PREPARED FOR FERNANDO GOLDSZTEIN

The Impact of Your Philanthropy

MARCH 2022

Harnessing the Power of Collaboration to Defeat Medulloblastoma

Children diagnosed with pediatric brain cancer need safe, effective therapies now. They do not have time to wait. According to the National Center for Health Statistics, brain tumors are the leading cause of cancer deaths in children and adolescents. Medulloblastoma is the most common pediatric malignant brain tumor.

The complexity of brain tumors, which grow and change with a child, is something Dr. Roger J. Packer, director of the Brain Tumor Institute at Children's National Hospital, and his team are working tirelessly to understand. In the past 40 years, we have made incredible strides and translated our knowledge into better survival rates. Today, these rates stand at 70% to 80% for children with medulloblastoma. Yet we must do more. This is especially true for the 20% to 30% of children who experience a recurrence of medulloblastoma. For these children, there is no effective treatment available. The vast majority will succumb to their disease.

The Cure Group Four Medulloblastoma Consortium is an international, multi-institutional research collaborative launched in spring 2021. Together, nine brain tumor laboratories throughout the United States and Canada are working synergistically to unravel one of the most aggressive and resistant forms of medulloblastoma — medulloblastoma group four (Group 4 MB)— and its molecular and immunologic underpinnings. Consortium collaborators share a bold vision: to harness and galvanize extensive research and clinical expertise to accelerate the development of a novel therapeutic treatment for children and teens with Group 4 MB. The consortium aims to do this within 18 to 24 months — not in the traditional three- to five-year timeline.

Medulloblastoma experts from Children's National, SickKids Toronto, Sanford Burnham Prebys Medical Discovery Institute, University of Florida, McMaster University and Children's Healthcare of Atlanta are:

- Using new technologies (including single cell sequencing and proteomics) to discover Group 4 MB's origins and drivers and to identify novel targets for molecularly targeted therapy;
- Discovering novel neoantigens that the immune system can target;
- Developing new, more informative mouse models (including humanized models) of the disease to expedite the development of therapies targeting the novel biologic underpinnings we are discovering; and

- Using these models to understand the interplay among the molecular determinants, the immune system and the tumor environment in Group 4 MB, which will help us better design immunologic approaches.

Research Progress in the First Six Months

Laboratories of Dr. Michael Taylor and Dr. Vijay Ramaswamy
SickKids Toronto | Toronto, Ontario, Canada

Bioinformaticians in the Taylor and Ramaswamy labs have analyzed the unpublished preliminary datasets referenced in the initial project plan (specifically 545 Group 3 and Group 4 MB datasets) to identify oncofetal antigens expressed in Group 4 MB tumors. These analyses led to a startling finding that hints toward the developmental origin of Group 4 MB. New data indicates that Group 4 MB likely arises due to failure of resident progenitor cells to differentiate and develop into healthy cells.

These findings bring forward a number of potential targets for medulloblastoma, one of which is OTX2. The SickKids teams tested this by knockdown of OTX2 in model systems and found that this relieves the differentiation blockade. This allows medulloblastoma cells to spontaneously proceed along normal developmental differentiation trajectories. The labs continue to investigate OTX2 and other potential targets.

The Taylor Lab also continues to sequence more Group 4 MB samples. The team has sent eight samples to the lab's sequencing facility to date; the lab has prepared an additional 40 samples for submission. Bioinformaticians will begin to analyze data as they receive them from the facility. This additional large dataset will allow the labs to confirm the possible developmental origin of Group 4 MB. They will then combine them with the previous datasets to find more targets that they can pass to the rest of the consortium for validation. This may lead to the development of new therapies.

Laboratory of Dr. Robert Wechsler-Reya
Sanford Burnham Prebys Medical Discovery Institute | San Diego, California

The laboratory's aims are to:

1) Identify the epigenetic vulnerabilities of Group 4 MB,
2) Determine whether epigenome-modifying drugs can enhance an immune response, and
3) Test whether chromatin regulators can function as a driver of tumor formation.

