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ORIGINAL PAPER

Distinct disease-risk groups in pediatric supratentorial and posterior fossa ependymomas

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Abstract No reliable classification is in clinical use for the therapeutic stratification of children with ependymoma, such that disease risk might be identified and patients treated to ensure a combination of maximal cure rates and minimal adverse therapeutic effects. This study has examined associations between clinicopathologic and cytogenetic variables and outcome in a trial cohort of children with ependymoma, with the aim of defining a practical scheme for stratifying this heterogeneous tumor. Intracranial ependymomas ($n = 146$) from children treated on the RT1 trial at St. Jude Children's Research Hospital were evaluated for the status of multiple pathological features. Interphase FISH (iFISH) defined the status of loci on chromosomes 1q (*EXO1*), 6q (*LATS1*) and 9, including 9p21 (*CDKN2A*). Data relating to these clinicopathological and cytogenetic variables were compared with survival data in order to model disease risk groups. Extent of surgical resection was a significant determinant of outcome in both supratentorial and infratentorial compartments. Tumor cell density and mitotic count were associated with outcome among children with posterior fossa ependymomas ($n = 119$). Among pathologic features, only brain invasion was associated with outcome in children with supratentorial ependymomas ($n = 27$). For posterior fossa tumors, gain of 1q was independently associated with outcome and in combination with clinicopathological variables defined both a two-tier and three-tier system of disease risk. Among children developing posterior fossa ependymomas treated with maximal surgical resection and conformal radiotherapy, key clinicopathological variables and chromosome 1q status can be used to define tiers of disease risk. In contrast, risk factors for pediatric supratentorial tumors are limited to sub-total resection and brain invasion.

Keywords Ependymoma · Chromosome 1q gain · Mitotic count · Outcome indicators

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Introduction

Ependymomas constitute approximately 10 % of childhood central nervous system (CNS) neoplasms, but this figure

rises to about 30 % among children aged less than 3 years [3, 17, 26]. The disease remains a considerable therapeutic challenge; progression-free survival for ependymoma is at best 60 % after 5 years [20, 21]. In addition, although improved functional outcomes have been observed among survivors of ependymoma treated with modern methods of surgery and radiotherapy [5], many remain at risk for adverse therapeutic effects that can be related to radiation dose and volume [19, 21]. Improving survival and reducing the late effects of therapy among children with ependymoma have become primary objectives in clinical trials and important considerations for designing novel therapeutic strategies.

Attempts to improve outcome for patients with ependymoma have been complicated by various factors. Fundamentally, this is a rare disease, which hampers the study of its biology and the interpretation of clinical studies based on small patient cohorts. Radiotherapy undeniably improves outcome in ependymoma [19, 20, 29], but a clear survival advantage to augmenting radiotherapy with a specific chemotherapeutic regimen has yet to be conclusively demonstrated [12, 16]. In addition, pathological classifications of ependymoma are difficult to apply and of uncertain clinical utility [8, 11]; few new trials recommend a grading scheme to stratify patients. In recent years, genetic studies of ependymoma have begun to improve our understanding of its biology and to suggest alternative approaches to defining disease risk [14, 15, 25, 32, 33], but no robust diagnostic or therapeutic molecular markers are yet in routine clinical use.

We report a detailed histopathological and cytogenetic analysis of intracranial ependymomas from children treated on the St. Jude Children's Research Hospital (SJCRH) "Phase II study of image-guided radiation therapy for pediatric CNS tumors and quantification of radiation-related CNS effects" (RT1) trial [21]. This trial utilized conformal radiotherapy following a rigorous approach to surgical resection and has the most favorable outcome data reported for pediatric ependymoma to date. Histopathological evaluation was undertaken with the aims of (1) studying associations between individual features and outcome, without the constraint of how these might be combined subjectively into a pathologic grade; and (2) determining concordance on scores for these features among three microscopists. The analyzed cytogenetic abnormalities were those shown in previous studies to be of prognostic significance; specifically, gain of chromosome 1q and copy number alterations on chromosomes 6 and 9 [4, 15, 18, 22]. Thus, our aim was to define, within an optimal therapeutic paradigm, a stratification scheme of disease risk based on robust clinical, pathological, and cytogenetic outcome indicators.

Methods

Patient cohort

The study cohort consisted of children ($n = 146$) treated at SJCRH between 1997 and 2008 for localized (non-metastatic) intracranial ependymoma [21]. Eligibility required no prior irradiation, but pre-irradiation chemotherapy was allowed. Minimum age at the time of irradiation was 12 months, and median age for the study cohort was 2.5 years, range 0.2–22.8 years (two patients aged >18 years). Gross total resection (GTR) was systematically attempted prior to irradiation and achieved in 81 % of patients. Two attempts at resection were undertaken for 27 % of those that achieved GTR. Post-operative neuroimaging was used to determine the extent of resection. In addition to post-operative neuroimaging performed within 72 h of resection, all patients underwent neuroimaging prior to radiotherapy to determine the extent of resection. GTR was defined as no evidence of residual disease by magnetic resonance imaging plus an operative report to indicate that no macroscopic tumor was visible upon completion of surgery. Any residual tumor did not extend beyond a diameter of 1 cm. Conformal radiation therapy included a clinical target volume margin of 1 cm surrounding the post-operative residual tumor and/or tumor bed. Total dose was 59.4 Gy, except for children under 18 months of age who received 54 Gy after GTR. Patients were systematically followed and evaluated according to guidelines previously reported [21]. Additional clinical characteristics are summarized in Table 1.

