Embryonal Central Nervous System Neoplasms Arising in Infants and Young Children

A Pediatric Brain Tumor Consortium Study

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Context.—Medulloblastomas (MBs) and atypical teratoid/rhabdoid tumors (AT/RTs) arising in infants and children can be difficult to distinguish; however, histologic characterization is prognostically important.

Objective.—To determine histologic and phenotypic markers associated with utility with progression-free survival (PFS) and overall survival (OS) in children younger than 3 years with MBs and AT/RTs.

Design.—We undertook a histologic and immunophenotypic study of MBs and AT/RTs arising in infants and children younger than 3 years treated in a Pediatric Brain Tumor Consortium study. The 41 girls and 55 boys ranged in age from 2 to 36 months at enrollment. These infants and children exhibited 51 MBs, 26 AT/RTs, and 24 other tumors (not further studied). Median follow-up of the patients was 17.2 months from diagnosis (range: 1.4–93 months).

Results.—Infants and children with AT/RT exhibited a statistically significant shorter PFS and OS when compared to infants and children with MBs (both $P < .001$). A lack of nuclear BAF47 immunohistochemical reactivity proved reliable in identifying AT/RTs. Among MBs, our data suggest an association of nodularity and prolonged PFS and OS, which must be independently confirmed. Anaplasia correlated with OTX2 reactivity and both OTX2 and moderate to severe anaplasia correlated with PFS but not with OS.

Conclusion.—Distinguishing AT/RT from MBs is clinically important. For expert neuropathologists, the diagnoses of AT/RT and MB can be reliably made from hematoxylin-eosin stains in the vast majority of cases. However, certain rare small cell variants of AT/RT can be confused with MB. We also found that immunohistochemical reactivity for BAF47 is clinically useful in distinguishing MBs from AT/RTs and for identifying certain small cell AT/RTs. Among MBs, nodularity may be an important prognostic factor for improved PFS and OS in infants and children.

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investigate the feasibility of the addition of regional therapy with intrathecal mafosfamide to an intensive regimen of systemic chemotherapy in children younger than 3 years at diagnosis with MB, PNETs, ependymoma with metastatic disease, or other primary intracranial embryonal tumors.

Medulloblastoma is designated grade IV by the World Health Organization and is one of the most common malignant solid tumors of infancy, but exhibits widely varying responses to radiation therapy and chemotherapy, responses that may vary by age, genetic abnormalities, and histologic features. The tumor has been the target of a large number of histologic and molecular studies, with 3 major variants commonly recognized: a nodular variant, a diffuse (or classic) variant, and a large cell/anaplastic variant, the last of which may or may not be a subset of diffuse tumors. Studies seem to indicate that therapeutic responses show a correlation with the morphologic variants, with nodular tumors having a significantly better than average prognosis and anaplastic variants having a much worse than average prognosis. Recent studies have suggested that OTX2 is overexpressed in MBs with anaplastic features and may represent an objective means of identifying anaplasia. Not clear is whether or not degrees of anaplasia or focal regions of anaplasia in a small biopsy sample are of prognostic significance in this age group. Tumors with nodularity frequently also demonstrate desmoplasia as identified by diffuse pericellular reticulin, however, not all cases with diffuse pericellular reticulin exhibit pale nodules. Also controversial in this age group is whether the nodular variant known as MB with extensive nodularity (MBEN) deserves a grade IV designation.

The AT/RT is an aggressive, malignant, embryonal tumor most commonly composed of epithelioid cells with round nuclei and prominent nucleoli with frequent mitotic figures. Often the tumor displays a spectrum of rhabdoid cells, epithelioid cells, and spindle cells. Genetically, it exhibits silencing mutations affecting the hSNF5/INI1 complex, also known as the BRG1-associated factors and called BAF47. Recently, it has been shown that the immunohistochemical loss of BAF47 reactivity identifies the vast majority of AT/RTs. Arising in the cerebrum and cerebellum, the tumor is often radiographically mistaken for CNS PNET or MB, based on location. The advent of the immunohistochemical detection of BAF47 has helped not only to clarify the morphologic spectrum of these tumors, but also to reveal that certain tumors may lack the epithelioid cytoplasm and exhibit small round cells with high nuclear to cytoplasmic ratios. Accordingly, recent studies have recommended that all cerebral and cerebellar tumors with embryonal features be studied immunohistochemically for BAF47 loss. Not only has this practice identified a widened spectrum of tumors now recognized to be AT/RT, but also there has been a recognition of a worse than average survival among these cryptic small round cell AT/RTs versus MBs, supporting the value of a pathologic distinction.

