Risk Assignment in Childhood Brain Tumors: The Emerging Role of Molecular and Biologic Classification

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Brain tumors as a group are the most common solid tumors of childhood and currently have the highest mortality rate. A major emphasis has historically been placed on stratifying therapy for these tumors based on histologic and clinical prognostic factors. However, with the increasing application of molecular approaches to refine the categorization of these tumors, it has become apparent that histologically comparable lesions may exhibit diverse patterns of gene expression and genomic alterations, which may correspond with important prognostic distinctions. This paper summarizes these observations and discusses how they are being applied in a preliminary fashion as a foundation for risk-adapted stratification of childhood brain tumor therapy.

Introduction

Central nervous system (CNS) neoplasms are the leading cause of death among childhood tumors [1,2]. Although improvements have been made in the survival of patients with certain tumor types, such as low-grade glioma and nondisseminated medulloblastoma, many other groups of CNS tumors continue to carry a poor prognosis. In addition, even among "favorable" tumor types, a substantial percentage of children ultimately succumb to disease progression. Moreover, children who survive their tumor commonly suffer morbidity from neurotoxic therapies that compromise their long-term quality of life. Accordingly, the overall goals for the treatment of childhood CNS neoplasms are to improve the prognosis of children with tumors that have been resistant to prior therapies, and improve the quality of life of those with treatment-responsive tumors using the results of previous studies as a platform upon which to build new, and hopefully improved, therapeutic strategies. To date, these goals have been pursued by tailoring therapy to target tumor subgroups that are defined solely by histologic and clinical criteria. However, increased utilization of molecular tools to study pediatric brain tumors has led to the recognition that broad groups of histologically similar lesions may actually encompass distinct subgroups that exhibit vastly different prognoses. The implication of this observation is that the historical approach for categorizing brain tumors on a purely histologic basis may need to evolve toward a more refined classification paradigm in which molecular features are employed to promote risk-adapted biologic stratification within individual histologic and clinical subgroups. This review summarizes recent results regarding medulloblastomas, infant brain tumors, and high-grade gliomas that indicate how molecular techniques may supplement histologic information to refine prognostic assessments, and discusses how these strategies are being examined and implemented in ongoing and planned therapeutic studies (Table 1).

Medulloblastoma/Primitive Neuroectodermal Tumor

Clinical factors, such as extent of residual tumor after resection, tumor location, and metastasis stage effectively divide patients with medulloblastoma/primitive neuroectodermal tumor (PNET) into distinct risk groups [3–6,7•]. Children with posterior fossa PNETs who exhibit minimal residual disease after surgery and have no evidence of tumor dissemination on cerebrospinal fluid cytologic examination and MRI are classified as "standard-risk." Such patients have a 70% to 80% 5-year progression-free survival after treatment with standard doses of radiotherapy (3600 cGy to the craniospinal axis and 5400 cGy to the posterior fossa), or with reduced doses of radiotherapy (2340 and 5400 cGy, respectively) in conjunction with adjuvant chemotherapy [3,8]. In contrast, high-risk patients have at best a 50% to 60% 5-year progression-free survival rate. However, these figures imply that there is still

Table 1. Potential prognostic factors for selected childhood brain tumors and COG studies designed to evaluate their prognostic significance

Tumor type	Marker	Potential prognostic effect	COG studies*
Medulloblastoma/PNET	TrkC	Favorable	ACNS0124, ACNS0125
	ErbB2	Adverse	A9961
	Multigene expression	Both	CCG-99701
Infant tumors	*INI1*	Adverse	P9934
	TrkC (PNET)	Favorable	ATRT concept
	ErbB2 (PNET)	Adverse	Infant M+ medullo concept
	Multigene expression	Both	
Gliomas	AGT levels	Adverse	ACNS0126
	MIB1 expression		A9952 (MIB1 only)
	p53 expression		

*All markers within a given tumor category are being examined in each study.
COG—Children's Oncology Group; PNET—primitive neuroectodermal tumor.

substantial uncertainty in the prognosis of individual children. In this context, recent institutional pilot studies have suggested that selected biologic markers may help to refine the accuracy of prognostic assessments.

Segal *et al*. [9] initially reported that high TrkC expression, as determined by Northern blot analysis, was associated with a favorable prognosis in patients with medulloblastoma/PNET. These findings were recently confirmed in an independent group of patients by Grotzer *et al*. [10••], using in situ hybridization analysis of formalin-fixed, paraffin-embedded tissues. In the latter study, 5-year progression-free survival was greater than 75% in 58 patients with elevated TrkC expression, but less than 50% in the 38 patients with low levels of expression ($P<0.00005$). This survival advantage was observed both among patients with favorable clinical risk factors and among those with adverse factors, supporting the independent prognostic utility of this marker.