The Wechsler-Reya lab has made significant progress in the first six months of the study. To identify novel epigenetic vulnerabilities of Group 4 MB cells, the team used a pooled CRISPR library (consisting of 9,280 sgRNAs targeting 643 epigenetic

modulators) to perform a screen in CHLA-01-MED cells, a cell line isolated from a Group 4 MB patient. This screen identified 84 genes that were significantly depleted in CHLA-01-MED cells after two weeks of growth in culture. These studies demonstrate the feasibility of performing a CRISPR screen of this type on medulloblastoma cells. Going forward, the Wechsler-Reya lab will perform similar screens using MBT375 cells (a Group 4 MB cell line generously shared by Dr. Sheila Singh at McMaster University; see her update on page 5) as well as two Group 4 MB patient-derived xenografts (PDX) the lab generated. The focus will be on genes identified as vulnerabilities in multiple Group 4 MB lines. Among those, the lab will prioritize genes they can target with existing FDA-approved or investigational drugs used to treat other cancer types. There are no pediatric cancer drugs currently approved for use.

These studies will allow the Wechsler-Reya lab to identify key regulators of Group 4 MB growth and survival as well as drugs that can inhibit tumor growth by targeting the epigenome. In addition to CRISPR screening, the lab has begun to perform high-throughput drug screening to identify chromatin-modifying drugs that affect immunogenicity. To this end, the lab team treated D283, another medulloblastoma cell line, with a custom library of 222 small molecule epigenetic regulators and evaluated cell viability and HLA expression by flow cytometry. At the highest concentration, many of the compounds had a strong impact on cell viability, but at lower concentrations, some compounds were able to upregulate HLA expression. HLA expression is critical to ensure that treatment agents, such as CAR T cells, will attach to cancerous cells.

Laboratory of Dr. Duane Mitchell
University of Florida | Gainesville, Florida

The Mitchell lab aims to evaluate the safety, efficacy and immunologic effects of adoptive cellular therapy targeting neoantigens and uniquely expressed tumor-associated antigens (TAAs) in preclinical models of Group 3 and Group 4 MB. These TAAs and neoantigens, identified by the Mitchell lab (RNA expression profiling), Rood lab (proteomics analysis) and Taylor Lab (single cell transcriptomics) represent a synergistic joining of multiple efforts. Tumor neoantigens also are a novel approach to tumor immunotherapy. The lab's second aim is to determine the capacity to isolate and expand Group 4 MB-specific memory T cells in vitro, drawn from patients with recurrent medulloblastoma who have received adoptive cellular therapy at the University of Florida Health Cancer Center.

Dr. Mitchell's lab continues to investigate tumor neoantigens in patients with Group 4 MB. The lab culled simple somatic mutations including single nucleotide variants and small insertions and deletions from the International Cancer Genome Consortium. The team found recurrent neoantigens in multiple patients. This data shows Group 4 MB patients have at least one targetable neoantigen. Taken together, these data indicate that precision medicine may be a good therapeutic option for patients.

There are very few medulloblastoma neoantigens as compared to other brain tumors. To expand the targeting antigen categories, the Mitchell lab continues to investigate the immunologic environment of medulloblastoma tumors. They derived gene signatures from a single cell RNAseq dataset of purified peripheral blood mononuclear cells. They then applied this to medulloblastoma patient microarray data. This investigation revealed that Group 4 MB did not trigger as robust an immune response when compared to the three other molecular subgroups of medulloblastoma. Using a custom antigen prediction pipeline, they identified potential Group 4 MB rejection antigens with important implications for the development of medulloblastoma cell therapies. The lab also is testing the immunologic effects of adoptive cellular therapy targeting neoantigens and uniquely expressed TAAs.

In the near future, the Mitchell lab aims to determine which neoantigens and TAAs are driving T-cell reactivity. They will achieve this through the analysis of RNAs encoding the individual neoantigens and TAAs in T-cell stimulation assays. This work, in total, should inform the next generation of immunotherapy protocols for children with Group 4 MB.

Laboratories of Dr. Catherine Bollard and Dr. Conrad Cruz
Children's National Hospital | Washington, D.C.

These laboratories' aims are to identify optimal T-cell antigen targets that can expand a tumor-specific T-cell product against intracellular oncofetal and repetitive element antigens identified in Group 4 MB. They also aim to optimize an expansion method that can produce both CAR-expressing and antigen-specific T cells that are cytolytic against (or destroy) Group 4 MB cells in vitro.

The work within the Bollard and Cruz labs to date has resulted in a unique method of T-cell expansion. They hope to publish this as a separate process manuscript.