The present study undertook an analysis of histologic, histochemical, and immunohistochemical features of these infantile embryonal tumors. The goal was to investigate whether or not a set of histologic markers are of diagnostic and prognostic utility. In order to accomplish this, we analyzed the interobserver reproducibility of the diagnoses issued by 3 neuropathologists with expertise in pediatric brain tumors and compared these diagnoses against the diagnoses issued by the originating institutional pathologists. We also assessed the utility of reticulin staining and immunohistochemical reactions for OTX2 and BAF47 as objective standards for predicting PFS and OS.

The results indicate that the diagnoses of MB, AT/RT, and PNET are valid and reproducible as determined by a 2 out of 3 consensus being at least 95%. The data also support the routine use of BAF47 immunoreactivity in analyzing small cell embryonal tumors, the use of OTX2 to characterize anaplasia in MBs, the identification of moderate to severe anaplasia in MBs, and the recognition of nodularity in MBs, all of which proved significant in characterizing prognosis in embryonal tumors of the pediatric CNS.

MATERIALS AND METHODS

Tumors in this study were embryonal neoplasms from infants and children younger than 3 years entered into the PBTC Protocol 001 (clinicaltrials.gov Identifier: NCT00042367), a pilot study that tested the utility of intrathecal mafosfamide to supplement intensive chemotherapy and radiation therapy of embryonal tumors and metastatic ependymomas. This study was performed with multi-institutional institutional review board approval. The diagnosis of the originating institution was considered the gold standard, as the institutional pathologists had full access to the clinical histories, the radiographic studies, and any ancillary diagnostic studies required to establish the diagnosis. The primary goal of the 3 neuropathologists (R.E.M., A.A., and V.R.) was to independently render a diagnosis based on hematoxylin-eosin stains of representative sections of tumors. A secondary goal was to characterize and/or quantitate the presence of a variety of histologic features often associated with pediatric embryonal neoplasms. For MBs, the presence of reticulin, OTX2 expression, and nodular, classic, anaplastic, and large cell morphologies were identified and recorded directly into a central, online database, ProtoTrak, maintained by the PBTC Operations and Biostatistics Center. Cases in which at least 2 of 3 reviewers identified a case as exhibiting anaplasia were subsequently evaluated for degree of anaplasia and extent of anaplasia by 1 reviewer (R.E.M.). Cases identified by at least 2 of 3 reviewers as being nodular were further analyzed for the diagnosis of MBEN by the same neuropathologist.

Medulloblastoma is a PNET defined by location in the cerebellum. The diffuse pattern of MB presents a histologic appearance of densely packed cells with round-to-oval or carrot-shaped hyperchromatic nuclei surrounded by scanty cytoplasm. The classic pattern is similarly characterized by a diffuse growth pattern populated by groups of monomorphic cells with round, regular nuclei in which the chromatin is less condensed; neuroblastic or Homer-Wright rosettes are most commonly encountered in this group. Although this definition is unrelated to the presence of reticulin, reticulin is only rarely encountered in these tumors. Nodular variants of MB exhibit many of the cytologic features of the classic variant; however, they vary by 2 distinctive features: pericellular reticulin in the monotonous zones and around the nodules (Figure 1, A and B). Nodules are composed of circumscribed collections of cells with neurocytic features, including round nuclei and relatively more cytoplasm than found in the surrounding tumor, and are occasionally associated with obvious streaming neuropil. Reticulin is absent in these nodules and mitotic activity is infrequent. Some tumors exhibit a profound collection of such nodules, many of which are associated with sweeping lines of neuropil and rare compressed regions of internodular cells. These MBENs are considered a subgroup of the nodular variant for this study.
Some MBs exhibit regions of pericellular reticulin, which may occur either focally or diffusely. Pericellular reticulin invariably occurs where tumor cells invade the leptomeninges. However, pericellular reticulin is more commonly a widespread phenomenon among the internodular cells of nodular MBs. In small biopsies, it is often not possible to distinguish between the 2 etiologies of pericellular reticulin. Therefore for the purposes of this study, reticulin was categorized as negative, multifocal, or diffuse, according to its distribution in the histologic sections, without regard to the presence of nodules (Figure 2), a common accompanying feature.