Pathological evaluation

Standard histological preparations from each tumor (formalin-fixed paraffin wax-embedded (FFPE) 5 μm sections stained with hematoxylin and eosin) were initially reviewed by one pathologist (DWE). The following features from the WHO classification of ependymomas were assessed: cell density, microvascular proliferation, necrosis, and mitotic count. A high cell density was recorded when tumor cells exhibited a high nuclear:cytoplasmic ratio across more than 25 % of the sectioned tumor's area (Fig. 1). Hyperplasia of mural or endothelial cells in intratumoral blood vessels was required for microvascular proliferation to be scored as present (Fig. 1). When observed, necrosis was scored positively irrespective of whether or not areas of necrotic tissue demonstrated circumferential palisading of tumor cells. A mitotic count was recorded per 10 high-powered fields (hpfs) and preferentially within areas of tumor with high cell density, and a mean value calculated for three series of 10 hpfs. When mitotic count was studied as a categorical variable, the

Table 1 Clinical characteristics of study cohort

	All patients ($n = 146$)	ST tumors ($n = 27$)	PF tumors ($n = 119$)
Median age (age range) at diagnosis (years)	2.5 (0.2–22.8)	5.6 (0.2–18.7)	2.3 (0.6–22.8)
Sex (male:female)	1.28:1	1.08:1	1.33:1
Gross total resection	118 (81 %)	24 (89 %)	94 (79 %)
Pattern of failure			
None	100 (68 %)	20 (74 %)	80 (67 %)
Local only	22 (15 %)	4 (15 %)	18 (15 %)
Metastatic only	17 (12 %)	2 (7 %)	15 (13 %)
Local and metastatic	7 (5 %)	1 (4 %)	6 (5 %)

integer just above the median value for the cohort (mitotic count = 4) was used as a cut-off value. Invasion was assessed where an interface between ependymoma and brain was evident in submitted tissue (all supratentorial tumors; only 9 % of posterior fossa tumors) and was defined as infiltration of adjacent CNS parenchyma by individual tumor cells (Fig. 1). Additionally, the presence of two morphological phenotypes was recorded (Fig. 1), one combining nodules of high cell density with a focal papillary or cerebriform architecture, the 'biphasic-cerebriform phenotype', and the other combining a network of fine capillaries with focal clear cell change, the 'vascular phenotype' [28]. Two other investigators (C.G. and J.M.K.) independently evaluated all tumors for the same pathological features.

Interphase fluorescence in situ hybridization (iFISH)

Multi-color iFISH was performed on 5 μm FFPE sections as previously described [7]. Probes were derived from BAC clones (BACPAC Resources, Oakland, CA) and labeled with either AlexaFluor-488 or rhodamine fluorochromes. Probes were co-denatured with target cells on a hotplate at 90 °C for 12 min. Slides were incubated overnight at 37 °C and then washed in 4 M urea/2×SSC at 25 °C for 1 min. Nuclei were counterstained with DAPI (200 ng/ml). The following BACs were used to assess copy number abnormalities at genetic loci of interest: *EXO1* at 1q43, RP11-610O24 (1p control, CTD-3241G19); *LATS1* at 6q25, CTD-2221P18 (6p control, RP11-945O22/RP11-186N7); *CDKN2A* at 9p21, RP11-149I2/RP11-145E5 (9q control, RP11-235C23).

Statistical analysis

Progression-free survival was defined as the interval between radiotherapy start date and date of progression or death, and overall survival was defined as the interval between radiotherapy start date and death. Patients without an event (progression-free survival or overall survival) were censored at the date of last follow-up. Progression-free and overall survival distributions were estimated using the Kaplan–Meier method and compared between two or more groups of patients using the log-rank test. To investigate associations between multiple covariates and progression-free survival or overall survival, Cox proportional hazards regression models were employed. For pathology review data from the three microscopists, descriptive statistics and graphical tools were used to present the level of consensus among reviewers on various pathological features. The Wilcoxon–Mann–Whitney test was used to compare the distribution of a continuous variable (e.g., mitotic count) at the levels of a binary factor of interest (e.g., cell density). Logistic regression models were used to investigate the association between a factor of interest and the likelihood of a 3-in-3 consensus on any pathological feature. *P* values were not adjusted for multiplicity.