In the next six months, Drs. Bollard and Cruz plan to test the function of these cells against the two cell lines, completing the requirements for year 1 deliverables. Potential synergy with the rest of the consortium lies in the identification of critical targets across the different groups. From this list, the Bollard and Cruz labs can choose another set of intracellular and extracellular antigens.

Laboratory of Dr. Yanxin Pei
Children's National Hospital | Washington, D.C.

The Pei laboratory aims are to generate a new transgenic mouse model of Group 4 MB and to identify neoantigens in the radiochemotherapy-resistant cells in Group 4 MB using PDX models.

To date, the Pei lab has examined whether overexpression of Tbr1 in Math1- or Tbr2-expressing neural progenitors can generate a more informative mouse model for Group 4 MB.

A small population of radiochemotherapy-resistant cells that ultimately progress to lethal tumors often mediate a medulloblastoma relapse. Immunotherapy, particularly the adoptive CAR T-cell therapy that targets the tumor-specific antigen, may be a promising strategy to eradicate radiochemotherapy-resistant cells. Collection of the radiochemotherapy-resistant cells that drive relapse has been impossible to date. This limits our ability to characterize the cells and identify neoantigens to generate CAR T cells. The lab is working to isolate the radiochemotherapy-resistant cells using novel PDX mouse models.

Laboratory of Dr. Brian Rood
Children's National Hospital | Washington, D.C.

The first aim of the Rood laboratory is to use functional CRISPR screening of differentially expressed proteins in Group 4 MB to identify proteins essential for tumor survival. Work is ongoing in available cell lines.

One of the limitations the lab has faced is the lack of a pure Group 4 MB cell line. To overcome this, they are now using a bona fide Group 4 MB cell line from Dr. Singh at McMaster University. The Rood team will establish CRISPR/Cas9 gene cell line knockouts in the new Group 4 MB line, as well as perform other experiments.

Once the new Group 4 MB cell line is in place, the lab aims to use a proteogenomic approach to identify tumor-specific proteins to serve as neoantigens for targeting T-cell immunotherapies. The lab has not begun work on this aim owing to a lack of Group 4 MB-specific reagents. They will identify tumor-specific neoantigens, almost all of which are unique to an individual tumor. The Rood lab will then share those neoantigen targets with the Mitchell lab for testing. Together, the two labs will carefully ensure that they are working synergistically with shared models.

Dr. Pei has implanted four Group 4 MB PDX lines in mice. Two of them have developed tumors. Two are still awaiting tumor growth. When the Rood lab receives the cells from Dr. Singh, the team will implant those as well. They will subject these five lines to the lab's recently published neoantigen identification pipeline.

Cure Group Four Medulloblastoma Consortium Expansion

Two laboratories joined the consortium in fall 2021.

Laboratory of Dr. Sheila Singh
McMaster University | Hamilton, Ontario, Canada

The Singh laboratory is evaluating the safety and preclinical efficacy of promising drug combinations that tackle medulloblastoma recurrence. Out of the four molecular subgroups of medulloblastoma, tumors belonging to Group 4 MB have the highest BMI1 expression. These increased levels correlate with increased invasiveness and reduced overall survival for patients. While several studies have provided insights into phenotypic and functional changes driven by the elevated levels of BMI1 in Group 4 MB, an effective targeting strategy remains elusive. The long latency of Group 4 MB relapse provides an ideal opportunity for therapeutic intervention with targeted therapies to prevent further disease progression.

In the proposed study, the Singh lab will evaluate the efficacy of PTC-596, a BMI1 function inhibitor, in Group 4 MBs alone and as a combination therapy. Additionally, we can rapidly deploy our high-throughput small molecule inhibitor screen in Group 4 MB — used recently in metastatic Group 3 MB — to identify strategies to limit or prevent the extent of leptomeningeal dissemination. Leveraging McMaster University's established expertise and infrastructure, the Singh lab is set to complete preclinical evaluation of PTC-596-based treatment regimens in Group 4 MB within a 12- to 24-month period. The laboratory will determine in vitro efficacy of PTC-596 against patient-derived Group 4 MB.

The lab will next perform in vitro and in vivo preclinical validation of combination therapies with inhibitors targeting BMI1 and PI3K for the development of rationally designed combinatorial treatment regimens. In the current aim, they propose to perform in vitro and in vivo preclinical validation of combinatorial therapies with inhibitors targeting BMI1, PI3K and PLK1 for the development of rationally designed combination treatment regimens.