Despite its powerful association with outcome, it is clear that outcome stratification by TrkC expression alone still carries a high rate of false positives and negatives. To address this limitation, Pomeroy *et al*. [11] incorporated a microarray-based analysis to assess expression of a large panel of genes to further refine outcome predictions. In preliminary experiments, gene expression profiles were generated for a series of 28 patients with medulloblastoma, supratentorial PNET, atypical teratoid/rhabdoid tumor (AT/RT), and malignant gliomas using Affymetrix HuGeneFL 6800 DNA microarrays, which can quantify the expression of more than 6800 genes. The medulloblastoma specimens were clearly segregated from the other malignant tumors and from normal cerebellum by their expression profiles. Gene expression profiles were then obtained from a cohort of 60 patients with medulloblastoma to determine whether expression of a specific subset of genes could be used to predict outcome. Using a variety of algorithms, a five-gene expression-based outcome predictor was derived and compared with clinical parameters for predicting survival. Patients predicted by the five-gene model to have favorable outcome had a 5-year overall survival of 85%, compared with only 22% for patients predicted to have a poor outcome ($P=0.0000057$; log rank analysis), representing by far the most significant predictor of medulloblastoma outcome currently available. Although TrkC was one of the genes in this model, which validates the prognostic data derived in the aforementioned studies, it was clear that analysis of TrkC expression alone did not predict survival as well as the multigene expression panel.

Although these results strongly suggest that multigene expression profiling can improve upon the prognostic assessments based on clinical parameters alone, it will be essential to validate the utility of this panel prospectively using an independent cohort of meticulously staged patients before this approach can be applied as a tool for treatment planning. A practical concern is that these analyses require large quantities of high-quality RNA and would demand standardized analysis conditions as a requirement for their application in the prospective stratification of medulloblastomas. The Children's Oncology Group (COG) will be assessing the feasibility of accomplishing these goals, in parallel with studies of TrkC expression, in two prospective standard-risk medulloblastoma pilot studies that are evaluating the safety of reductions in the dose of craniospinal radiotherapy (ACNS0124) and in the volume of the posterior fossa boost dose (ACNS0125). The expression profiling studies will be conducted within the context of the ACNS01B4 biology study.

In addition to TrkC and the array-based five-gene panel, there are likely to be other molecules that can further refine the stratification of medulloblastoma/PNET. Gilbertson *et al*. [12,13] have reported studies of two patient cohorts that support a role for high levels of ErbB2 receptor expression in aggressive disease behavior. First, in a preliminary study of 47 cases from the recently closed International Society of Pediatric Oncology (SIOP)/PNET III trial, only 57% of cases with 50% or more ErbB2 immunoreactive tumor cells were disease-free 5 years after diagnosis, compared with 87% of

patients with less than 50% ErbB2 immunopositive tumor cells (P=0.02). Second, in a cohort of 41 patients with medulloblastoma, the authors observed seven deaths within 5 years of diagnosis among nine patients with "high" ErbB2-expressing tumors. In contrast, only 12 deaths occurred among 31 patients with "low" expressing tumors (P=0.003). Importantly, when patients with favorable clinical risk factors were analyzed, ErbB2 receptor expression also enabled the separation of patients with poor outcomes from those with excellent long-term survival [14]. As with the above analysis approaches, it will be important to validate these results in an independent, meticulously staged cohort prior to their application for therapeutic stratification. To address this issue, analysis of ErbB2 expression will be included along with TrkC analysis and expression profiling in the ACNS0124 and ACNS0125 studies for newly diagnosed meduloblastoma.

Two other approaches that are being evaluated in pilot studies within the COG to better characterize these tumors include serial analysis of gene expression (SAGE) and comparative genomic hybridization (CGH). SAGE accurately quantifies gene transcript number in a cell population [15]. The method is designed to clone and isolate a 10-base-pair tag at a defined position in the transcript. Ten bases of random sequence are enough to differentiate more than 1 million possible combinations. A SAGE library is a collection of these tags cloned into a sequencing vector for easy counting. There are several advantages to this type of analysis compared with microarray-based gene expression profiling. The first is that it is not necessary to have a hybridization probe for each gene to be assayed, and by sequencing sufficient tags, nearly every expressed gene is assayed. An additional advantage is that digital data are easier to process and deal with statistically. Also, SAGE data are absolute; transcript levels are not relative to another sample or compared with a standard. Each transcript assayed can be expressed as a fraction of the total transcripts counted and comparisons made electronically between all available libraries. Meaningful comparisons can be made between SAGE libraries constructed at different times or in different laboratories, making cumulative data sets possible. This technique thereby provides a hypothesis-generating strategy for identifying novel candidate genes that can distinguish between different classes of tumors, which can then be further evaluated with more focused analyses that target the genes of interest.

