PROGRESS UPDATE SPRING 2022

Recent Advances in DIPG Research and Care

The Dana-Farber Campaign *Defy Cancer*

Dana-Farber Cancer Institute | The Jimmy Fund

Katherine Warren, MD, Clinical Director, Pediatric Neuro-Oncology

Mariella Filbin, MD, PhD, Research Co-Director, Pediatric Neuro-Oncology Program

Introduction

At Dana-Farber Cancer Institute, specialists in pediatric neuro-oncology are committed to finding better treatments for diffuse intrinsic pontine glioma (DIPG). This aggressive, high-grade brain tumor affects up to 300 children a year in the United States, and it is almost universally fatal. Under the leadership of **Katherine Warren, MD,** and **Mariella Filbin, MD, PhD**, a team of physician-scientists are partnering across disciplines and institutional walls to develop urgently needed therapies for patients with this disease. Their efforts have yielded several key insights, and we are grateful for your partnership in advancing our understanding of DIPG.

Illuminating a Complex Biology

INTERNATIONAL SEQUENCING EFFORT YIELDS NEW INSIGHTS

Filbin is partnering with an international team to assemble an unprecedented repository of rare high-grade glioma samples, including DIPG. This effort will enable them to better understand the genetic underpinnings of these tumors, potentially opening unexplored avenues to treatment.

The team has collected tissue samples of DIPGs and a range of other high-grade gliomas. To date, they have sequenced 50 of these samples using a spectrum of technological platforms, from single-cell RNA sequencing, in which scientists resolve tissue into individual cells and sequence each cell's whole RNA to determine which genes are active, to whole exome sequencing, a broader strategy that conducts a wide sweep for cancer genes in tumors.

Pons: Located in the brainstem, the pons control many of the body's most vital functions such as breathing, blood pressure, and heart rate.

Filbin and her colleagues are comparing the molecular and behavioral features of different high-grade gliomas to DIPG, as well as samples from various age groups, from infants to adults. Filbin and her team also explored DIPG samples from a variety of locations within the brain and spinal cord, instead of just the pons (see sidebar). "We used to look at the pons only, but now we are looking at tumors in the thalamus with the same mutations as found in DIPG, and also further down the spinal cord," Filbin explained.

In addition, Filbin and her team used "spatial transcriptomics," a method that sequences genes on an intact tumor tissue slide, to see which normal cells were close to which tumor cells. This data can be leveraged to learn how tumor cells hijack normal brain structures for their own purpose, and how these interactions can be blocked. Through their efforts, the researchers have amassed an unprecedented dataset they can use to help identify the subtle

Pratiti (Mimi) Bandopadhayay, MBBS, PhD

Rameen Beroukhim, MD, PhD

Keith Ligon, MD, PhD, Director, Center for Patient Derived Models

Organoids: tumor cells from surgical specimens and biopsies grown in the lab as 3D structures, which recapitulate the tumor's native environment more faithfully than two-dimensional cell cultures. This key development has significantly advanced the study of cancer biology and the development of patient-specific drug screening methods.

Patient-derived xenografts (PDXs): surgical grafts of human tumor tissues into mice. Once established, PDXs faithfully recapitulate the genetic complexity of human cancers and offer an elegant platform in which to test new therapeutic agents.

similarities and differences between each malignancy. Insights from this work could shed light on treatment strategies for other pediatric brain tumors, as well as clarify the underlying drivers of DIPG's more unique characteristics.

While data analysis is ongoing, early findings suggest that high-grade gliomas that harbor mutations in H3K27M, which is the case in 80% of DIPGs, have different molecular features depending on the patient's age, suggesting that these malignancies evolve over time. This also indicates that teenagers and adults with H2K27M-mutated high-grade gliomas may require a different treatment approach than children with the same condition.