Laboratory of Dr. Toby MacDonald
Aflac Children's Hospital of Atlanta | Atlanta, Georgia

Dr. MacDonald's laboratory is exploring the utility of a new drug called ONC206, which may stop cancer cells from growing, in the treatment of Group 4 MB. The lab will determine the efficacy of combining ONC206 with an AKT inhibitor against Group 4 MB cells in vitro and determine efficacy of ONC206 +/- AKT inhibitor for treatment of Group 4 MB in vivo.

Recent discoveries have led to the clinical development of oral, brain-penetrant imipridones including ONC201, a selective dopamine receptor D2 (DRD2) antagonist3, and the ONC201 homologue ONC206. These agents exhibit selective cell killing of cancer cells and spare normal tissue. The MacDonald lab has shown in Sonic Hedgehog (SHH) and Group 3 MB cells that another key mechanism by which ONC206 exerts its activity is by activating the mitochondrial protease ClpP. This leads to apoptosis selectively in tumor cells by inducing the integrative stress response signaling pathway. Notably, the lab confirmed that ONC206 treatment in vivo achieves therapeutic concentrations in the brain of transgenic SmoA1 MB mice (a SHH-driven MB model), results in tumor regression and prolongs survival by more than threefold.

In the small population of surviving medulloblastoma cells, the MacDonald lab observed increased activated PI3K/AKT, suggesting that this pathway could mediate drug resistance in some cells and may be a target for combined drug therapy with ONC206. Although the majority of these studies have been conducted in non-Group 4 MB cells, the team has observed a similar response in patient-derived relapsed Group 4 MB cells using ONC206. Thus, ONC206 should be effective for Group 4 MB.

In a recently completed clinical trial in adults with relapsed malignant glioma, ONC201 showed unprecedented results for this disease, with up to 35% of patients surviving 2+ years after relapse. ONC206, which we have found to be 15 to 30 times more potent than ONC201 against medulloblastoma, is currently undergoing first-in human phase I assessment in recurrent adult brain tumors (NCT04541082). A new clinical trial using ONC201 recently opened for children with newly diagnosed malignant glioma and relapsed malignant brain tumors (NCT04732065). We believe preclinical testing of ONC206 in Group 4 MB models will support clinical evaluation of ONC206 in patients with recurrent Group 4 MB.

On the Consortium's Horizon

Liquid Biopsy to Monitor a Child's Medulloblastoma

Over the coming months, Children's National will investigate the use of liquid biopsy as a way to monitor how a child's medulloblastoma is responding to treatment and develop a tool to detect tumor growth as an early indicator of recurrence.

Dr. Javad Nazarian's laboratory in the Brain Tumor Institute established a liquid biopsy platform to detect mutations associated with diffuse intrinsic pontine gliomas (DIPG), a deadly childhood brain stem cancer. They were the first to demonstrate the feasibility of liquid biopsy in both subtyping a child's cancer as well as its ability to monitor tumor response in a clinical setting. Tumor DNA found in children's blood and cerebrospinal fluid allowed investigators to reliably determine a DIPG's growth or the tumor's treatment response. Children's National believes that measuring and monitoring brain tumor biomarkers through the extraction of tumor DNA from a child's blood and cerebrospinal is far less invasive that the traditional brain tissue biopsies. These require surgical intervention and very often damage the child's brain tissue.

Leveraging Philanthropy to Accelerate Discovery

Children's National is building on your generosity and that of others to revolutionize care for children with brain tumors. In fall 2021, an anonymous family made a significant philanthropic investment in the Brain Tumor Institute. Their investment supports:

- Providing vital research infrastructure and additional laboratory personnel to push the Brain Tumor Institute to new frontiers through innovative clinical trials — This ensures dedicated staffing resources to develop and implement IRB protocols and participant enrollment for new clinical trials. Staff are critical to developing studies, entering patients into studies and following them carefully. Their presence ensures that studies can open quickly, and new treatments are available to children in need.
- Building research infrastructure and the rare brain tumor research program — Children's National recently recruited physician scientist Dr. Adriana Fonseca to the Brain Tumor Institute to formalize and lead our Brain Tumor Institute Rare Pediatric Brain Tumor program. We also are actively recruiting a clinical research coordinator to work alongside Dr. Fonseca to advance initiatives focused on developing novel immunotherapies and molecularly targeted therapies for rare brain tumor types.
- Immunotherapy research program infrastructure — These investments in the laboratories of Dr. Brian Rood and Dr. Eugene Hwang will bolster ongoing medulloblastoma research initiatives by providing vital staffing and equipment resources. These will help accelerate discoveries to target the molecular and immunologic underpinnings of aggressive brain tumors.