The large cell variant (Figure 3) of MB is defined in the literature as a tumor composed of tumor cells with large, round, vesicular nuclei, prominent nucleoli, and variably abundant eosinophilic cytoplasm. Anaplasia (Figure 4) is identified by a mitotically active tumor that both demonstrates elongated hyperchromatic nuclei that are densely crowded and exhibit characteristic nuclear wrapping against adjacent tumor cells and demonstrates abundant individual cell necrosis that also aggregates into geographic regions of necrosis. Tumors with features of both large cells and severe anaplastic cells often occur together, lending the group the name “large cell/anaplastic.”

No consensus definition for moderate dysplasia exists, but our experience indicates that tumors that lack the full features of severe dysplasia most commonly demonstrate frequent individual cell necrosis. Therefore our working definition of moderate anaplasia differs from severe anaplasia by the absence of geographic necrosis but features a “starry-sky” pattern of apoptosis dotting the profusion of small tumor cells. Similarly, no consensus definition exists concerning mild anaplasia. Therefore, our working definition was that mild anaplasia lacks both geographic necrosis and single-cell apoptosis but does exhibit nuclear molding and hyperchromasia. Tumors with round regular nuclei and a high overall cellular density were considered to lack anaplasia. The degree of anaplasia was based on the most severe component identified; its distribution was graded as absent, focal, or diffuse.

Atypical teratoid/rhabdoid tumor is a tumor characterized by a polymorphous cytologic phenotype (Figure 5A), which encompasses rhabdoid cells and expresses both neural and
Figure 4. A, Severely anaplastic medulloblastomas (MBs) with dark, pleomorphic nuclei overlapping adjacent tumor cells. B, Severely anaplastic MBs with frequent apoptotic nuclei and geographic necrosis. C, Moderately anaplastic MBs with starry-sky apoptosis. D, Mildly anaplastic MBs with hyperchromatic nuclei and rare apoptotic cells (hematoxylin-eosin, original magnifications ×40 [A and B] and ×20 [C and D]).

Figure 5. A, Epithelioid features and large, round, open nuclei with prominent nucleoli are features of the atypical teratoid/rhabdoid tumors. B, Scattered islands of immunoreactivity without obvious differentiation are also found (hematoxylin-eosin, original magnification ×40 [A]; glial fibrillary acidic protein, original magnification ×40 [B]).
Table 1. Comparison of Diagnostic Agreement Among Reference Pathologists Reviewing Hematoxylin-Eosin Slides of Representative Blocks of Tumor From the Entire Cohort of 96 Patients Versus the Referring Institutions’ Diagnoses as Either Medulloblastoma (MB) or Atypical Teratoid/Rhabdoid Tumor (AT/RT)

<table>
<thead>
<tr>
<th>Institutional Diagnosis</th>
<th>Pathologist 1</th>
<th>Pathologist 2</th>
<th>Pathologist 3</th>
<th>3 of 3, No. (%)</th>
<th>At Least 2 of 3, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB (n = 42)</td>
<td>45</td>
<td>39</td>
<td>40</td>
<td>38 (90.5)</td>
<td>40 (95.2)</td>
</tr>
<tr>
<td>AT/RT (n = 26)</td>
<td>23</td>
<td>29</td>
<td>24</td>
<td>15 (57.7)</td>
<td>23 (88.5)</td>
</tr>
</tbody>
</table>

nonneuroepithelial immunophenotypic markers, including not only a lack of immunoreactivity for the IN11/BAF47 gene product, but possibly also scattered islands of immunoreactivity for glial fibrillary acidic protein (Figure 5B), epithelial membrane antigen, and smooth muscle actin, among others.

**Histochecmical Methods**

The Wilder method to detect the presence of reticulin was used to define presence or absence of desmoplasmia.

