The Dana-Farber Campaign *Defy Cancer*

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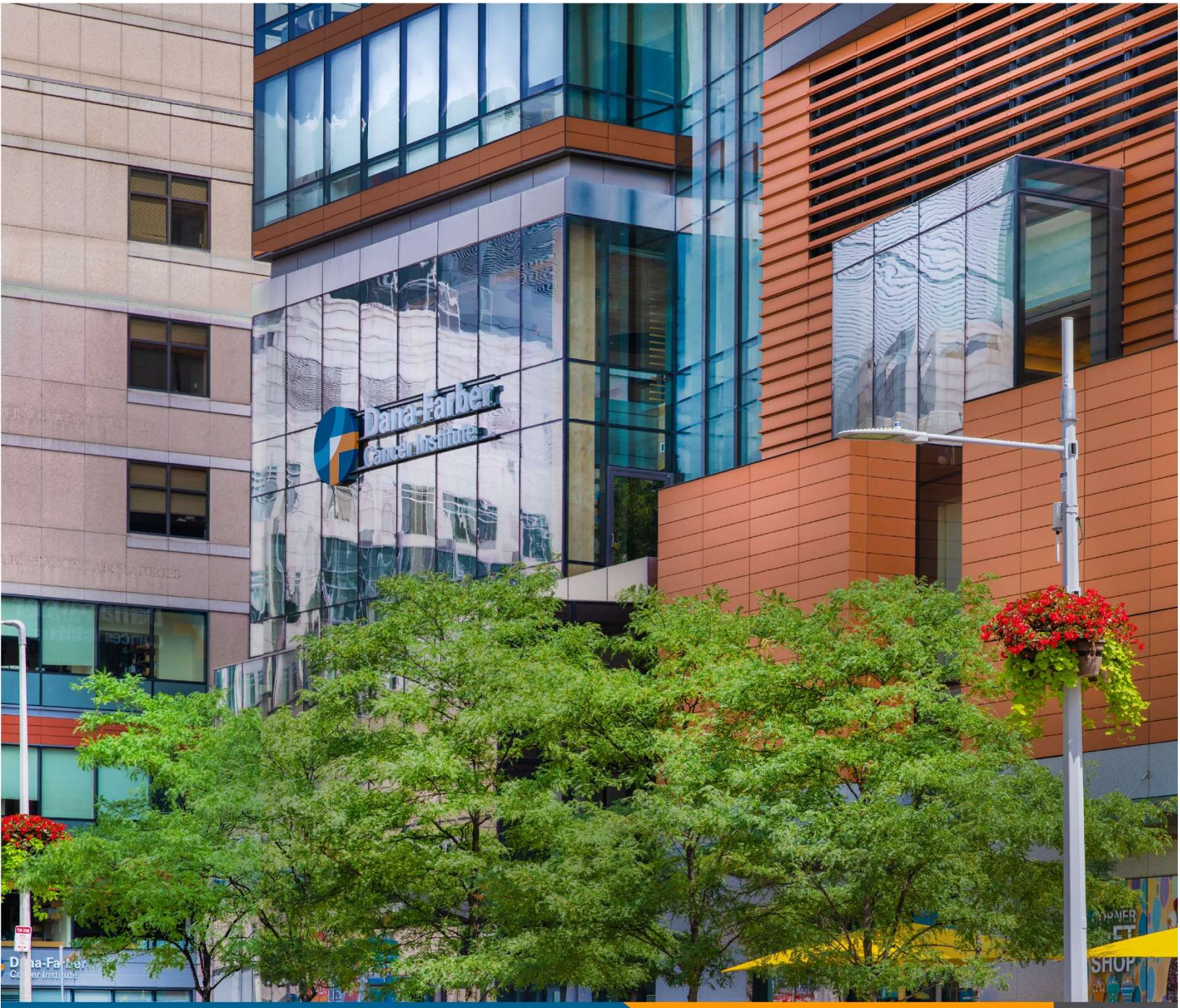


2023 PROGRESS UPDATE

Recent Advances in Diffuse Intrinsic Pontine Glioma Research and Care

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

Introduction

Diffuse intrinsic pontine glioma (DIPG) is one of the most difficult to treat childhood tumors, and our world-class team at Dana-Farber Cancer Institute is driving efforts to develop better therapies as rapidly as possible. Thanks to your support, our team has made remarkable contributions to the field this year, including a major publication that illuminates the structural variants that give rise to DIPG. On behalf of our patients and their families, thank you for your generous partnership in this important work.