- Developing new minimally invasive neurosurgical techniques to remove medulloblastoma tumors — Dr. Robert Keating and his team recently developed a hyperspectral camera capable of seeing dozens of wavelengths simultaneously to detect the unique spectral "fingerprint" of a tumor and its boundaries. The surgeon receives the information in real-time, providing them with the ability to see beyond the visible spectrum. Developing a safe and reliable way to detect the boundaries of brain tumors ensures that surgeons can remove the tumor entirely in one procedure. It also protects against irreparable brain damage by ensuring surgeons do not remove healthy tissue.
- High- and low-intensity focused ultrasound (HIFU and LIFU) projects — The pediatric brain tumor community is rapidly developing a deeper knowledge of the molecular underpinnings of brain tumors. The molecules that initiate and drive a tumor's progression are targets we can aim for when treating a child. The protective blood-brain barrier, however, is a shield that stops us from delivering the novel agents, including immunotherapy, that could prove lifesaving. In the next six months, Children's National will have the ability to safely disrupt the blood-brain barrier using HIFU and LIFU and open clinical trials that are the first of their kind in the world. This non-invasive, outpatient approach promises to fundamentally change how we care for children with brain tumors.

In winter 2021, another anonymous family made a multimillion-dollar gift to further the Brain Tumor Institute's work. Their generosity will:

- Move forward work within the Pei laboratory, which focuses on generation of diverse preclinical mouse models of medulloblastoma, elucidation of the molecular mechanisms of metastasis, therapeutic resistance and tumor relapse, and exploring new approaches to enhance delivery of therapeutic drugs to overcome the blood-brain barrier in aggressive brain tumors.
- Allow us to advance work being done as part of our collaboration with the Pacific Pediatric Neuro-Oncology Consortium (PNOC). PNOC is an international consortium dedicated to bringing new treatments to children with brain tumors. Together, we are expediting work on a new screening platform using cells derived directly from tumor biopsy. We aim to determine what drugs and agents are most likely to be effective in treating an individual tumor.

Both of these families share your commitment to eradicating pediatric brain tumors. Philanthropy is collectively strengthening research efforts, advancing clinical trials and deepening our understanding of brain tumor genetics, allowing us to move faster and into areas we never thought possible. Together, you are transforming the landscape of brain tumor care and research and propelling us toward a cure for brain cancers.

With Gratitude

Uncovering new treatments and a cure for medulloblastoma will only be possible through the strength and work of many hands and minds. Your trust and generosity empowers the Cure Group Four Medulloblastoma Consortium to collaborate in new, promising ways. Thank you for partnering alongside us as we push closer to answers for the children and families who turn to us for care.

Appendix 1: Financial Summary

Since March 2021, we have secured a **$6,117,000.18** in gifts, grants and multi-year pledge commitments in support of the Cure Group Four Medulloblastoma Consortium. This places us at more than **61% of our $10,000,000** goal.

Year 1 Expenses

Budget Category	Amount
Personnel costs (salaries and benefits)	$212,465
Animal costs (Pei animal purchase and maintenance)	$34,900
Supplies ($24,790 for Pei lab/DNA/spectrometry and $74,845 for Cruz lab)	$99,635
Rood CRISPR screens and sequencing	$20,000
Executive Advisory Board honorariums	$8,000
Annual consortium meeting	$15,000
Packer Discretionary Fund	$54,100
Sanford Burnham Prebys Medical Discovery Institute subcontract (to fund consortium research activities)	$194,000
SickKids subcontract (to support consortium project)	$521,900
University of Florida subcontract (to fund consortium project scope)	$167,000
McMaster University subcontract (to complete consortium research activities)	$75,000
Aflac Children's Hospital of Atlanta subcontract (to fund consortium project scope)	$65,000
Total	**$1,467,000**