For immunohistochemical assays, formalin-fixed, paraffin-embedded sections were cut at 5 μm, deparaffinized in xylene, and brought to water through graded alcohols. Endogenous peroxidases were blocked in 3% H₂O₂ in dH₂O at room temperature for 10 minutes. For anti-OTX2 immunohistochemistry, all sections were incubated in citrate buffer, pH 6.1, for 20 minutes at 100°C, blocked with 10% normal rabbit serum for 1 hour, and incubated with OTX2-specific goat polyclonal antibody (dilution 1:80; AF1979, R&D, Minneapolis, Minnesota) for 45 minutes at room temperature, followed by 30 minutes of incubation with biotinylated rabbit anti-goat secondary antibody at 1:200 dilution (BA5000, Vector Laboratories, Burlingame, California). Detection of antibody binding was performed using Vectastain Elite ABC reagent (PK-7100, Vector Laboratories) according to the protocol. For the slides to be tested for BAF47 expression, the slides were placed in preheated Tris/EDTA, pH 9.0, and heated for 20 minutes in a 100°C water bath. Solutions and slides were cooled for 20 minutes, washed in deionized water, and placed in Tris-buffered saline, pH 7.5. Primary antibody mouse monoclonal BAF47 (1:200; B-D Biosciences, San Jose, California) or negative control reagent mouse IgG was applied and incubated for 1 hour at room temperature. Detection of antibody binding was performed using horseradish peroxidase-conjugated anti-mouse Envision Plus (Dako, Carpinteria, California) using diaminobenzidine. All slides were washed with tap water, hematoxylin or OS werestain was applied, followed by dehydraulation with absolute alcohol, clearing with xylene, and coverlapping with a permanent mounting media.

**Statistical Methods**

Descriptive statistics were used to assess the interrater agreement on diagnostic and diagnostic feature calls. Agreement of diagnostic calls by the 3 pathologists was assessed independently and with respect to institutional diagnosis calls. Associations of diagnostic value of consensus calls with OS and PFS distributions were investigated. PFS was defined as the interval from initiation of treatment to the earliest indication of disease progression or death on study for patients who failed. OS was defined as the interval from initiation of treatment to death. Data from patients with failure for PFS or OS were censored at off-study or at the patient’s last date of follow-up. Distributions of PFS and OS were estimated using the Kaplan-Meier method and compared using the log-rank test. An exact Cochran-Armitage trend test was used to compare degree of anaplasia with the likelihood of OTX2 presence. No multiplicity correction was used for the P values reported in this study, and because of the number of tests performed, statistical significance was called only if the P value was <.002.

**RESULTS**

The patients, 41 girls and 55 boys, represented in this study ranged in age from 2 to 36 months at the time of enrollment. Median follow-up of the patients was 17.2 months from diagnosis (range: 1.4–93 months).

**Case Identification**

Table 1 represents the diagnoses rendered by each pathologist compared to the treating institution’s original diagnoses of MB or AT/RT (Table 1). As the central reviewers were not provided with site of origin, MB, PNET, and pineoblastoma (PB) were combined into a single diagnostic category. Of 96 patients treated on PBTC-001 protocol with pathology data, the institutional pathologists reported 42 having MB. Of these 42 patients, the 3 central pathologists had perfect consensus on MB/PNET/PB for 38 patients (90.5%), and had at least 2-of-3 consensus for 40 cases (95.2%). One case was diagnosed as AT/RT by 2 of the 3 central pathologists, and 1 case was diagnosed as pleomorphic xanthoastrocytoma by 2 of the 3 central pathologists. Among the 54 cases for which the institutional diagnosis was not MB, 5 cases were diagnosed as MB/PNET/PB by all 3 central pathologists, and 3 cases were diagnosed as MB/PNET/PB by 2 of the 3 central pathologists. The treatment protocols were based on the diagnoses rendered by the treating institutions and no changes in diagnoses were made based on the results of these studies.

The 2-of-3 consensus data are slightly misleading in that diagnoses of MB were rendered on more than 42 cases by the central reviewers shown in Table 1. When viewed from the perspective of the central pathologists who were reviewing the entire cohort of 96 cases, there were 55 cases of MB/PNET/PB. Out of these 55 patients, the 3 central pathologists had perfect consensus on MB/PNET/PB for 43 patients (78%), and at least 2-of-3 consensus for 50 cases (91%) indicating that there were an additional 13 cases for which we derived the diagnosis of MB/PNET/PB that were either PNETs or PBs by the institutional diagnosis. More informative for the purposes of this study are the 5 non-MB/PNET/PB diagnoses on cases in which the institutional diagnosis was not MB, PNET, or PB. For these 5 cases, the differential diagnoses included AT/RT, malignant lymphoma, pleomorphic xanthoastrocytoma, and glioblastoma.