Similarly, studies are in progress to develop sensitive methods for identifying genomic alterations in medulloblastoma and supratentorial PNET, as well as other pediatric CNS tumors, using array CGH [16]. This modification of conventional CGH has been developed to replace normal metaphase spreads, as a platform, with large stretches of human DNA (100–200 kb) packaged in replicable units called bacterial artificial chromosomes. These bacterial artificial chromosomes are selected in such a way that they are evenly distributed throughout the human chromosomes and can be arranged in an array to facilitate subsequent analysis after hybridization. Depending on the number of bacterial artificial chromosomes selected, the entire genome can be represented in a single array. The advantages of bacterial artificial chromosome-array CGH include improved resolution, greater reproducibility, availability of direct reagents for subsequent identification of the candidate genes in the minimally deleted regions, and increased throughput.

Infant Embryonal Tumors

Embryonal tumors, such as PNET, account for 30% to 40% of malignant CNS tumors that occur during the first 3 years of life. Although infant PNETs have a poorer prognosis than those that arise in older patients [3,6,17,18], recent studies suggest that outcome in infants with these tumors may be influenced by several of the molecular factors that appear to be prognostically important in older children [11,13]. In addition to the above factors, it has become apparent during the past decade that a subset of tumors previously classified as PNETs actually constitute a distinct group, the AT/RTs, which have had an extremely poor prognosis with conventional therapies [19]. These tumors have some histologic similarities to medulloblastoma/PNETs, but have distinct cytogenetic and molecular features (Fig. 1). In particular, AT/RTs characteristically have monosomy or deletion of chromosome 22, whereas medulloblastoma/PNETs often demonstrate an isochromosome 17q [19,20]. Although these abnormalities are not diagnostic, they are helpful for distinguishing PNETs from AT/RTs. More recently, a region of chromosome 22 (22q11.2) has been identified to contain the *hSNF5/INI1* tumor suppressor gene [20,21••,22]. Loss or inactivation of this gene appears to be the primary genetic event leading to the development of rhabdoid tumors in the brain, as well as the kidney and soft tissues [21••,22]. Germline mutations of *INI1* have been reported in children with apparent AT/RTs, as well as in children with two primary tumors [21••,23,24].

Biegel *et al*. [25] recently summarized the results of their deletion and mutation analysis of a series of 107 rhabdoid tumors obtained from different anatomic sites. Homozygous deletion of *INI1*, as determined by interphase fluorescence in situ hybridization, was detected in approximately 10% of cases, but was most common among extrarenal rhabdoid tumors with apparently balanced cytogenetic translocations involving chromosome band 22q11.2. Monosomy 22, or loss of one copy of 22q11.2, was most frequent among CNS AT/RTs, whereas mitotic recombination or loss and duplication of chromosome 22, resulting in loss of heterozygosity, was more frequent among renal rhabdoid tumors. Mutations of *INI1* were detected in 80 cases. In two tumors, two different mutations were identified, whereas in the other 78 tumors, a single mutation was observed. The mutations were distributed throughout