H3K27M mutations occur in 80% of DIPGs.

RNA sequencing is used to learn the exact order of the building blocks that make up all RNA molecules in a cell. RNA sequencing studies are used uncover which genes are expressed (turned on) in different types of cells and how these genes are expressed.

Spatial transcriptomics maps tumors in intact tissue, offering a more nuanced rendering of different cell types and their spatial relationships.

Differentiation is 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.

Lab Studies Decode a Complex Biology

Laboratory research is the first step in drug discovery, as it offers a low-risk setting to study tumor biology and test novel therapies. Our multidisciplinary team of basic research scientists and clinicians are collecting tissue samples and building sophisticated research models to better understand DIPGs' underlying molecular makeup. Ultimately, through their studies, they aim to identify unique vulnerabilities that can be targeted with drugs.

SEQUENCING STUDY SHEDS NEW LIGHT ON DIPGS

Historically, DIPGs have presented significant barriers to genetic study. Not only are they located deep in the brain, where they are hard to remove, they are also rare, making tissue samples scarce. On their own, no single institution can accumulate enough tumor specimens to make statistically significant research findings, and so **Mariella Filbin, MD, PhD**, has formed partnerships with other cancer centers in Austria, England, and Germany to build a shared large bank of rare high-grade gliomas.

Leveraging this tumor bank, the team analyzed 50 high-grade glioma samples with **H3K27M mutations** (see sidebar) from patients aged 2 – 68. As published in the December 2022 *Nature Genetics*, the team used single-cell **RNA sequencing** as well as a novel sequencing method called **spatial transcriptomics** (see sidebar), to reveal the cellular landscape of DIPG and other high-glioma samples in unprecedented detail.

Comparing data from adult and pediatric tumors, the researchers found that gliomas from older patients harbored more mesenchymal-like cells, a group of partially **differentiated** (see sidebar) stromal cells that could mature into different cell types in the body, such as bone, cartilage, muscle, fat, and other supportive tissues. This type of cell can be targeted with existing small

Pratiti (Mimi) Bandopadhayay, MBBS, PhD

molecule inhibitors, presenting a potential treatment avenue for adults with high-grade gliomas.

Meanwhile, the pediatric gliomas harbored stem cells called oligodendroglial precursor cells (OPC), a subtype of glial cell that had not yet undergone differentiation. Tumors that had spread beyond the brain stem had even more OPC-like cells, suggesting that these cells could serve as a marker of more aggressive disease. Filbin is now exploring if therapies that induce differentiation—forcing the OPC cells out of their stem-like state and, by extension, halting tumor growth—might be effective against this subtype.

The team also discovered that one of the dominant cell types present across all tumors were astrocyte-like cells, a type of glial cell that supports and protects neurons. This finding, which was only visible through spatial transcriptomics, demonstrates the importance of using many different sequencing methods to fully capture the complex cellular makeup of high-grade gliomas. Ultimately, this study lays groundwork for the design of future research models and therapeutic development.

FOXR2: SCIENTISTS IDENTIFY A KEY GENE

Physician-scientists at Dana-Farber and the Broad Institute of MIT and Harvard have discovered that a gene called FOXR2, which is normally turned off in most tissues in the body, is activated in at least 70% of cancer types and 8% of all individual tumors. The study, led by **Pratiti (Mimi) Bandopadhayay, MBBS, PhD,** and published in the September 2022 *Cancer Research*, may help scientists better understand how a wide variety of cancers develop.

As Bandopadhayay's team analyzed the genome sequences of a type of pediatric brain cancer called diffuse midline gliomas, which also encompasses DIPGs, they found that this cancer showed abnormal expression of FOXR2. This gene, located on the X chromosome, encodes a transcription factor normally expressed only in the testis, so this was a surprising discovery.