AT/RT proved to have a strong trend of recognizability and resulted in reasonably accurate diagnoses (Table 1). The institutional pathologists reported 26 patients having AT/RT. Out of these 26 patients, the 3 central pathologists had perfect consensus on AT/RT for 15 patients (57.7%) and had at least 2-of-3 consensus for 23 cases (96.2%). For the remaining 3 cases, the differential diagnostic considerations from the central reviewers included malignant CNS tumor and MB/PNET/PB. Other diagnoses made by the central reviewers included CNS germ cell tumor, choroid plexus carcinoma, anaplastic ependymoma, and malignant meningioma, all of which reflect the epithelial and occasionally papillary appearances manifested by the tumor.
At the conclusion of the study, 27 patients with MB and 9 with AT/RT were alive.

**Medulloblastoma—Anaplasia and Nodularity**

The qualitative assessment of the identification of nodular status, classical growth pattern, diffuse growth pattern, presence of anaplastic component, and presence of large cell component all proved to produce close to perfect consensus, with a minimum 2-of-3 consensus being at least 95% in any of these evaluations (Table 2). Anaplasia was identified by at least 2 of the reviewers in 14 (25.5%) of 55 MB samples submitted for review.

Based on an agreement of 2 of 3 neuropathologists (Table 3), those patients whose tumors exhibited any anaplastic features (mild to severe) included those with diffuse large cell/anaplastic morphology (n = 11); nodular with large cell/anaplastic morphology (n = 12); and diffuse with anaplasia morphology (n = 7). The findings of an analysis of the 3 central reviewers who characterized anaplasia as simply present or absent suggested that anaplasia was not associated with OS or PFS in this age group (Table 3). However, analysis based on degree of anaplasia suggests that patients with tumors exhibiting moderate or severe anaplasia (Figure 5) may have a worse prognosis (Table 3). Similarly, MB patients whose tumors exhibited OTX2 immunoreactivity may have a worse prognosis. OTX2 immunoreactivity was compared against degree of anaplasia via an exact Cochran-Armitage trend test that demonstrated a P value of <.001, which suggests that as anaplasia gets more severe, there is a higher likelihood of OTX2 presence. We also undertook to further characterize anaplasia by extent of tumor involved. No further associations with survival distributions were suggested among these patients by subgrouping according to absent anaplasia (n = 20), focal anaplasia (n = 17), or diffuse anaplasia (n = 7).

Figure 6, A and B, suggests that nodularity may be a positive prognostic factor. Because MBEN is morphologically distinctive, we further investigated the survival associations among patients whose tumors exhibited nodularity by comparing survival of MBEN (n = 11) versus nodular MB (n = 8) and found insignificant differences in PFS (P = .08) and OS (P = .74; Table 3).

We further analyzed effect on survival of both nodular patterns versus diffuse MBs (n = 21), in which no nodular growth pattern was discernible (Figure 7, A and B). Among these growth patterns, tumors with any nodularity exhibited a better OS (P = .004; Table 3). In contrast, the presence of fine reticulin-staining fibers coursing through the tumor, a finding that commonly accompanies nodular MBs in older children and adults, did not exhibit a statistically significant effect on either PFS (P = .13) or OS (P = .89). In this age group, desmoplasia, as identified by reticulin-positive fibers in the tumor, is biologically distinctive from nodularity (Figure 3). However, in adults, nodular tumors commonly display reticulin-positive fibers; we found that in this age group many diffuse tumors also demonstrate pericellular reticulin. Thus, the presence of nodularity is a prognostically beneficial finding, but the presence of reticulin-staining fibers is not. However, this may represent our lack of distinction as to the source of pericellular reticulin from cases with leptomeningeal infiltration; it is difficult to know how much weight to place on this finding.

**Atypical Teratoid/Rhabdoid Tumor**

As a result of the previous studies of Haberler et al., BAF47 immunohistochemistry was performed on 32 tumors for which at least 1 pathologist made a diagnosis of MB; BAF47 reactivity was diffuse in 17 cases, multifocal in 14 cases, and absent in 1 case. The diagnosis of the BAF47-negative MB (60775; shown in Figure 8, A and B) was agreed upon by 2 of the 3 central reviewers; it had no epithelial elements and was histologically identical to a diffuse MB without anaplasia. To compare the immunoreactivity of BAF47 in these tumors versus the AT/RTs, a sample of 6 AT/RTs as diagnosed by the originating institution were analyzed for BAF47 expression; BAF47 was absent in 5 of 6 (Table 4). The remaining case diagnosed by the outside institution as AT/RT (107487; Figure 9, A and B) was diagnosed as PNET by 2 of 3 central reviewers. Survival analysis based on the diagnoses of the originating institutions revealed significant differences in PFS and OS (P < .001 and P < .001, respectively) with regard to making a diagnosis of MB versus AT/RT (Figure 10).