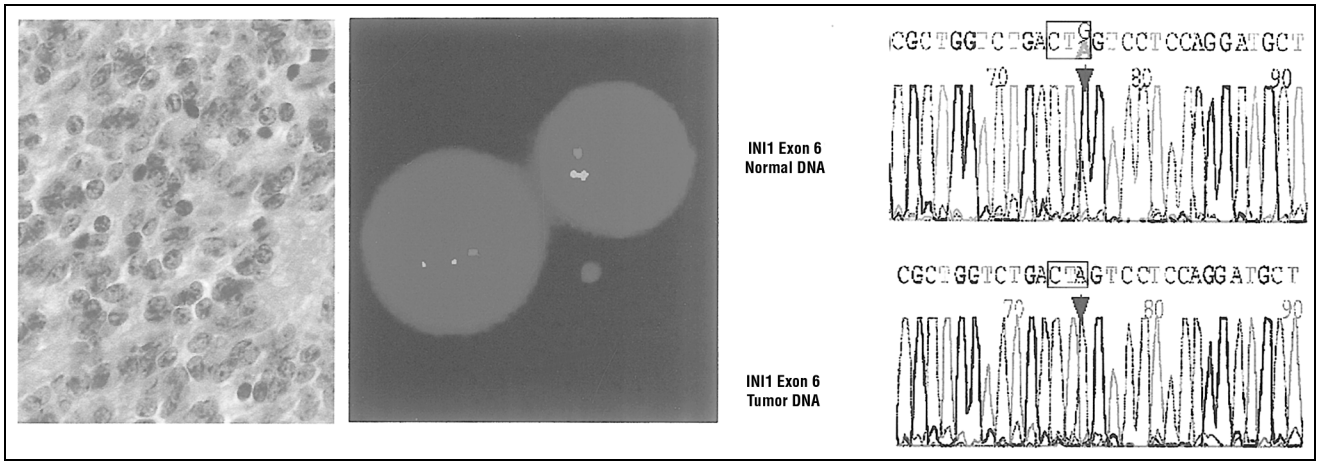

Figure 1. Analysis of an atypical teratoid/rhabdoid tumor (AT/RT) that was initially classified as a primitive neuroectodermal tumor (PNET). **Left**, Hematoxylin and eosin-stained section, showing a poorly differentiated tumor that on initial examination was considered to be a PNET. Subsequent evaluation led to a revised diagnosis of AT/RT. **Middle**, Fluorescence in situ hybridization analysis demonstrates a deletion of chromosome 22. **Right**, *INI1* mutation analysis shows a single base-pair change in this lesion.

the nine coding exons of the gene, although there did appear to be hot spots (exon 9), and some exons (3 and 8) were under-represented. Most mutations were nonsense mutations that coded for a novel stop codon, and predicted premature truncation of the protein. There did not appear to be any difference in the type of mutation observed in CNS atypical teratoid tumors with mixed histologic features, or pure rhabdoid tumors of the brain. Decreased expression of *INI1*, either at the RNA or protein level, has also been observed in a small percentage of tumors without coding sequence mutations. Although approximately 80% of rhabdoid tumors analyzed had deletions and/or mutations of the *INI1* gene, this percentage varied greatly depending on the type of specimen obtained. A much lower percentage of mutations was detected in formalin-fixed and paraffin-embedded samples than from flash-frozen specimens.

The potential utility of cytogenetic and molecular techniques in the evaluation of infants with embryonal tumors is highlighted by the fact that even skilled pathologists often have difficulties distinguishing AT/RTs from other histologically similar lesions. AT/RTs of the CNS are frequently misclassified initially as medulloblastoma/PNET or choroid plexus carcinoma, and less commonly as ependymoma and germ cell tumor [19,26]. Although it was initially thought that the finding of *INI1* mutations in such tumors might indicate lack of specificity of the marker [27], a more recent report that incorporated expert neuropathology review has indicated that *INI1* mutations are extremely uncommon in PNETs, suggesting that an *INI1*-mutated embryonal tumor is probably an AT/RT until proven otherwise [28].

The importance of identifying such tumors prospectively is highlighted by the fact that their prognosis is dramatically worse than infant PNETs [19,26]. Although a logical consequence of this fact is that these two groups of tumors should be treated differently, an essential first step is to achieve a reliable way of making the diagnosis in a clinically applicable fashion, rather than as a research tool. Because AT/RTs are identified by a combination of expert neuropathologic review, cytogenetic studies, fluorescence in situ hybridization using an *INI1*-specific probe, and mutation analysis of the *INI1* gene (Fig. 1) rather than a single test, the feasibility of applying these techniques in a rapid, prospective fashion in a large cohort remains to be confirmed. A new COG study for infants with malignant brain tumors is being developed to address this challenge, incorporating a single site (the COG Infant Tumor/Medulloblastoma Resource Laboratory) as a central facility for performing cytogenetic and molecular genetic testing in conjunction with expert neuropathologic review. In preliminary studies, this site demonstrated the feasibility of performing this analysis within a 3-week time frame. This biologically based stratification scheme would be coupled with distinctive therapeutic approaches for children with AT/RTs versus those with PNETs, which would permit the prospective evaluation of innovative treatment strategies.