WHOLE-GENOME SEQUENCING REVEALS NEW DRIVERS IN DIPGS

Pratiti (Mimi) Bandopadhayay, MBBS, PhD, **Rameen Beroukhim, MD, PhD**, and **Keith Ligon, MD, PhD**, analyzed whole-genome sequencing data from approximately 200 pediatric high-grade gliomas, including more than 100 DIPGs. The group applied new methods to study structural variants, which occur when two parts of the genome not commonly next to each other come together. They discovered more about the mutations that help form DIPGs, including histone mutations and other cooperating genes, and the team is now studying these changes, which may help lead to new therapeutic approaches.

While mutations commonly affect genes that cause tumors to grow, other parts of the genome that do *not* code for proteins may also help lead to DIPG. To uncover these drivers, Bandopadhayay and her lab have been working to identify non-coding RNAs that cooperate with histone mutations to help form DIPGs. Their hope is that these studies will help them further understand the mechanisms through which histone mutations induce DIPG.

USING MODELS TO DECODE DIPGS

By studying cells and tissues taken from patients in laboratory models, researchers are able to better understand the underlying biology of cancer and predict how patients with specific diseases might respond to treatment. Under the direction of Ligon, the Center for Patient Derived Models creates patient-derived cell lines, organoids, xenografts (PDX models, see sidebars), and other types of models for all forms of cancer and metastatic tumors.

Warren is partnering with Ligon to leverage this remarkable repository of research platforms to learn more about DIPG. Ligon and his team are creating a range of cell lines and mouse models of this rare disease, and then characterizing the genetic subtypes for future study. Ultimately, these models will serve as a powerful resource for investigators seeking to better

Kee Kiat (Aaron) Yeo, MD

understand the genetic makeup of this complex disease and to screen promising new therapies prior to clinical trials.

The program is already accelerating novel research. For example, pediatric neuro-oncologist **Kee Kiat (Aaron) Yeo, MD**, is partnering with Ligon to study how the genetics and behavior of IDH-mutant gliomas, DIPGs among them, in adolescent and young adult patients might differ from those found in pediatric patients. By leveraging years of clinical and genomic data from patients across age groups, the investigators are making research inroads that could reveal more about how best to treat each population.

Bringing Laboratory Insights to Patients

DIPG ALL-IN INITATIVE REIMAGINES DIPG CLINICAL TRIALS

Warren is leading a nationwide research consortium meant to optimize clinical trials for DIPG. The DIPG ALL-In Initiative, centered at Dana-Farber, supports preclinical studies that will inform patient trials by increasing cross-institutional collaboration between scientists working on similar or interrelated research, as well as translating preclinical findings into clinical trials. The consortium consists of six hospitals across the United States, led by investigators with particular expertise and research in DIPG.

Pharmacokinetics: the study of how the body responds to a drug, including its absorption, distribution, and metabolism.

Pharmacodynamics: the study of how the drug responds to a living organism, such as mouse models. This helps to determine proper dosing and potential side effects.

As part of this initiative, Warren and her team are performing preclinical studies of the chemotherapy azacytidine combined with panobinostat, a drug that inhibits the activity of an enzyme called HDAC, which is overexpressed in cancer cells. They are evaluating the combination's activity in mouse models, including its pharmacokinetic and pharmacodynamic (see sidebar) effects, in order to optimize clinical trial design.

PPM1D: A NEW THERAPEUTIC STRATEGY COMES TO LIGHT

In the February 2022 *Nature Communications,* Bandopadhayay, Beroukhim, and Ligon published the first in-depth analysis of PPM1D, a key mutational driver of DIPG and other high-grade gliomas. PPM1D is known to play a role in DNA damage repair (see sidebar), but few studies have interrogated how this gene can go awry to propagate DIPG.

DNA repair genes: genes that give rise to proteins responsible for identifying and repairing errors that develop in the DNA, often during DNA replication. When DNA repair genes are not functioning properly, mutations can accumulate in the genome and ultimately lead to some forms of cancer.

Using mouse models, the investigators demonstrated that PPM1D plays a foundational role in glioma development and intensifies its progression. Furthermore, they found that PPM1D mutations occur in the p53 pathway, which can be targeted with MDM2 inhibitors.