**COMMENT**

Among infants and children less than 5 years, CNS tumors affect more than 1300 each year in the United

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**Table 2. Comparison of Diagnostic Agreement Among Reference Pathologists for Various Descriptors in Medulloblastomas**

<table>
<thead>
<tr>
<th>Variable of Interest</th>
<th>3-of-3 Consensus, %</th>
<th>At Least 2-of-3 Consensus, %</th>
</tr>
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<tbody>
<tr>
<td>Classic component</td>
<td>21.4</td>
<td>97.6</td>
</tr>
<tr>
<td>Diffuse component</td>
<td>45.2</td>
<td>100</td>
</tr>
<tr>
<td>Apoptoses present</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td>Large cell component</td>
<td>57.1</td>
<td>100</td>
</tr>
<tr>
<td>Anaplastic component</td>
<td>47.6</td>
<td>100</td>
</tr>
<tr>
<td>Necrosis present</td>
<td>64.3</td>
<td>100</td>
</tr>
<tr>
<td>Nodular status</td>
<td>64.3</td>
<td>97.6</td>
</tr>
</tbody>
</table>

| Abbreviations: AT/RT, atypical teratoid/rhabdoid tumor; LCA, large cell/anaplastic; MB, medulloblastoma; Nod, nodular; OS, overall survival; PFS, progression-free survival.

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**Table 3. Log-Rank Test P Values to Compare Various Subgroups and Pathologic Features**

<table>
<thead>
<tr>
<th>Variable of Interest</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplasia versus no anaplasia</td>
<td>.29</td>
<td>.79</td>
</tr>
<tr>
<td>Absent versus focal versus diffuse</td>
<td>.57</td>
<td>.90</td>
</tr>
<tr>
<td>None/mild versus moderate/severe</td>
<td>.02</td>
<td>.25</td>
</tr>
<tr>
<td>Nodularity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None versus not extensive versus extensive</td>
<td>.01</td>
<td>.01</td>
</tr>
<tr>
<td>(None or not extensive) versus extensive</td>
<td>.005</td>
<td>.03</td>
</tr>
<tr>
<td>None versus (not extensive or extensive)</td>
<td>.02</td>
<td>.004</td>
</tr>
<tr>
<td>Reticulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative versus multifocal versus diffuse</td>
<td>.13</td>
<td>.89</td>
</tr>
<tr>
<td>Large cell/anaplastic versus nodular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCA/–Nod. – versus LCA+/Nod. – versus LCA/–Nod. + versus LCA+/Nod. +</td>
<td>.02</td>
<td>.01</td>
</tr>
<tr>
<td>LCA/–Nod. + versus others</td>
<td>&lt;.001</td>
<td>.02</td>
</tr>
<tr>
<td>LCA versus others</td>
<td>.01</td>
<td>.001</td>
</tr>
<tr>
<td>Diagnostic category</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB versus AT/RT</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
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States; 320 of these tumors will be embryonal tumors, \(^1\) MB and AT/RT being the most common types. These 2 tumor types have diagnostic overlap.\(^{22,30}\) Because diagnostic variants of MB seem to have widely variable prognostic implications,\(^{5,12,23}\) proper classification is important. The PBTC drug trial 001, investigating the utility of intrathecal ifosfamide to supplement intensive chemotherapy and radiation therapy in infants and children younger than 3 years with embryonal tumors and metastatic ependymomas, offered a resource whereby these tumors and the features used to evaluate them could be further studied.

The patients entered into this protocol exhibited tumors that represented the spectrum of embryonal tumors of childhood, including MB, AT/RT, PB, CNS PNET, ependymoblastoma, and choroid plexus carcinoma. In the present study, we first undertook to determine the reproducibility of making histologic distinctions between MB and AT/RT. Next, we proceeded to analyze the reliability of characterization of histologic features used to characterize the embryonal tumors in general and MBs specifically.