Although the information provided by the above techniques as well as the expression profiling and CGH studies noted earlier promise to refine the treatment of infant brain tumors, it is important to emphasize that the close association of this tumor type with a potentially inherited genetic alteration raises important issues for affected patients, their families, and the physicians involved in their treatment and genetic evaluation. These issues center around the need for providing options for genetic testing and counseling of parents who may have other children at risk, and for determining subsequent tumor risk in children who may survive their initial disease.

High-grade Gliomas

High-grade astrocytomas are among the most common and deadly brain tumors of childhood, with most patients dying of tumor progression within 2 to 3 years of diagnosis

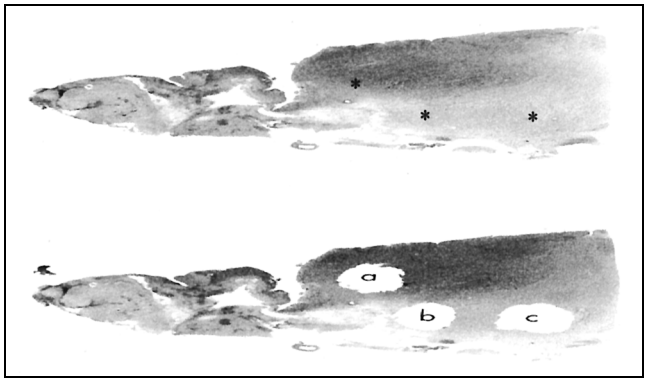

Figure 2. Top, Topographic tissue selection illustrating targets (*asterisks*) identified from a hematoxylin and eosin-stained section. These were excised by microdissection from an unstained serial section. **Bottom**, The accuracy of sample selection is confirmed by staining of post-topographically selected slides.

despite treatment with a combination of surgery, radiotherapy, and conventional chemotherapy [29–31]. However, 20% to 30% of patients respond favorably to conventional therapy and survive for more than 5 years without disease progression. The basis for these widely differing outcomes has remained enigmatic, even taking into account known prognostic factors, such as patient age, extent of tumor resection, mode of adjuvant therapy, and histologic subgrouping (*ie*, anaplastic astrocytoma [grade 3] versus glioblastoma multiforme [grade 4]) [29–31]. Accordingly, there is a need to identify biologic markers that supplement clinical and histologic information to refine therapeutic decision making. In contrast to the extensive work done to characterize the patterns of genetic alterations in adult high-grade gliomas [32••,33], limited information is available on the molecular features of pediatric gliomas. Institutional studies by Pollack *et al*. [34•,35,36•] and others [37–40] suggested that pediatric malignant gliomas may differ on a molecular basis from primary adult high-grade gliomas. In studies incorporating an institutional patient cohort, Pollack *et al*. [34•] also noted that the presence of *TP53* mutations was associated with an adverse prognosis, implying that the molecular genetic features of these tumors might provide a biologic basis for refining outcome predictions and stratifying therapy. These studies also indicated a strong association between MIB-1 proliferation index and outcome as assessed by labeling the Ki-67 antigen using the MIB-1 antibody [41]. However, the small size of these cohorts as well as the association between marker status and histology precluded determining whether these markers could provide data that had independent prognostic utility.

Children's Cancer Group (CCG) study B975 was initiated to more conclusively evaluate the association between biologic and molecular genetic features and outcome in pediatric high-grade gliomas. This study incorporated the multi-institutional cohort of CCG-945, the largest clinical study of pediatric high-grade gliomas to date. The large size of the cohort, coupled with the availability of central neuropathology review and comprehensive clinical data, provided a unique opportunity to address issues of molecular etiology and prognostic factors for these tumors. The markers to be examined in these studies were selected from a panel of putative markers that had been evaluated in preliminary studies with institutional cohorts of childhood gliomas [34•,35,36•,41], including a number of genes implicated in pathways of adult glial neoplasia. These approaches incorporated a combination of microdissection-based topographic genotyping with loss of heterozygosity and gene sequence analysis and immunohistochemical assessment. An example of the topographic selection of a glioma specimen is shown in Figure 2.

Tumor specimens were obtained on 179 children enrolled on this study [42], making this the largest collection of microdissected glioma specimens accrued from a consistently treated patient cohort, as well as the largest group of childhood malignant glioma specimens ever assembled. Although this study remains in progress, a review of its results to date is summarized below.

One goal of this study was to evaluate the association between proliferation index and outcome in children with malignant gliomas. As an initial step to assess prognostic utility of this marker, the cohort was divided into three groups: those with proliferation indices less than 18% (below the median), those with indices between 18% and 36% (the next highest quartile), and those with indices of 36% or more (the highest quartile). Five-year event-free survivals in these three groups were 44% ± 6% (n=71), 30% ± 8% (n=33), and 10% ± 6% (n=29), respectively (P<0.0001, log rank test) [42].