In the lab, Bandopadhayay, Beroukhim, and Ligon are studying the utility of MDM2 inhibition in PDX models of PPM1D-driven DIPG and other high-grade gliomas. These efforts will help them ascertain the best dosing strategy as well as the resistance mechanisms that arise. "Eventually tumors learn to grow through these interventions," Beroukhim explained, "so we're exploring how to stop that." Insights from this work could set the stage for early phase clinical trials.

In addition, the investigators are partnering with the Broad Institute of MIT and Harvard to more deeply interrogate the function of PPM1D. Because the gene modifies a range of other proteins in a given cell, researchers hope to illuminate which modifications are crucial for basic human functioning and which drive tumor development. To answer this question, Bandopadhayay, Beroukhim, and their colleagues are leveraging advanced technology to edit the genome to create a single point mutation—an approach that more accurately reflects the after-effects of a mutation and helps investigators more easily identify the mutational networks driving cancer.

This important first step in drug discovery enables our researchers to better pinpoint which proteins could safely be inhibited so that cancerous activities are halted, while preserving those processes that are crucial for survival.

Joelle Straehla, MD

USING NANOPARTICLES TO DELIVER DRUGS TO THE BRAIN

The blood-brain barrier surrounds the brain and prevents harmful toxins and bacteria in the bloodstream from entering this vital organ. What evolved as a lifesaving defense, however, also blocks many drugs from reaching the brain. **Joelle Straehla, MD,** is working with Warren to develop a drug delivery system that strikes a crucial balance while infiltrating the blood-brain barrier, selectively targeting tumor cells while sparing the healthy ones. The answer lays in nanoparticles, which can be designed to carry drugs or drug combinations directly to the cell type of interest—a compelling prospect for hard-to-reach targets such as brain tumor cells.

Focusing primarily on the most aggressive forms of pediatric brain cancer—high-grade gliomas, like DIPG, and pediatric medulloblastoma—Straehla tested a broad range of nanoparticles in cell lines and microfluidic models of the blood-brain barrier. She identified several that had powerful interactions with the tumor cells, including a nanoparticle formulation of panobinostat, which was particularly effective against DIPG in preclinical studies. Straehla will begin testing this formulation in mouse models in spring 2022.

Jun Qi, PhD

In partnership with Bandopadhayay, Straehla is also using CRISPR technology (see sidebar) to more deeply investigate a nanoparticle that demonstrated strong uptake in pediatric high-grade glioma cell lines. Her work will help answer fundamental questions about how nanoparticles enter tumor cells, and serves as a crucial first step in determining whether this drug carrier is a potential candidate for clinical trial.

EPIGENETIC RESEARCH OPENS NEW AVENUES TO TREATMENT

Advances in genomic medicine have brought enormous benefits to adults with historically intractable cancers, but because childhood cancers have fewer genetic alterations readily amenable to drug targeting, precision treatments have been less beneficial for young patients. Thanks to recent scientific and technological advances, however, researchers have begun to understand another factor that plays a large role in childhood malignancy: epigenetics. This field of study refers to changes in the way genetic material is read and processed, rather than changes to the DNA itself. Understanding these mechanisms and how they can be manipulated for therapeutic benefit is the key to the next generation of treatments for our youngest patients.

CRISPR is a state-of-the-art genome editing tool that enables scientists to modify the genetic makeup of living cells with astonishing speed and efficiency, facilitating experiments that would not have been feasible using previous techniques.

To explore this burgeoning field of study, Filbin partnered with **Jun Qi, PhD**, to design novel epigenetic therapies that target the unique drivers of DIPG. Filbin and Qi used CRISPR technology to systematically delete a library of potential epigenetic targets from DIPG cell lines to ascertain whether they are necessary for tumor survival. This effort yielded a potential target in a particular chromatin complex (see sidebars) that, when deleted, forced DIPG cells to differentiate (see sidebar), ultimately killing them off.

Chromatin complex: a family of proteins that carry out modifications in chromatin structure. These regulate the turning on and off of genes for cell- and tissue-specific purposes. Malfunctions of the chromatin remodeling system have been implicated in many cancers and neurodevelopmental disorders.