First, it was important to determine if the morphologic features used by the central reviewers could be replicated among the pathologists conducting the central review. Among the factors examined, both nodular morphology and anaplastic features proved to be reliable and reproducible. In a number of cases, the central neuropathology review committee disagreed with the diagnosis submitted by the original institution. Because the central reviewers were blinded as to anatomic site, these discrepancies appeared to be related to site in the misclassification of MB as PNET. In a reevaluation of the results in which all cases classified as PNET were recombined with tumors classified as MB and PB, the number of disagreements diminished, both between the central reviewers and the submitting institutions and the central neuropathology review committee.

Figure 6. A, Progression-free survival estimates by degrees of anaplasia (medulloblastoma patients, \(P = .02\)). B, Overall survival by degrees of anaplasia (medulloblastoma patients, \(P = .25\)).

Figure 7. A, Progression-free survival estimates by degrees of nodularity (medulloblastoma patients, \(P = .02\)). B, Overall survival by degrees of nodularity (medulloblastoma patients, \(P = .01\)).
among the central reviewers themselves. The data support the historical contention that knowledge of tumor location is essential in making a diagnosis using light microscopy.

**Desmoplasia**

Medulloblastomas with nodules are currently classified by the World Health Organization using the compound term “desmoplastic/nodular.” These tumors are characterized by nodular reticulin-free zones (‘pale islands’) surrounded by densely packed, highly proliferative cells with hyperchromatic and moderately pleomorphic nuclei which produce a dense intercellular reticulin fiber network.” The definition indicates that the presence of intercellular reticulin without nodules should not be considered a desmoplastic/nodular variant. The present study undertook an analysis of the effect of the presence of nodules alone and desmoplasia alone and found that nodules, regardless of the presence of reticulin or anaplastic content, were associated with a good OS.

Nodular MBs (Figure 1) comprised 19 of 42 MBs (45%) in the present study, a rate similar to the 57% rate previously reported in infants. The nodular MB variant, MBEN, is a tumor of infancy that is known to be associated with a good outcome. Some have suggested that the nodular tumors are more likely to be lateral, thus more accessible to a complete resection and therefore having a better associated survival. However, other reports suggest the biologic response of MBENs to therapy may be different from that of other MBs; McManamy and colleagues hypothesized that the limited growth potential of nodular MBs was related to their differentiation capacity.

An attempt was made to determine if the findings of the present study were somehow a drug treatment–related effect versus an age-related effect, that is, whether there were more MBEN types in this cohort compared to other MB treatment trials because MBENs are predominately infantile tumors. The rarity of this tumor in patients older than 3 years makes such a comparison difficult and not readily found in the literature. Furthermore, the lack of radiation therapy in this trial (and other recent trials involving infants) also poses a problem in the comparison. The initial hopeful report of Giangaspero and colleagues all involved children younger than 4 years, and the reports of Eberhardt et al, Grill and colleagues, Rutkowski and colleagues, and McManamy and colleagues all emphasize the good prognostic effect of MBEN histology in infants.

**Figure 8.** A, Tumor from patient 60775 reveals no epithelioid or rhabdoid features by hematoxylin-eosin. B, Negative BAF47 reactivity is noted in the tumor with intact endothelial cell immunoreactivity (hematoxylin-eosin, original magnification ×40 [A]; anti-BAF47, original magnification ×40 [B]).

**Figure 9.** A, Tumor from patient 107487 diagnosed by institution as atypical teratoid/rhabdoid tumor. B, Diffuse BAF47 immunoreactivity is present (hematoxylin-eosin, original magnification ×40 [A]; BAF47, original magnification ×40 [B]).
infants. Less sanguine reports involved children of older age, although McManamy and colleagues noted only one example of MBEN arising in a child older than 3 years. In the absence of an evaluation of MBEN histology and prognosis occurring in older children, it seems sufficiently conservative to say that MBEN histology occurring in infants is predictive of good PFS and OS when confronted with the intensive therapeutic regimen of this protocol. In contrast, the presence of reticulin, a finding frequently associated with nodular phenotype, was not associated with a good prognosis, a finding incorporated into the World Health Organization definition as well as supported by Lamont et al and by McManamy and colleagues.