To look for traces of the gene in other cancers, the team combed through cancer databases, analyzed human cell cultures, and sequenced tumors from animal models of cancer. They found that some of the most common pediatric and adult cancers—including osteosarcoma, melanoma, and non-small cell lung cancer—also showed FOXR2 expression, as did diffuse midline glioma tumors taken from the **Dana-Farber Tumor Bank** (see

The **Dana-Farber Tumor Bank** is a repository of leftover human tissue that has been removed during a medical procedure such as an operation, a biopsy, or a blood test. This extra tissue is not needed for patient diagnosis or treatment. Patients can opt in to donate the tissue to the Tumor Bank, where scientists use it to study disease and find better ways to diagnose, prevent, and treat cancer.

Rameen Beroukhim, MD, PhD

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

sidebar). The team also confirmed that many types of childhood tumors rely on FOXR2 to grow.

By studying mice with and without activated FOXR2, the team discovered that the gene boosts the growth of brain tumors, including diffuse midline gliomas. They also found that most cancer cells activate the gene through a mechanism involving a chemical modification that removes methyl groups from a gene. Further experiments revealed that a group of transcription factors called ETS were overexpressed when FOXR2 was active, suggesting that these two classes of transcription factors work in concert to drive tumor formation.

Bandopadhayay is continuing to investigate the relationship between ETS and FOXR2 to better understand how the gene is activated. The team is also working with chemists and structural biologists to explore how to target this gene as a possible new cancer treatment. “The fact that this gene is normally switched off in most tissues means that we might be able to target it in a way that doesn’t cause a lot of side effects,” said Bandopadhayay.

FIRST IN-DEPTH SURVEY OF GENOMIC MIX-UPS IN PEDIATRIC HIGH-GRADE GLIOMAS

Bandopadhayay led a collaboration of scientists in the United States, Canada, and Europe to provide the first large-scale accounting of structural variants—the most extensive type of genomic alterations—in pediatric high-grade gliomas, including DIPG. The findings, published in the August 2022 *Nature Cancer,* will be critical to understanding how the disease develops and whether some treatments work better in certain patients. Ultimately, researchers hope the information can point them to vulnerabilities in the disease’s genetic circuitry that can be exploited by new therapies.

Bandopadhayay worked with **Rameen Beroukhim, MD, PhD, Frank Dubois, MD**, and **Keith Ligon, MD, PhD**, to perform whole genome sequencing in tumor tissue from 179 children with high-grade gliomas. The sequencing enabled Dubois to search the cells for structural variants—and he discovered that pediatric high-grade gliomas can be split into two types based on their pattern of structural variants. “One is marked by the presence of a large number of complex structural variants, with a lot of genetic code in the wrong location,” Dubois explained. “The other has just a few structural variants, one or two pieces of code out of place, activating one specific cancer gene.”

Kee Kiat (Aaron) Yeo, MD

Karen Wright, MD, MS

Indeed, these structural variants could affect high-grade glioma cells in a variety of ways, generating abnormal proteins or, more often, influencing genetic expression. At least 10% of the gliomas had a structural variant that supercharged activity in MYC, a gene that is often altered in cancer. This was a surprising discovery because MYC was not previously thought to play a role in these cancers.

The discovery of different patterns of structural variants also offers clues into how these gliomas form in the first place. For example, previous research has established that high-grade gliomas often harbor mutations in a group of genes responsible for histones (proteins around which DNA is wrapped). The team's findings suggest ways that different histone mutations might uniquely inform the initiation and progression of these tumors. Researchers also found that of the two pediatric high-grade glioma subtypes they identified, those with more complex structural variants tend to be more aggressive. The team is now exploring whether the treatments currently in use confer differing degrees of effectiveness against the two subtypes.

"The more complex structural variants there are, the more disruptions to the genome, the more cells are stressed," Bandopadhayay explained. "We're starting to consider how this turmoil might introduce vulnerabilities that can be taken advantage of with new therapies. These cells are 'living on the edge'; it may not take much to push them over."

IDH MUTATIONS IN ADOLESCENT GLIOMAS

Next-generation sequencing is a set of techniques designed to unveil the complete genetic makeup of a sample (such as a tumor, a tissue, or even a whole organism) in a short amount of time and at a fraction of the cost associated with older sequencing methods.

