

**MOLECULAR ANALYSIS OF DIFFUSE INTRINSIC  
PONTINE GLIOMAS**

**Principal Investigator: Alberto Broniscer, M.D.  
Co-Principal Investigator: Suzanne J. Baker Ph.D.  
St. Jude Children's Research Hospital**

**CONTACT INFORMATION**

**Principal Investigator**

**Alberto Broniscer, M.D.**  
Associate Member, Department of Oncology  
St. Jude Children's Research Hospital  
262 Danny Thomas Place Mail Stop 260  
Memphis, TN 38105  
Tel: (901) 495-4925  
FAX: (901) 521-9005  
E-mail: [.broniscer@stjude.org](mailto:.broniscer@stjude.org)

**Co-Principal Investigator**

**Suzanne J. Baker, Ph.D.**  
Associate Member, Department of Developmental Neurobiology  
St. Jude Children's Research Hospital  
262 Danny Thomas Place  
Memphis, TN 38105  
Tel: (901) 495-2254  
FAX: (901) 595-2270  
E-mail: [suzanne.baker@stjude.org](mailto:suzanne.baker@stjude.org)

**Researcher responsible for financial matters**

**Amar Gajjar, M.D.**  
Full Member, Department of Oncology  
St. Jude Children's Research Hospital  
262 Danny Thomas Place Mail Stop 260  
Memphis, TN 38105  
Tel: (901) 495-2615  
FAX: (901) 521-9005  
E-mail: [.gajjar@stjude.org](mailto:.gajjar@stjude.org)

## **EXECUTIVE SUMMARY**

Brainstem gliomas constitute 10 to 15% of all brain tumors in children. Diffuse intrinsic pontine gliomas (DIPG) account for 80% of all brainstem tumors. The treatment of children with DIPG represents a formidable challenge because of the critical location of these tumors, and their refractoriness to therapy. Surgery to confirm the diagnosis is only recommended for patients with atypical tumors by magnetic resonance imaging (MRI). Radiation therapy (RT) is the mainstay of therapy. Although 60% to 80% of affected patients benefit from RT, the clinical improvement is generally temporary. Less than 10% of children with DIPG survive more than 3 years from diagnosis. Standard chemotherapy has not been beneficial in the treatment of children with this cancer.

Since surgery to obtain a sample of DIPGs is rarely required, very little is known about the biology of these tumors. Recently, new treatment approaches combining biologic agents (synthetic antibodies or medications called small-molecule inhibitors) during and/or after RT have been used in the treatment of children with DIPG. These new drugs target specific molecules or signaling pathways within tumors; however, since little is known about the molecular and genetic abnormalities associated with DIPG, it has been impossible to elaborate on important therapeutic issues such as the selection of the most appropriate drug(s) to be used, or which patients would most benefit from a specific drug.

In this multi-institutional study, we are prospectively collecting tumor and constitutional tissue samples from patients with DIPG and other types of brainstem gliomas either during therapy or at autopsy to perform an extensive analysis of the genetic make-up of these tumors. Our hypothesis is that the better understanding of the genetic abnormalities underlying DIPGs will provide the tools to design better and specific treatments for affected children. Such broad and extensive analysis has never been done before because of lack of appropriate tissue samples.

We have been conducting this prospective study at our institution since June 2006. To date, tumor tissue samples with or without control normal tissue (blood and/or normal brain) have been obtained from 23 research participants. Tissue samples were obtained at autopsy in 21 patients (one case at diagnosis and the remainder after tumor progression). Tissue was obtained at diagnosis in 4 research participants. One research participant had tumor samples collected at diagnosis and after tumor progression. Only 3 autopsies took place at our institution. The remaining procedures have occurred at different hospitals in 11 different states (Alabama, Arkansas, Florida, Georgia, Massachusetts, Missouri, New York, North Carolina, Ohio, Rhode Island, and Tennessee).

All available tumor tissue samples have been reviewed by Dr. David W. Ellison, who is a co-investigator in this study and an international expert in the field. The tissue samples will be analyzed at the laboratory of Dr. Suzanne Baker, Ph.D., who has extensive expertise in genetic and molecular analysis of tumor samples. Dr. Baker's laboratory has already analyzed the quality of DNA obtained from all available tissue samples. The extracted DNA has been of excellent quality and suitable for the proposed genetic studies. We have not yet completed the analysis of quality of RNA.