Anaplasia
To our knowledge, no previous studies have analyzed the interobserver reproducibility of anaplasia as a histologic feature. The highly aggressive large cell variant of MB was first reported by Giangaspero and colleagues, who also described the poor OS associated with the presence of anaplasia and large cell morphology and its association with MYC oncogenic amplification. Additional reports have indicated no prognostic difference among cases with large cell morphology versus anaplastic morphology, and, indeed, many tumors exhibited large cell morphology intermixed with areas of anaplastic morphology, thus leading to the conjoined term anaplastic/large cell MB. Although anaplasia was associated with a significantly worse survival in this study, our initial analysis suggested that its impact was not independent of nonnodular morphology. This discrepancy was further investigated. Using a working definition of moderate anaplasia as an MB with starry-sky apoptosis but lacking geographic necrosis and mild anaplasia as a tumor with nuclear molding but lacking both starry-sky apoptosis and geographic necrosis, a reanalysis of our cases subsequently supported the historic findings that moderate and severe anaplasia inferred a worse PFS and OS.

We also examined the antigenic expression of OTX2, a developmentally regulated transcription factor that has recently been described to be overexpressed in anaplastic MBs. OTX2 immunoreactivity correlated with anaplasia in this cohort of patients and was associated with a strong trend toward a poor PFS, suggesting a need for a larger study of the prognostic application of this immunohistochemical marker in diffuse MB.

Atypical Teratoid/Rhabdoid Tumors
Previous studies have identified the difficulty in diagnosing AT/RT and in distinguishing the tumor from MB. The propensities of the tumor to exhibit both neuronal and glial markers (as well as epithelial markers) plus its lesser-recognized ability to mimic the features of diffuse MB compound this difficulty. We sought to determine the interobserver variation among a group of expert neuropathologists in making these diagnoses and to study the immunoreactivity profile among a group of AT/RTs and MBs. As noted in Table 1, the 3-of-3 concordance among MBs was clearly better than the concordance seen when diagnosing AT/RTs. However, when 2-of-3 consensus was examined, the diagnostic variance between MBs and AT/RTs diminished considerably. MB and other “small blue cell” tumors frequently entered into the differential diagnosis of AT/RT. Thus, the subjective distinction of an AT/RT from other small blue cell tumors is prone to error. The BAF47 antibody has proven its utility in objectively identifying the nonexpressing AT/RTs from other expressing tumors in the differential diagnosis with rare exception. Therefore, among the AT/RTs and MBs studied, we performed BAF47 staining on a sample of 6

Table 4. Medulloblastomas (MBs) and Atypical Teratoid/Rhabdoid Tumors (AT/RTs) With Diagnoses by Referees and BAF47 for the Conflicting Cases (at Least 1 Atypical Teratoid/Rhabdoid Call)

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Institutional Call</th>
<th>BAF47</th>
<th>Call by Pathologist 1</th>
<th>Call by Pathologist 2</th>
<th>Call by Pathologist 3</th>
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<td>AT/RT</td>
<td>MM</td>
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<tr>
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<td>AT/RT</td>
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<tr>
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<tr>
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<tr>
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</tr>
<tr>
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<td>AT/RT</td>
<td>Diffuse</td>
<td>PNET</td>
<td>PNET</td>
<td>PNET</td>
</tr>
<tr>
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<td>MB</td>
<td>Multifocal</td>
<td>MB</td>
<td>AT/RT</td>
<td>MB</td>
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<tr>
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<td>Diffuse</td>
<td>PNET</td>
<td>PNET</td>
<td>PNET</td>
</tr>
</tbody>
</table>

Abbreviations: CNSNeo, central nervous system neoplasm, not otherwise specified; CPC, choroid plexus carcinoma; MM, malignant melanoma; PNET, primitive neuroectodermal tumor.
AT/RTs as well as on 32 MBs. In support of the findings by Kraus and colleagues and by Edgar and Rosenblum, 5 of 6 AT/RTs (as diagnosed by the institutional pathologists) demonstrated negative immunoreactivity, as did 1 diffuse MB, possibly a cryptic AT/RT (Figure 8, A and B). This MB was lacking in both anaplastic features and cytologic features of epithelioid or rhabdoid differentiation. The literature has indicated that the otherwise typical-appearing MBs with diffuse histology, but negative expression of BAF47, all proved to have poor clinical outcomes, and were best considered AT/RTs. However, to date, no studies have examined the polyphenotypic profiles of the small cell AT/RTs seen in the more typical examples.

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References