografts (PDXs) of *MYCN*-amplified neuroblastoma that we have implemented. Importantly, use of MLN8237 in neuroblastoma has been developed by Dr. Mossé and is in clinical testing through the NANT (New Approaches to Neuroblastoma Therapy) Consortium (1). ***Therefore, while the short-term goal of this proposal is to provide the preclinical rational for this combination therapy in diverse models of MYCN-amplified neuroblastoma including unique PDXs, and to demonstrate the underlying mechanism(s) of ABT-199/MLN8237 sensitivity, the long-term goal is to bring this combination therapy into clinical trials in patients with MYCN-amplified neuroblastoma at The Children's Hospital of Philadelphia.***

## Specific Aims

Less than half of children with high-risk neuroblastoma survive. Targeted therapies are now being explored in neuroblastoma, with ALK inhibitors being developed for a subset of patients with ALK-driven tumors. However, MYCN pathway inhibitors have proved difficult to develop. Thus, strategies aimed at blocking MYCN must use principles such as synthetic lethality and other alternative approaches to develop rational approaches. To this point, based on data from a large drug screen in conjunction with gene expression analysis in multiple neuroblastoma data sets, we hypothesize that *MYCN*-amplified neuroblastomas are specifically sensitive to the combination of the BCL-2 inhibitor ABT-199 and the Aurora Kinase A inhibitor, MLN8237.

**Specific Aim 1. Define the sensitivity of ABT-199 and MLN8237 in neuroblastoma.** Our preliminary data demonstrates combining ABT-199 and MLN8237 in *MYCN*-amplified neuroblastoma is highly effective and specific compared to *MYCN* wild-type neuroblastoma. We will further characterize this differential sensitivity.

**Specific Aim 2. Characterize the mechanism of sensitivity to dual inhibition with ABT-199 and MLN8237 in MYCN-amplified neuroblastoma.** Our preliminary data demonstrates *MYCN*-amplified neuroblastoma are sensitive to ABT-263 through MYCN-induced NOXA, and retains sensitivity to ABT-199 through high BCL2/BCL-xL ratios. Furthermore, *MYCN*-amplified neuroblastomas demonstrate potent sensitivity to the combination of ABT-199 and MLN8237 through synergistic apoptosis. In this Aim, we will explore the underpinnings to the enhanced sensitivity of the combination by concentrating on the convergence on BCL-2 family members that govern apoptosis.

**Specific Aim 3. Determine the efficacy of combined inhibition with ABT-199 and MLN8237 *in vivo* using new neuroblastoma-specific patient-derived xenograft models**. Bringing novel pediatric therapies to the clinic requires rigorous preclinical evaluation. These experiments will provide the preclinical justification required to move this combination to the clinic.