Our proposed budget will finance the initial SNP and gene expression microarray studies of the tissue samples collected so far.

## **RESEARCH PROPOSAL**

### **Hypothesis**

Our primary hypothesis in this project is that we may be able to design more efficacious treatments for children with diffuse intrinsic pontine gliomas (DIPG) by better understanding the biology of these tumors. Therefore, our goal is to prospectively collect tumor and normal tissue samples (brain and/or peripheral blood) from children with DIPG either at diagnosis (if clinically indicated) or after death (autopsy). Tumor and constitutional DNA and RNA will be extracted from tissue samples. Thereafter, we will perform extensive genome-wide analysis of both DNA and RNA using single nucleotide polymorphism (SNP) arrays and Affymetrix gene expression-profiling, respectively. SNP array is the most sophisticated technique to detect chromosomal gains and losses. Affymetrix gene expression-profiling provides detailed information about expression patterns of RNA within tumors, and may indicate particular pathways or groups of genes that play an important role in DIPG.

This work will provide the essential foundation for future studies beyond the scope of this proposal including validation and more in-depth analysis of candidate target genes identified by genomic and gene expression studies.

### **Objectives**

To perform genome-wide analysis of DNA gains and losses, and RNA expression in tumor samples and normal tissue in patients with DIPG

- To identify regions of genomic gain or loss using SNP arrays
- To investigate genome-wide expression patterns of RNA derived from tumor samples and normal tissue via Affymetrix gene expression-profiling

## Background Information

DIPG accounts for about 10% of all pediatric central nervous system neoplasms.<sup>1</sup> The diagnosis of DIPG is almost uniformly based on typical appearance demonstrated by magnetic resonance imaging (MRI) which precludes the need for histologic confirmation.<sup>1</sup> The typical aspect of DIPG by MRI consists of an intraaxial, pontine-based, infiltrative lesion that causes mass effect on surrounding structures such as the fourth ventricle and the basilar artery.

Children with DIPG have a dismal prognosis with long-term survival less than 10% despite treatment with high doses of local RT alone or combined with several different regimens of conventional chemotherapy.<sup>1</sup> Surgery for histologic confirmation is only recommended for those patients with radiologically atypical neoplasms (e.g., those with features suggestive of other tumor histologies, or localized tumors).

Since conventional chemotherapy has demonstrated no benefit in the treatment of patients with DIPG, several recent clinical trials have been testing the usefulness of small-molecule inhibitors (tyrosine kinase inhibitors) in the treatment of such patients. The underlying principle for the use of these inhibitors is their ability to target molecules or pathways that are pivotal in the formation of cancer. Clinical trials in adults with different types of cancer (e.g., non-small cell lung cancer) which used small-molecule inhibitors have shown that specific genetic or molecular characteristics of these cancers may directly impact the response to treatment among histologically identical tumors.<sup>2,3</sup>

Very little is known about the biology of DIPG since histologic confirmation is rarely recommended in such patients. Six studies reported limited analyses in 63 tumor samples derived from patients with DIPG<sup>4-9</sup>. In these studies, half of the tumor samples studied harbored a *TP53* mutation, and in one study 57% of the tumor samples displayed loss of heterozygosity (LOH) of the short arm of chromosome 17.<sup>4</sup> Mutation of *PTEN*, an important tumor-suppressor gene localized in the long arm of chromosome 10, was detected in only one tumor analyzed,<sup>7</sup> and approximately 43% of all cases in one series demonstrated allelic losses in the long arm of chromosome 10.<sup>4</sup> A recent Pediatric Brain Tumor Consortium study that analyzed 28 tumor samples of patients with DIPG demonstrated a correlation between histologic grade and epidermal growth factor receptor (EGFR) protein expression.<sup>9</sup> While two of 12 World Health Organization (WHO) grade II gliomas had detectable protein expression, seven of nine WHO grade III and all WHO grade IV tumors had EGFR protein detected. *EGFR* amplification was detected in one of four WHO grade III and in two of four WHO grade IV neoplasms. None of the nine WHO grade II tumor samples analyzed displayed *EGFR* gene amplification.

