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USING ZEBRAFISH TO ACCELERATE DIPG DRUG DEVELOPMENT

Translational
DIPG, Childhood (Brain Cancer)
Affiliation: ISPNO Cure Fund
Requested Amount: $50,000

LAY SUMMARY

A critical barrier to improvements in diffuse intrinsic pontine glioma (DIPG) therapy is a lack of new drugs that have a broad therapeutic index. In DIPG, validation of new therapeutics is hampered by the long time (6 months) that most human DIPG tumors take to kill mice. This means that it can take 9-12 months to complete an efficacy study of a single candidate drug. To address this bottleneck, in collaboration with the laboratory of Dr. Jeffrey Mumm, who is an expert in zebrafish development, we have established human DIPG orthotopic xenografts in zebrafish. Because these fish are tiny, yet have central nervous systems analogous to those of humans, and are translucent, we can monitor the fluorescently-tagged human cells in real time as they grow and migrate in the developing fish. We have already established that our human DIPG cells to live and invade through the fish brain for up to five days, and we propose to further validate these studies, focusing on injecting zebrafish embryos with human cells to facilitate the use of these fish for rapid drug screening. Successful completion of this project will allow us to bypass one of the major impediments to progress in DIPG therapy.

EXECUTIVE SUMMARY

One of the major barriers to progress in DIPG is the long latency time that DIPG tumors take to kill mice. In vitro screening can identify many promising therapies (such as panobinostat), but if these drugs do not penetrate the brain or are highly toxic, they are destined to fail in clinical trials. The long duration of time most DIPG orthotopic xenografts take to kill mice means that survival studies of a single agent can take 9 to 12 months to complete. Combination studies of potential active drugs becomes virtually impossible to accomplish. Luciferase or MRI imaging can in part identify a response. However, there are agents such as bevacizumab which can improve the MRI appearance in human patients without affecting overall survival. Luciferase imaging of highly infiltrative DIPG tumors may additionally have false positive results. Many tumor cells join the rostral migratory stream and end up in the rodent olfactory bulb. However these cells are not contributing to the major cause of death in these animals. To address this major roadblock in DIPG drug development, we propose to use zebrafish bearing human DIPG xenografts as an intermediate step to determine in vivo efficacy and toxicity.

Developing zebrafish (Danio rerio) larvae are translucent and tiny. At just a few days post fertilization (dpf) they are free swimming and have fully formed central nervous systems, including a pons. They do not develop T-cells until approximately 10-14 dpf and so do not reject human xenografts until that time. We have demonstrated that fluorescently transduced human DIPG cells that we inject into the 4 dpf zebrafish brain persist and invade into the surrounding zebrafish brain. Furthermore, the Mumm lab has developed transgenic zebrafish in which the microglia/macrophages are fluorescently tagged. This allows us to observe in real time, in a living vertebrate, how DIPG cells interact with microglia/macrophages. The role of these innate immune cells in promoting tumor growth is an area of increasing investigation. In figure 1, we show live animal confocal imaging of fluorescently-tagged DIPG cells in a living zebrafish brain 2 days after and 5 days after injection. In figure 2 we show preliminary time-lapse living animal confocal experiments in which fluorescently tagged zebrafish microglia/macrophages (red) are migrating between and appearing to interact with DIPG cells (green). Our strong preliminary data demonstrates the feasibility of our approach.

We further propose to pioneer earlier developmental injection of fluorescently tagged DIPG cells into 1000 cell stage zebrafish blastocysts. A recent manuscript described the injection of human GBM cells into this stage zebrafish embryo and found that human GBM cells migrated along with zebrafish neural precursors and populated the developing brain over the course of the next several days. Injecting into blastocysts is advantageous because it allows for potential mechanization of the procedure, which would further facilitate the development of this model for high-throughput screening of novel drugs. The human GBM tumors grew and invaded further into the developing zebrafish brain, forcing the animals to be sacrificed approximately six days after injection.

The scientific merit of the proposal rests on the need to develop better screening systems to accelerate DIPG drug development and our ability to study the interaction of the zebrafish innate immune system with transplanted human DIPG cells. In addition to initial characterization of the ability of human DIPG cells to recruit microglia/macrophages, we also propose to determine if deletion of these native immune cells decreases the ability of DIPG cells to grow and invade in the zebrafish brain. The Mumm lab has transgenic zebrafish in which it is possible to selectively ablate macrophages/microglia using metronidazole. We will first inject human cells, and after 24 hours we will ablate the microglia/macrophages to determine if loss of these cells reduces the ability of the DIPG cells to grow in the zebrafish brain.

Since zebrafish larvae are approximately the size of a comma in this sentence, thousands of larvae could be seeded in 96 well plates and used for drug testing. Drugs are added to the fish water, and would need to demonstrate the ability to penetrate into the brain of the fish. Plate readers could read the fluorescence of the human DIPG cells in the zebrafish brain before and after drug treatment. Toxic effects of drugs on a rapidly developing vertebrate organism analogous to a growing human child could be identified by monitoring treated fish for survival and teratogenicity.

Our proposal will leverage the DIPG expertise of the Raabe lab with the Mumm lab's expertise in zebrafish biology to create a model for rapid in vivo testing of novel DIPG therapeutics. Our project is feasible, as demonstrated by our preliminary data and the work of others undertaking analogous xenografts of adult GBM. At the end of the granting period, we anticipate submitting a manuscript describing the ability of our six human DIPG cell lines (2 pre-treatment biopsy-derived, 4 autopsy derived, 1 ACRV1 mutant, all H3F3A mutant) to survive and migrate after injection into zebrafish blastocysts. We will also report on the ability of our DIPG cells to recruit zebrafish microglia/macrophages and will determine the effect of ablation of microglia/macrophages on DIPG tumor cell growth. The CSN has the opportunity to jumpstart a project that could pay enormous dividends for DIPG research. Instead of waiting 6 months to learn if a drug kills DIPG cells in vivo, we can know in 6 days. If our pilot experiments demonstrate an important interaction between DIPG cells and the immune system, our system can be critical for rapid evaluation of drugs that alter this interaction.

DESCRIPTION OF RESEARCH PROPOSAL

Hypothesis: We hypothesize that we will be able to grow human DIPG cells in developing zebrafish and that this system will be useful for studying the role of the innate immune system in promoting DIPG tumor growth.

We will test this hypothesis in two **Specific Aims:**