While many pediatric gliomas are driven by alterations in the BRAF pathway, a significant proportion of older children and young adolescents have "adult-type" gliomas with IDH1/2 mutations. These mutations typically lead to more aggressive tumors and poorer prognosis. While IDH1/2 mutations were previously thought to be rare in pediatric patients, recent data suggests these may occur at a significantly higher frequency, especially among adolescents. Importantly, the prognostic significance of IDH1/2 mutations in children remains unclear.

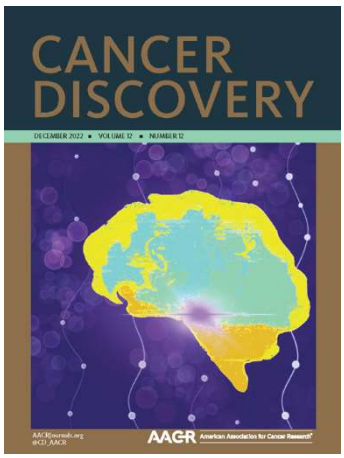
Kee Kiat (Aaron) Yeo, MD, and **Karen Wright, MD**, in collaboration with other physician-scientists across the country, led a study that systematically evaluated the rate of IDH1/2 mutation among 851 patients with pediatric gliomas who underwent **next-generation sequencing** (see sidebar). Of the 851 patients studied, 277 had high-grade gliomas, including DIPG. They found that, in both high-grade and low-grade cases, approximately 10% of pediatric gliomas harbor IDH mutations—a rate that rose to 20% in those ages 15-21.

Jun Qi, PhD

The study, published in the January 2023 *Neuro Oncology,* could lay the groundwork for a new treatment protocol for adolescent patients with IDH-mutated gliomas. Yeo suspects they may benefit from the same treatments that adult patients receive, given that the disease's genetic makeup is similar. He hopes that better defining this overlooked population will ultimately raise awareness among pediatric and adult neuro-oncologists alike and that, in the future, they will be included in clinical trials testing new therapies for IDH-mutant gliomas.

Paving the Way for Better Therapies

Lab research enriches our knowledge of DIPG tumor biology, revealing novel therapeutic approaches that could make a difference in the lives of our patients. Dana-Farber's physician-scientists are continuously testing experimental therapies to ensure these drugs can be administered safely and effectively, with the goal of bringing these findings into the clinic.



BAF COMPLEX: A NOVEL EPIGENETIC VULNERABILITY

For decades, cancer research has focused on how changes in DNA sequence, called genetic mutations, drive cancer. But cancer can also be spurred by epigenetic factors, or errors in the way genetic material is read and processed by cells. In recent years, Filbin discovered that epigenetics is a particularly compelling area of study for DIPG, which is sustained by an epigenetic error, one in which DIPG cells are kept frozen in a stem-like state. This means the cancer cells never mature through the lifecycle, continuously replicating without dying off. Now, Filbin is partnering with **Jun Qi, PhD**, to explore how these cells might be coaxed through the differentiation process, opening the door to a new class of cancer-fighting drugs.

In a study published in the December 2022 *Cancer Discovery,* Filbin and Qi used CRISPR technology to delete a library of epigenetic targets in H3K27M-mutated glioma cell lines, DIPG among them, to ascertain which ones were necessary for tumor survival. This effort brought a promising dependency to light: the BAF chromatin complex, a network of proteins that regulate the expression of genes. When proteins in this complex were deleted, DIPG cells were forced to differentiate and, ultimately, died. Filbin, Qi, and collaborators were then able to replicate these effects in **patient-derived xenograft models** (PDX models, see sidebar), which indicated that this target could have therapeutic benefit.

Patient-derived xenograft (PDX) models are surgical grafts of human tumor tissues into mice. Once established, PDXs faithfully recapitulate the genetic complexity of human cancers and offer a platform in which to test new therapeutic agents.