Whereas very little is known about the biology of infiltrative gliomas (WHO grade II to IV), and in particular of DIPG in children,<sup>1</sup> a great deal of information is available about molecular and genetic abnormalities that occur in their adult counterparts.<sup>10</sup> Several recent reports have used microarray technology to perform an unbiased and large-scale analysis of genome-wide DNA abnormalities and RNA expression in infiltrative gliomas in adults, particularly glioblastoma multiforme.<sup>11-28</sup> As expected, groups of tumors that were classified by traditional histologic subtypes and grades of malignancy can also be grouped together by characteristic gene-expression signatures and changes in genomic structure. Additionally, array technologies identified

subsets of tumors that share gene-expression signatures and genomic alterations that were not identified by traditional pathologic classification. This unbiased approach to explore changes in the genome and transcriptome is a promising avenue of investigation that should lead to discovery of previously unknown genetic abnormalities that contribute to tumorigenesis, as well as classification of tumors that may reflect prognosis or response to specific treatment regimens. Whereas few studies to date have reported similar investigations for gliomas in children,<sup>29, 30</sup> no such analysis has ever been conducted in patients with DIPG.

Since little progress has been made in the treatment of patients with DIPG in the past 2 decades, we hypothesize that a better understanding of the biology of such tumors will contribute to the design of therapies that potentially can impact the grim prognosis of affected patients.

Histologic confirmation is only recommended in cases where the diagnosis of DIPG is uncertain, and even in those cases, the acquisition of samples for research purposes is complicated because of the difficulty in getting tissue from such critical structure as the brainstem. Therefore, we assume that the majority of tumor samples obtained in this study will stem from autopsies.

Before we initiated this study, there was a concern about the suitability of tumor tissue obtained at autopsy in patients with DIPG for this genome-wide analysis of DNA abnormalities, and RNA and protein expression as envisioned in this protocol. As a proof of principle, we obtained postmortem tumor and normal brain tissue (cerebellum) from one of our patients who died of DIPG back in 2005. Tumor and normal brain samples were collected approximately 6 hours after death. No extraordinary measures were taken to preserve tissue. DNA and RNA were isolated from tumor and normal brain following standard procedures. The DNA obtained from both tumor and normal brain in this patient was of good quality. The RNA demonstrated some minor degradation, but was still suitable for Affymetrix gene expression microarray analysis. Based on this promising preliminary result, we proceeded with the collection of autopsy material for analysis.

### **Research Design**

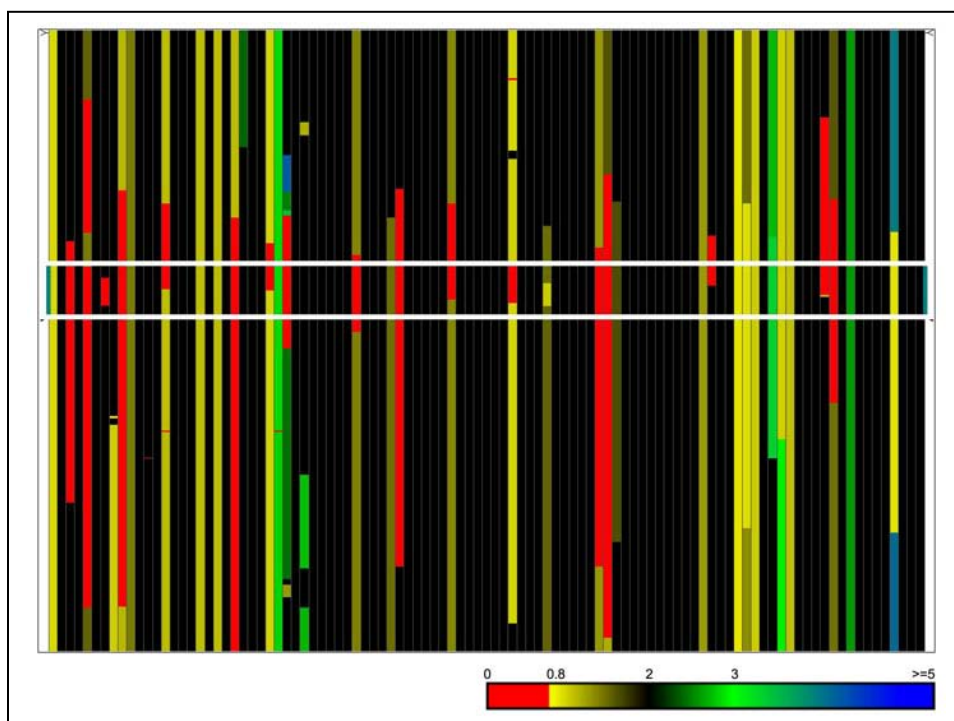
We have established an efficient mechanism to successfully collect tissue samples in different locations throughout the US, particularly at autopsy. In most cases so far we also have normal tissue which will serve as control for our experiments. We have already collected 23 tumor samples from patients with DIPG since May 2005. This protocol (NBTP02 Comprehensive Molecular Analysis of Tumor Samples Derived from Patients with Diffuse Brainstem Glioma – a Pilot Study) has been approved by the Institutional Review Board of our institution and has been opened since June 2006.