As expected, there was a strong association between proliferation index and histopathologic grade, with a significant difference in the mean proliferation indices among tumors classified as anaplastic astrocytoma (AA: 18.2 ± 2.31) versus glioblastoma multiforme (GBM: 29.2 ± 2.82, P=0.004, student's t test). Despite this correlation between histology and proliferation, MIB-1 labeling had an independent association with outcome (P<0.0001, log rank test stratified for histology), and this was also apparent after adjusting for the extent of resection, age, or sex in multivariate regression modeling (P<0.0005), or when performing the multivariate analysis using the diagnosis established on central pathology review (P<0.005). The association between MIB-1 labeling index and outcome was evident among tumors classified as AA (P=0.002) (Fig. 3A) and those classified as GBM (P=0.002) (Fig. 3B). Although there was no evidence from multivariate modeling analysis that a discrete cut-point for MIB-1 index value existed that could identify a "good-risk" group of anaplastic astrocytomas or glioblastomas, it was clear that MIB-1 labeling identified an extremely poor-risk group of tumors with proliferative indices greater than approximately 36%, in which the probability of long-term survival was less than 5%, sub-

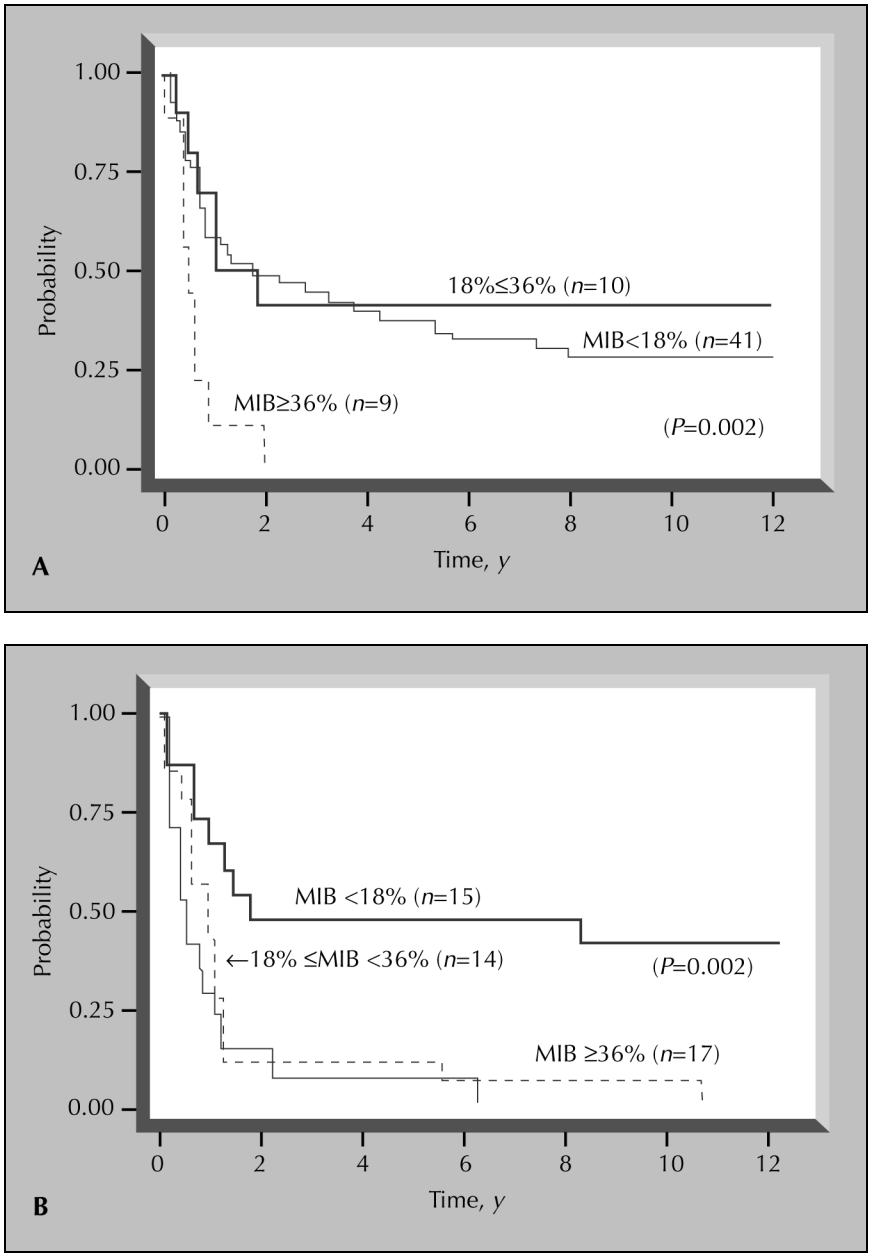

Figure 3. Event-free survival in children with anaplastic astrocytoma (**A**) and glioblastoma multiforme (**B**) was strongly associated with MIB-1 labeling indices.