William Hahn, MD, PhD, Executive Vice President and Chief Operating Officer; William Rosenberg Professor of Medicine, Harvard Medical School

Kimberly Stegmaier, MD, Co-Director, Hematologic Malignancy Center; Ted Williams Chair at Dana-Farber

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

Having already developed an inhibitor and a degrader—a therapy that destroys rather than disables its target—against this chromatin complex, Qi is now working to modify these drugs so they can cross the **blood-brain barrier** (see sidebar). His work could bring an entirely new class of drugs into the clinic, opening a new chapter for epigenetic therapies in pediatric neuro-oncology, and would mark a major advance for young patients who have not been able to reap the benefits of traditional precision drugs.

VRK1: A THERAPEUTIC TARGET COMES TO LIGHT

DIPG, like many other pediatric cancers, has few driver mutations to target directly, so investigators are seeking out genetic dependencies—those genes that support cancer cell survival but do not drive cancer directly—as potential Achilles' heels. To uncover potential dependencies to target, Filbin drew on the Cancer Dependency Map and the Pediatric Cancer Dependency Map, which form a comprehensive catalogue of genetic vulnerabilities across all adult and pediatric cancers, including DIPG.

As reported in the October 2022 *JCI Insight,* Filbin found that adult and pediatric central nervous system tumors, including DIPGs, depend on the VRK1 enzyme for survival. Deleting VRK1 in cell lines and mouse models led to cancer cell death, suggesting that the enzyme could serve as a drug target. Now, Filbin is working with **William Hahn, MD, PhD, Kimberly Stegmaier, MD**, and industry partners to develop a small molecule inhibitor against the enzyme, with the hopes of bringing the drug to clinical trial.

Supporting Patients in the Clinic

Dana-Farber medical oncologists are running clinical trials to bring urgently needed new therapies to patients with DIPG. These trials are contributing to a body of research that enhances patient care at the Institute and beyond.

LAROTRECTINIB IN THE CLINIC

A small subset of DIPGs is driven by NTRK fusions, rare genetic mutations that occur across many different cancers. In 2018, the Food and Drug Administration (FDA) approved the NTRK inhibitor larotrectinib for adults and children with solid tumors driven by the fusion. Now, **Susan Chi, MD**, is leading a multicenter phase I clinical trial testing the drug in pediatric patients with newly diagnosed high-grade gliomas that harbor NTRK fusions. Through this study, she aims to ascertain whether this drug, when administered on its

The **blood-brain barrier** is a protective boundary that surrounds the brain and prevents harmful toxins and bacteria in the blood stream from entering the vital organ. What evolved as a life-saving defense, however, also blocks many drugs from reaching the brain, creating a major problem in treating brain

The Dana-Farber Campaign

Defy Cancer

In May 2021, Dana-Farber announced **The Dana-Farber Campaign,** our ambitious, multiyear $2 billion fundraising initiative to prevent, treat, and Defy Cancer by accelerating **revolutionary science, extraordinary care, exceptional expertise, and essential opportunities.**

own, may treat these tumors just as well as the standard of care. In addition, the study aims to explore the safety of the drug when administered in combination with chemotherapy and or radiation. A second trial is also in development testing a specific targeted inhibitor for pediatric patients with newly diagnosed high-grade gliomas that harbor ALK fusions, set to open in late 2023.

