



**A report on the Pediatric Low-Grade Astrocytoma Program
Prepared for the Lauren's First and Goal Foundation
October 5, 2009**

Thank you

The continued generosity of the Lauren's First and Goal Foundation has allowed the PLGA Program to expand its innovative laboratory and clinical research efforts with the singular goal of conquering pediatric low-grade astrocytomas. In concert with an impressive array of leading multi-disciplinary researchers and clinicians based at Dana-Farber, across the United States and around the world, Drs. Charles Stiles and Mark Kieran have been able to greatly advance their efforts toward creating a PLGA-specific tissue bank, identifying new drug-susceptible targets in LGA, developing laboratory models to evaluate the activities of drugs against these targets, and launching a clinical trial to test a potential new therapy.

Progress related to the three research segments of the PLGA Program

Research Segment One: Create a tissue bank to cement program infrastructure

Access to high-quality tumor tissue samples remains critical to meeting our research goals. Due to the limited pediatric LGA diagnoses in North America (only approximately 700 cases per year) and the infrequency with which the tumors are resected (only half of the 70 cases at Dana-Farber/Children's Hospital Boston), we have been reaching out internationally to establish relationships with physicians in major population centers around the world. These partnerships will prove vital to helping us to gather thousands of new tissue samples. In addition, we anticipate that these relationships could also lead to future recruitment of their LGA patients into clinical trials that test new targeted therapeutics.

Specifically, fresh surgical isolates of tumors are being frozen and banked in Cairo for imminent shipment here, pending Institutional Review Board approval. We also took the first steps toward forging a partnership with Dr. Memet Ozek, Chief of Pediatric Neurosurgery at Marmara University Medical Center in Istanbul.

In addition, Drs. Stiles and Kieran traveled to Beijing in October 2008 to meet Dr. Liwei Zhang, Chief of the Department of Neurosurgery at Beijing's Tian Tan Hospital, and to discuss the PLGA Program with Dr. Zhang and his colleagues. Our counterparts in Beijing agreed to collaborate with Dana-Farber and have sent a pediatric neurosurgeon from Tian Tan Hospital to Boston for a one-year research sabbatical. We are happy to report that Dr. Yongji Tian arrived at DFCI in July and has begun his training in the laboratory of PLGA Program neuropathologist Dr. Keith Ligon, with whom he will learn molecular technologies and exchange information on the diagnosis and clinical management of LGA tumors. We expect that Dr. Tian will return to Beijing at the end of his sabbatical armed with the tools needed to analyze tumor samples from his patients and to thus continue the collaboration with the PLGA Program.

In addition to this strong international effort to gather new PLGA tissue samples, we have also expanded our tissue banking work to include collaborations with centers that are able to share their patients' paraffin-preserved, or archived, tumor tissue samples. We are able to utilize this archived tissue due to our proven success in analyzing archived tumor tissue samples via Dana-Farber's innovative OncoMap technology as mentioned in Research Segment Two, below. These synergies include Dr. Stewart Goldman at Chicago's Children's Memorial Hospital and Dr. Charles Eberhardt at Johns Hopkins University School of Medicine. With their involvement, we will gather grade II astrocytoma tumor samples for inclusion in the next round of OncoMap analysis.

Research Segment Two: Determine the genetic lesions of PLGA

Within the past year, we developed a novel Fluorescence in situ Hybridization (FISH) assay to detect chromosomal rearrangements in pediatric LGA tumors. Using this technique, we identified an important activating duplication in the BRAF gene in archived, paraffin-preserved tissue samples. We detected BRAF duplication in all pilocytic astrocytomas analyzed, while duplication events were rarely detected in non-pilocytic gliomas. In contrast, we found a distinct BRAF mutation called BRAF^{V600E} in one-third of non-pilocytic gliomas.

We expect to publish these important findings soon; a manuscript reporting these results is currently under review. Our group presented these results at a research meeting in Nottingham, England in June, and will present at both a Society for Neuro-Oncology and an AACR meeting this fall.

Importantly, the BRAF tests can be done under CLIA-certified conditions so that individual test results can be included as part of the patient's medical record. These test results give neuropathologists new objective, diagnostic tools to distinguish classic pilocytic tumors from other non-pilocytic tumors. The tests also identify patients with the specific BRAF mutation who would likely benefit from drugs that are currently being developed to target activated BRAF. We will soon offer the BRAF tests to all children who are suffering from LGAs, including those who are not patients at DFCI/CHB, and those with the BRAF mutation will be eligible to participate in future clinical trials that will test the BRAF therapeutics under development in the laboratory.

In the coming year, we plan to run another round of OncoMap tests on a panel of grade II LGAs to identify other, less common, genetic mutations that may cause this disease in tumors with normal BRAF. These mutations may also prove critical drug targets for a population of LGA patients.

Research Segment Three: Develop mouse models to test potential new therapies preclinically

Over the past year, we have developed new mouse brain tumor models that show high penetrance (meaning that 100% of mice develop tumors) and short latency (meaning that tumors arise within 2-4 weeks), making them very good models for preclinical drug testing. These models incorporate the two principal types of activating mutations of BRAF: the V600E point mutation and the duplication. These mice will be invaluable in testing BRAF compounds before they are used in clinical trials for children with BRAF LGAs.

The launch of the RAD001 clinical trial

We are also excited to share with the Lauren's First and Goal Foundation the news of a clinical trial underway for children with low-grade gliomas. An important pathway that has been implicated in pediatric LGGs is mTOR, a central relay site within the cell that, when activated, results in increased proliferation, cell migration and angiogenesis. RAD001 is a new oral mTOR inhibitor that has

demonstrated excellent inhibition of this pathway at clinically achievable doses. The drug is exceedingly well tolerated and is currently used to reduce the risk of solid organ transplant rejection. We have launched a formal multi-institutional clinical trial of RAD001 in children with recurrent or progressive LGGs after standard treatment, and patients are currently being accrued for this landmark study.

Future goals

To date, we have focused our attention on finding LGA-associated mutations, such as BRAF, in a class of molecules known as protein kinases because these kinases are easy to inhibit with drugs. In the coming year, we aim to broaden our portfolio of drug targets by looking beyond protein kinases at other important regulators of cell growth. Transcription factors, for example, control many important cellular processes, but are not currently the focus of many drug development efforts because they are difficult to target. A particular transcription factor known as Olig2 is of interest to us, however, because it is present in high levels in LGA tumors but is not widely present in normal areas of the brain. Olig2 is required in normal neural stem cells during the development of the brain to regulate division of the cells. We hypothesize that blocking the action of Olig2 in LGAs will stop the growth of these tumors. PLGA Program scientists are currently exploring creative ways to target this key regulator of stem cells in the hope of devising a new therapy for pediatric LGAs.

Conclusion

In just two years, the PLGA Program has made remarkable progress in understanding a set of diseases that had not benefited from any major scientific findings or new treatments for more than two decades. We attribute the program's growth to the Lauren's First and Goal Foundation and to other likeminded philanthropists who make this groundbreaking work possible. Renewed funding from your organization will provide our world-class faculty members with the flexible resources necessary to fulfill the PLGA Program's vital goals. On behalf of the entire program, and especially the young patients and family members who will ultimately benefit from your investment, thank you so much for all that you do in the fight against PLGA.