stantially worse than in the overall cohort. Moreover, the analysis also identified a subgroup of patients with extremely low MIB indices (< 5%), which corresponded to a subset of tumors in which the institutional diagnosis was found to have been discordant based on an independent, blinded central review. Generally, such cases were reclassified as atypical low-grade gliomas. Based on these results, it was concluded that MIB-1 labeling index was not only a useful adjunct for guiding the process of histologic classification, but also an important independent prognostic marker for refining the accuracy of histologically based prognostic assessments.

A second marker studied in this cohort was alteration in the p53 pathway, as determined both by direct mutational genotyping and p53 immunohistochemistry. To date, the status of *TP53* mutations in exons 5 to 8 has been assessed in 121 tumors, and p53 immunohistochemistry completed in 115. Forty tumors (33.1%) had mutations of *TP53* within exons 5 to 8, and four had p53 overexpression. Tumors with p53 overexpression and/or *TP53* mutations exhibited a substantially worse event-free survival than those lacking these features [43]. Five-year event-free survival was 43% ± 6% in the 74 tumors with low levels of p53 expression versus 17% + 6% in 41 tumors with p53 overexpression (P=0.0001). A weaker association was observed between *TP53* mutations and outcome. Five-year event-free survival was 38% ± 5% in the 81 tumors without mutations of exons 5 to 8 versus 23% ± 7% in the 40 with mutations (P=0.09).

Because it was recognized that the genotyping approach used would not detect mutations in areas of the *TP53* gene other than exons 5 to 8 or alterations of other genes that might deregulate p53 function, the data derived

from *TP53* mutation analysis was also examined in conjunction with information regarding p53 overexpression in an either/or fashion. This analysis indicated that the presence of either *TP53* mutations or p53 overexpression was an adverse prognostic factor in the overall group. Among the 109 tumors that had been evaluated for both parameters, the 41 that exhibited neither p53 overexpression nor *TP53* mutations had a 5-year event-free survival of 46% ± 8% versus 22% ± 5% in the 68 tumors that had either of these features (P=0.007).

In contrast to previous results in adult high-grade gliomas, this analysis also demonstrated that p53 mutations and overexpression were significantly more common in grade 4 than grade 3 tumors. Whereas p53 overexpression was detected in 58% of glioblastomas, it was apparent in only 26% of anaplastic astrocytomas and 21% of other grade 3 gliomas (P<0.002). Despite the strong association between p53 alterations and histology, this marker was independently associated with outcome after stratifying for histology (P=0.006). In the 22 glioblastomas with p53 overexpression, 2-year event-free survival was only 9.1% ± 6% versus 44% ± 12% in the 16 without p53 overexpression (P=0.047).

A second clinical factor that was strongly associated with p53 status was patient age. Among tumors with a centrally reviewed diagnosis of either anaplastic astrocytoma or glioblastoma multiforme, mutations were observed in only two of 17 lesions (11.8%) from children younger than 3 years of age, compared with 24 of 60 lesions (40%) from children between 3 and 18 years of age (P=0.04) [44].

Taken together, the results of this analysis indicated not only an association between p53 status and histology, but also a strong association between p53 status and outcome, independent of tumor histology, which differs from results in adult malignant gliomas. These data, in conjunction with other recent results [36•], support the contention that childhood and adult gliomas may exhibit distinct pathways of tumor progression. In addition, the finding that children younger than 3 years of age had a significantly lower frequency of *TP53* mutations than older children raises the issue of whether the tumors in such patients may have arisen by a molecular pathway distinct from that in older patients. A trend towards more favorable outcomes among young children than older children with malignant gliomas has been noted by other groups [17], which may reflect a greater sensitivity of the postoperative residual disease to adjuvant therapy associated with a normal p53 pathway.