EXISTING THERAPY BENEFITS PATIENTS WITH DIPG

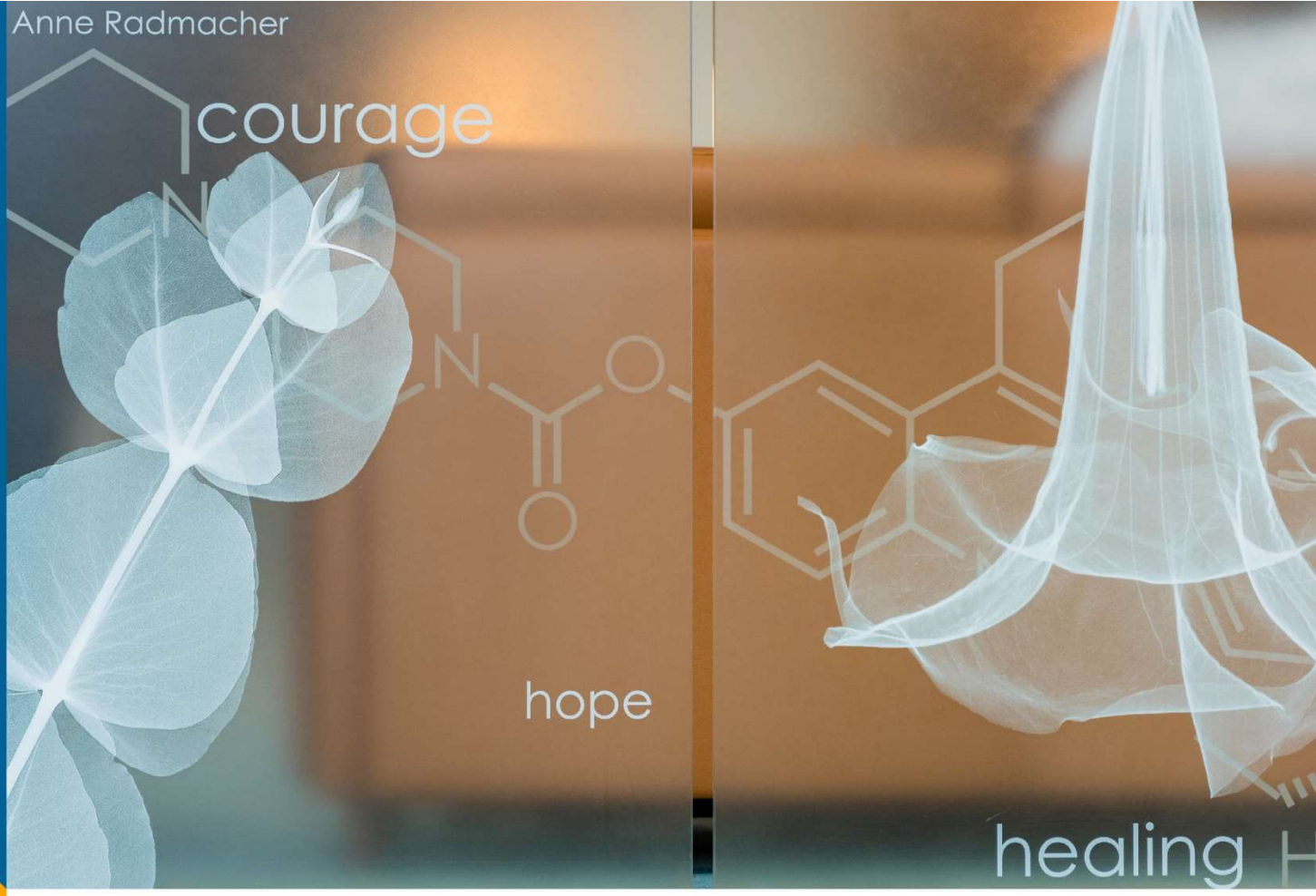
One method for discovering novel treatments is to expose DIPGs to a panel of existing drugs that are safe and effective against other cancers. Called a drug screen, this strategy circumvents the lengthy, uncertain process of designing a novel therapy. Using this method, Filbin identified a drug that showed remarkable promise against DIPG, both in cell lines and in mouse models. The treatment, avapritinib, is a kinase inhibitor that is already FDA approved for adult patients with gastrointestinal stromal tumors (GIST). The drug fulfilled two major prerequisites for brain cancer therapeutics: it largely spared normal cells and crossed the blood-brain barrier.

These findings set the stage for a clinical trial, which is already benefiting eight young patients with DIPG. Thus far, more than 50% have shown a response to the drug, a major advance for a disease that is largely resistant to precision therapies. This breakthrough could also yield crucial insights into the fundamental biology of the disease, which Filbin is currently exploring in the lab.

The Impact of Your Philanthropy

Through your support, Dana-Farber's translational and clinical research teams are pursuing a wide range of therapeutic strategies that will shape the future of care for children with DIPG. Thanks to your generosity, our physician-scientists are already making inroads against this aggressive, complex malignancy. On their behalf, and on behalf of our patients, thank you for making possible this important work.

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 22 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 #4 cancer center in the world by *Newsweek* in its World's Best Specialized Hospitals ranking.

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Ten percent of the gift will support the Faculty Research Fund to advance the Institute's research mission.