Targeting DIPG through Combining a Super-activator (MCB-613) of Steroid Receptor Co-activators with Radiation: an in vivo Study in Patient-derived Intra-brain Stem Xenograft Models

Section 2: Executive Summary

The objective of this application is to demonstrate that combining MCB-613, a small molecule stimulator of the oncogenic steroid receptor co-activator (SRC), with ionizing radiation would synergistically kill tumor cells of diffuse intrinsic pontine glioma (DIPG) *in vivo* and significantly prolong survival times in patient tumor-derived orthotopic (intra-brain stem) xenograft (**PDOX**) mouse models. This proposal is a follow-up study from a preclinical analysis of MCB-613 on DIPGs that was generously funded by the ***Cure Starts Now Foundation*** and ***DIPG Collective*** (2015-2017). Our goal is to establish strong preclinical rational to support a rapid initiation of MCB-613 clinical trials in children with DIPG.

DIPG is the most lethal childhood cancer, and virtually all children with this disease die within 1-2 years of diagnosis. In our previous study that was generously funded by the ***Cure Starts Now Foundation*** and the ***DIPG Collective***, we have shown that MCB-613 can overcome some major challenges for the development of new therapies for DIPG using our panel of intra-brain stem PDOX models of DIPG, including ***1)*** strong anti-proliferative activities *in vitro* in traditional monolayer cells as well as in neurospheres (enriched with the putative cancer stem cells), ***2)*** low toxicity to normal cells in NOD/SCID mice that were treated for 4 weeks; ***3)*** capability of passing through the blood brain barrier (**BBB**) to reach xenograft tumor cells *in vivo* in mouse brain stems; ***4)*** significant prolongation of animal survival times (the gold standard of therapeutic efficacy) in a DIPG PDOX model acting as single agent; and ***5)*** synergistic killing of DIPG xenograft cells and significant prolongation of animal survival times when combined with fractionated radiation. There were, however, some missing data that are critically needed to move MCB-613 into clinical trials. When administered as single agent, MCB-613 was active only in 1/4 DIPG models and the synergistic cell killing was only demonstrated in one DIPG model. Fortunately, it was also during the previous funding period that we have identified the "under treatment" (i.e., short and insufficient drug exposure time of 14 days) as one of the causes of tumor progression.

Our central **hypothesis** for this proposal is that ***1)*** Increase the length of MCB-613 treatment time and in combination with clinically relevant fractionated ionizing radiation will synergistically kill DIPG cells *in vivo* in intra-brain stem DIPG xenograft models and significantly prolong animal survival times compared to mice treated with MCB-613 and with radiation alone; and ***2)*** analyzing multiple DIPG xenografts that exhibited differential responses would facilitate the discovery of new diagnostic marker(s) for patient selection. To test our hypothesis, we will utilize our established PDOX models of DIPG to accomplish the following **Specific Aims**: ***1)*** Demonstrate that the combined treatment of intra-brain stem DIPG xenografts with extended MCB-613 treatment (> 4 weeks) and fractionated radiation (2 Gy/day x 5 days) will synergistic improve therapeutic efficacy and significantly prolonged animal survival times. ***2)*** Understand mechanisms of the synergistic cell killing induced by the combination of MCB-613 and XRT in DIPG cells. 3) Identify the cellular and molecular cause of therapy resistance toward the combined MCB-613 and XRT treatment.

This proposal is innovative on several levels. Firstly, our panel of intra-brain stem DIPG PDOX mouse models is derived from terminal-stage DIPG patients; consequently, they represent therapy-resistant disease, which is in desperate need of new therapies. These models provide us with unprecedented opportunities to study tumor biology and test new therapies *in vivo* in a microenvironment closest to human DIPGs. Secondly, our research proposal is the evaluating the therapeutic efficacy of MCB-613, a novel compound with a novel mechanism of action. This drug crosses the BBB and accumulates in brain tissue, thus allowing MCB-613 to research the tumor site. Indeed, MCB-613 has already shown promising anti-tumor activities against DIPG cells both *in vitro* and *in vivo*. Most importantly, since our treatment approach combines MCB-613 with radiotherapy, the standard treatment for DIPGs, our chances of rapid translation of the drug into clinical trials are greatly improved.