It should be noted that these observations do not support reduction of therapy based on "favorable" p53 status, but rather indicate that prognosis in children with malignant gliomas may be significantly influenced by molecular genetic factors, independent of other known prognostic factors, and that such molecular factors may help to refine prognostic assessments. In that context, these results call attention to a sizeable subgroup of children with high-grade gliomas who had an almost uniformly unfavorable prognosis following treatment with surgery, radiotherapy, and conventional chemotherapy, who may be appropriate candidates for more intensive or innovative therapeutic approaches. The demonstration that such features can influence long-term response to conventional chemotherapy and radiotherapy has provided an impetus for including their analysis in the prospective evaluation of novel therapeutic strategies for these tumors, as in the newly developed COG high-grade glioma study, ACNS0126.

In addition to proliferation and p53 status, a variety of other markers are currently being examined for their potential association with outcome in children with malignant gliomas, based on their association with disease progression in adult gliomas. These include *EGFR* amplification, which is a hallmark of primary glioblastomas in adults, but appears to be much less common in childhood gliomas [35,39]; *PTEN* deletions, which have been associated with an unfavorable outcome in an initial institutional pilot study from the Mayo Clinic [38]; and alterations of cell cycle–control genes, such as *CDKN2A*, $p14^{ARF}$, and *CDK4* [32••,33]. In contrast to the potentially adverse prognostic effects of the above factors, studies involving adult high-grade gliomas indicate that deletions of chromosomes 1p and 19q may identify a subgroup of tumors with oligodendroglial features that have a generally favorable prognosis [45•]. In this regard, Pollack *et al.* [46] reported a case of an infant who presented with a disseminated malignant glioma with multiple leptomeningeal metastases, which was classified as an anaplastic astrocytoma by multiple neuropathologists, but was later noted to have 1p/19q deletions. Notwithstanding the anticipated dismal prognosis, this child exhibited a complete radiologic remission after only two cycles of multiagent chemotherapy with ifosphamide, vincristine, carboplatin, and etoposide. Because of this patient's young age, he was watched expectantly without irradiation upon completion of maintenance chemotherapy. He remains progression-free 4 years later, having never received radiotherapy [46]. The 1p/19q deletions were only appreciated in retrospect, because these studies were not in routine use at the time of his diagnosis. However, it is conceivable, based on these observations, that this pattern of genetic changes, which has heretofore not been reported as a prognostic factor in childhood malignant gliomas, may also signal a favorable treatment response profile in these tumors. This potential association is currently being evaluated in the B975 patient cohort. In view of the cognitive risks of irradiation in young children and the potential for improvement in functional outcome if this modality can be deferred [17], there would be a clear benefit to identifying prospectively those patients who may be safely managed with chemotherapy alone.

In addition to the above molecular markers, other biologic factors associated with drug resistance mechanisms have been reported to influence prognosis in malignant gliomas. In particular, several studies have noted that the response of

malignant gliomas to alkylating agents, such as nitrosoureas and temozolomide, may be strongly influenced by expression of enzymes that contribute to drug resistance mechanisms, such as alkylguanine alkyltransferase and mismatch repair proteins [47,48,49•]. In several institutional studies, tumors with alkylguanine alkyltransferase overexpression have been observed to have a worse prognosis for event-free survival after treatment with nitrosoureas [47,49•], and tumors with mismatch repair deficiency have shown a worse response to temozolomide [48]. Because these agents comprise an important element in the therapy of malignant gliomas, the evaluation of their markers is being undertaken in a prospective fashion in the ACNS0126 high-grade glioma study.

Conclusions

Molecular analysis approaches hold promise in refining the management of children with malignant brain tumors, although the optimal application of these data requires that several conditions be met. An important initial step in the utilization of these tools to guide clinical decision-making involves translating these technologies from the research setting into clinically applicable diagnostic studies that can be performed rapidly and reliably on surgically obtained tumor specimens. Secondly, the validity of these prognostic correlates must be confirmed in an independent, preferably prospective, cohort of patients. Studies designed to address both of these challenges for several types of pediatric brain tumors are currently either in progress or under development within the Children's Oncology Group. A third challenge that awaits accomplishment of the first two involves demonstrating that biologic stratification can support risk-adapted therapeutic stratification that will improve the outcome of children with brain tumors. The realization of this long-range goal will require the identification of novel therapeutic strategies that hold promise for improving outcome in tumor subgroups, such as AT/RTs, that have been resistant to conventional therapies. An equally important aspect of this goal will involve the continuation of ongoing efforts to cautiously decrease the intensity of potentially neurotoxic therapies in order to reduce the morbidity of treatment in tumors that have particularly favorable risk factors.

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