High molecular weight DNA suitable for the SNP array analysis has already been extracted from all tumor samples collected so far. The extraction and analysis of quality of RNA of these tumor samples is still ongoing.

We will determine changes in genomic copy number and LOH using the Affymetrix Genome-Wide Human SNP Array 6.0, a single array containing 1.8 million genetic markers with a median inter-marker distance of 680bp. It features more than 906,600 SNPs, and more than 946,000 probes for detection of copy number variation.

Our studies will utilize 23 samples of DIPG collected so far and 12 samples obtained from patients with focal brainstem tumors (juvenile pilocytic astrocytoma). For genomic analyses, all samples will include matched normal DNA from the patients. We will also evaluate gene expression signatures using Affymetrix Human Genome U133 Plus 2.0 arrays, a single array covering over 47,000 transcripts and variants including approximately 39,000 well-characterized human genes. These studies will be performed on tumor samples only.

Further, we will be able to compare the results from DIPG to a large data collection from pediatric high-grade gliomas located outside of the brainstem, which is available as part of an ongoing project in Dr Baker's laboratory. She has a team with the required bioinformatics and biostatistical expertise in place to evaluate this type of complex data. Analysis of genomic and gene expression data will allow identification of candidate target genes important in DIPG. For example, in results from the pediatric high-grade glioma study, recurrent overlapping deletions within the *CDKN2A* locus readily identified it as an important tumor suppressor in pediatric high-grade glioma (Figure 1, SJ Baker, unpublished results).



**Figure 1.** Composite heatmap of copy number on chromosome 9p for 101 pediatric HGG. The white box indicates the location of *CDKN2A/2B*. Inferred homozygous deletions are red, hemizygous deletions, gold shades, copy number 2 is black.

We have successfully collected the tumor samples, recovered high quality DNA, and have assembled a team with the required clinical, neuropathological, molecular, and biostatistical expertise to complete the proposed studies and generate an unprecedented comprehensive molecular view of DIPG. Our expectation is to start the molecular analysis in the next 3 months.

## References

1. Broniscer A, Gajjar A. Supratentorial high-grade astrocytoma and diffuse brainstem glioma: two challenges for the pediatric oncologist. *The Oncologist* 2004;9:197-206
2. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129-2139
3. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497-1500
4. Louis DN, Rubio MP, Correa KM et al. Molecular genetics of pediatric brain stem gliomas. Application of PCR techniques to small and archival brain tumor specimens. *J Neuropathol Exp Neurol* 1993;52:507-515
5. Zhang S, Feng X, Koga H et al. p53 gene mutations in pontine gliomas of juvenile onset. *Biochem Biophys Res Commun* 1993;196:851-857
6. Sure U, Ruedi D, Tachibana O et al. Determination of p53 mutations, EGFR overexpression, and loss of p16 expression in pediatric glioblastomas. *J Neuropathol Exp Neurol* 1997;56:782-789
7. Cheng Y, Ng HK, Zhang SF et al. Genetic alterations in pediatric high-grade astrocytomas. *Hum Pathol* 1999;30:1284-1290
8. Sung T, Miller DC, Hayes RL et al. Preferential inactivation of the p53 tumor suppressor pathway and lack of EGFR amplification distinguish *de novo* high grade pediatric astrocytomas from *de novo* adult astrocytomas. *Brain Pathol* 2000;10:249-259
9. Gilbertson RJ, Hill DA, Hernan R et al. ERBB1 is amplified and overexpressed in high-grade diffusely infiltrative pediatric brain stem glioma. *Clin Cancer Res* 2003;9:3620-3624
10. Maher EA, Furnari FB, Bachoo RM, et al. Malignant glioma: genetics and biology of a grave matter. *Genes Dev* 2001;15:1311-1333
11. Sallinen SL, Sallinen PK, Haapasalo HK, et al. Identification of differentially expressed genes in human gliomas by DNA microarray and tissue chip techniques. *Cancer Res* 2000;60:6617-6622
12. Rickman DS, Bobek MP, Misek DE, et al. Distinctive molecular profiles of high-grade and low-grade gliomas based on oligonucleotide microarray analysis. *Cancer Res* 2001;61:6885-6891
13. Markert JM, Fuller CM, Gillespie GY, et al. Differential gene expression profiling in human brain tumors. *Physiol Genomics* 2001;5:21-33
14. Kim S, Dougherty ER, Shmulevich I, et al. Identification of combination gene sets for glioma classification. *Mol Cancer Ther* 2002;1:1229-1236
15. Nutt CL, Mani DR, Betensky RA, et al. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res* 2003;63:1602-1607
16. Mischel PS, Shai R, Shi T, et al. Identification of molecular subtypes of glioblastoma by gene expression profiling. *Oncogene* 2003;22:2361-2373
17. Shai R, Shi T, Kremen TJ, et al. Gene expression profiling identifies molecular subtypes of gliomas. *Oncogene* 2003;22:4918-4923