Importantly, Qi developed an inhibitor and a degrader—a therapy that destroys rather than disables its target—against this chromatin complex, and both interventions showed promise in cell lines and in mouse models. They then tested these drugs in combination with standard DIPG therapies to even stronger effect. Filbin and Qi are now working to adapt these drugs to cross the blood-brain barrier. If successful, they aim to partner with Warren to test the drug in a clinical trial.

Differentiation: the process through which cells mature and become more specialized. For example, blood stem cells divide to form more blood stem cells, or differentiate into one of three types of blood cells: white blood cells, which fight infection; red blood cells, which carry oxygen; and platelets, which help the blood to clot.

The partnership between Filbin and Qi continues to bear fruit, as they have identified 14 other potential chromatin-related targets, which they are currently validating. Qi and his team aim to develop drugs against the ones that show promise in cell lines and mouse models.

Susan Chi, MD, Deputy Director, Pediatric Neuro-Oncology

In the Clinic

NTRK INHIBITOR PUT TO THE TEST IN PILOT TRIAL

NTRK fusions are rare genetic mutations found in many different types of cancer, including a small subset of DIPGs, which results in the production of an overactive, cancer-driving protein. In 2018, the Food and Drug Administration approved the NTRK inhibitor larotrectinib for adults and children with solid tumors driven by the fusion. In preclinical trials, the drug has shown similar promise in pediatric NTRK-driven high-grade gliomas.

On the strength of these initial studies, **Susan Chi, MD**, launched a phase I clinical trial testing larotrectinib in pediatric patients with newly diagnosed high-grade gliomas like DIPG. The study aims to enroll a total of 15 patients, including a smaller surgical cohort who will receive the treatment prior to tumor resection.

Alongside the trial, the resected tumors are studied in the lab to help elucidate how the therapy alters DIPG's molecular profile. Using this information, investigators hope to identify biomarkers of response that could help clinicians identify the best candidates to receive larotrectinib. Their efforts could also reveal previously unknown mechanisms of resistance, which could serve as potential therapeutic targets to be inhibited in combination with larotrectinib to prolong its effect.

LARGE-SCALE DRUG SCREEN RESULTS IN PROMISING TRIAL

Drug screens work by exposing cancer cells to hundreds or thousands of compounds in the lab. The compounds that prove best at killing tumor cells are then earmarked for further study. Filbin has identified several promising treatment avenues by performing such drug screens on DIPG cell lines.

As one example, Filbin tested drugs from the National Cancer Institute's compound library, a repository of approximately 3,000 agents thought to have anti-cancer effects. Filbin also performed a drug screen using CRISPR technology to test the effect of epigenetic therapies in DIPG cell lines. Combining these approaches led Filbin to identify several novel targets in DIPG, one of which is already being tested in patients with DIPG and has shown promising results in two patients on this clinical trial.

Through her work, Filbin aims to understand the function of these novel targets in DIPG, which could point the way to biomarkers that predict patients' responses. Filbin and her team are also exploring how these novel targets might be combined to boost their efficacy and circumvent drug resistance, which is much more common in single-drug approaches. Filbin aims to bring the best combinations forward into clinical trials for patients with DIPG, starting at Dana-Farber, and then expanding to national and international clinical trials.

The Impact of Your Philanthropy

DIPG is an intractable disease that urgently requires novel treatment strategies. To expedite the translation of promising therapies to patient bedsides, our expert investigators have launched ambitious research initiatives that illuminate the biology of this difficult malignancy. Your longstanding support has been critical to shepherding their research from their earliest pilot studies to clinical trials. On behalf of our patients and their families, thank you for helping to make this important work possible.

Report written by Caroline de Lacvivier.

Dana-Farber Cancer Institute has been the top-ranked cancer hospital in New England by *U.S. News & World Report* for 21 consecutive years, and is the only cancer center in the country ranked in the top 4 for both adult and pediatric cancer programs.

Dana-Farber Cancer Institute was named the #3 cancer center in the world by *Newsweek* in its World's Best Specialized Hospitals ranking.

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