18. Sasaki T, Arai H, Beppu T, Ogasawara K. Detection of gene amplification and deletion in high-grade gliomas using a genome DNA microarray (GenoSensor Array 300). *Brain Tumor Pathol* 2003;20:59-63
19. Nakahara Y, Shiraishi T, Okamoto H, et al. Detrended fluctuation analysis of genome-wide copy number profiles of glioblastomas using array-based comparative genomic hybridization. *Neuro-oncol* 2004;6:281-289
20. Suzuki T, Maruno M, Wada K, et al. Genetic analysis of human glioblastomas using a genomic microarray system. *Brain Tumor Pathol* 2004;21:27-34
21. Freije WA, Castro-Vargas FE, Fang Z, et al. Gene expression profiling of gliomas strongly predicts survival. *Cancer Res* 2004;64:6503-6510
22. Hoelzinger DB, Mariani L, Weis J, et al. Gene expression profile of glioblastoma multiforme invasive phenotype points to new therapeutic targets. *Neoplasia* 2005;7:7-16
23. Nigro JM, Misra A, Zhang L, et al. Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. *Cancer Res* 2005;65:1678-1686
24. Liang Y, Diehn M, Watson N, et al. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci U S A* 2005;102:5814-5819
25. Misra A, Pellarin M, Nigro J, et al. Array comparative genomic hybridization identifies genetic subgroups in grade 4 human astrocytoma. *Clin Cancer Res* 2005;11:2907-2918
26. Bredel M, Bredel C, Juric D, et al. High-resolution genome-wide mapping of genetic alterations in human glial brain tumors. *Cancer Res* 2005;65:4088-4096
27. Mehrian Shai R, Reichardt JK, Ya-Hsuan H, et al. Robustness of gene expression profiling in glioma specimen samplings and derived cell lines. *Brain Res Mol Brain Res* 2005;136:99-103
28. Qi ZY, Hui GZ, Li Y, et al. cDNA microarray in isolation of novel differentially expressed genes related to human glioma and clone of a novel full-length gene. *Chin Med J (Engl)* 2005;118:799-805
29. Khatua S, Peterson KM, Brown KM, et al. Overexpression of the EGFR/FKBP12/HIF-2alpha pathway identified in childhood astrocytomas by angiogenesis gene profiling. *Cancer Res* 2003;63:1865-1870
30. Wong KK, Chang YM, Tsang YT, et al. Expression analysis of juvenile pilocytic astrocytomas by oligonucleotide microarray reveals two potential subgroups. *Cancer Res* 2005;65:76-84

## BUDGET

**Affymetrix Genome-Wide Human SNP Array 6.0:** 70 samples (35 pairs of tumor/normal DNA) x \$420/SNP array 6.0 = \$29,400.

**Affymetrix Human Genome U133 Plus 2.0 arrays:** 35 samples x \$550/U133 Plus 2 array experiment = \$19,250.

**Total cost= \$48,650**