Results

Clinicopathological variables and outcome

Progression-free survival and overall survival 5 years following diagnosis for the entire cohort were 68.9 ± 4.1 and 82.6 ± 3.4 %, respectively. Progression-free survival (and overall survival) for children with supratentorial tumors was slightly better than that for children with posterior fossa tumors (Supp. Figure 1), though this difference was not significant (progression-free survival, $P = 0.11$, log-rank; overall survival, $P = 0.44$, log-rank). In contrast, there was a clear association between a favorable outcome and GTR; progression-free survival and overall survival were significantly better for completely resected tumors across all patients ($P < 0.0001$, log-rank) and among children with posterior fossa ependymomas alone (both variables, $P < 0.0001$, log-rank; Supp. Figure 2; Table 2).

Among children with supratentorial ependymomas ($n = 27$), a GTR had been achieved for all those alive and disease-free ($n = 20$; Table 1). GTR could not be achieved in 3 of 27 cases, and all experienced relapse after radiotherapy. Of seven children with recurrent supratentorial disease, five had tumors characterized by infiltration of adjacent parenchyma. Only one other supratentorial ependymoma, which had been totally excised from a child now disease-free, showed brain invasion. Brain infiltration was the only histological variable significantly associated with outcome in supratentorial disease (progression-free

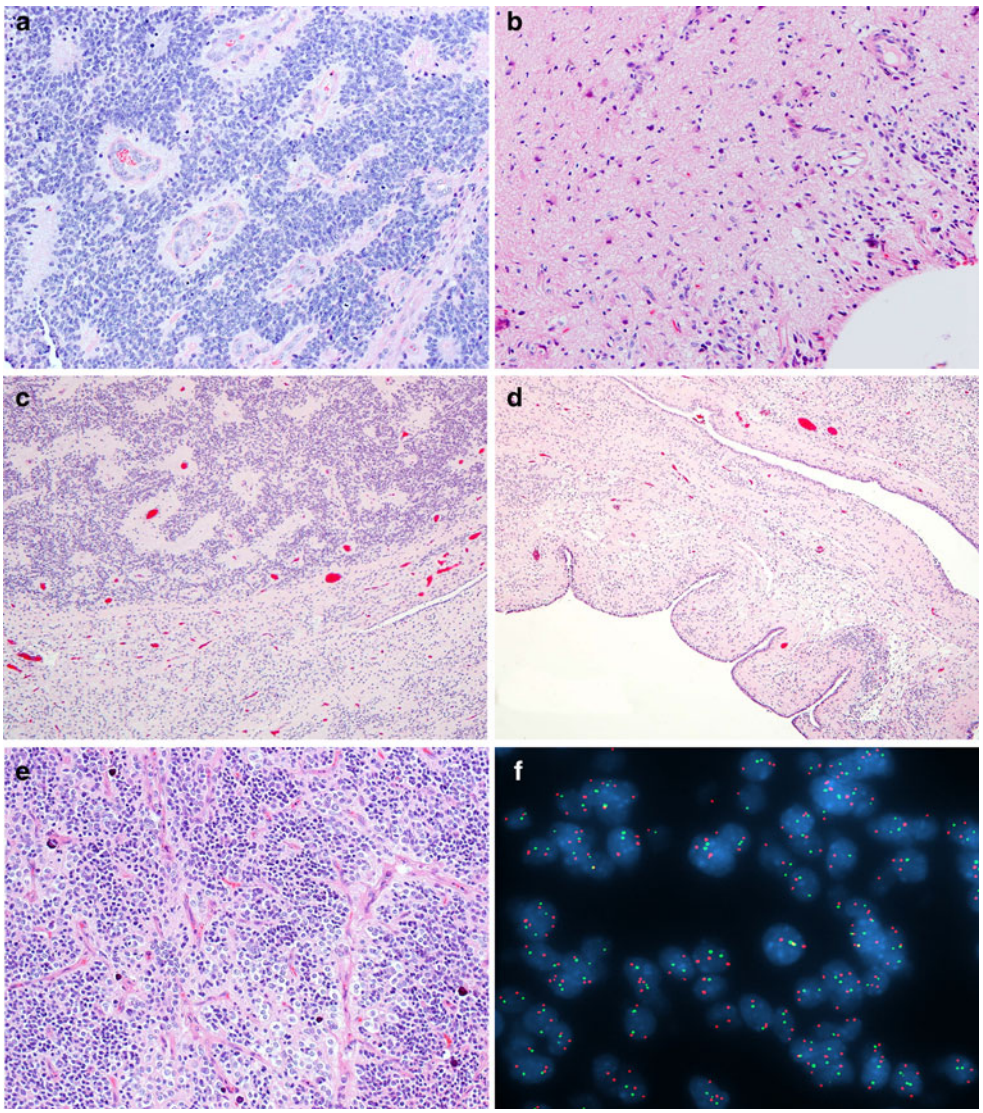


Fig. 1 Histopathology and molecular cytogenetics of ependymoma (**a**–**e** hematoxylin and eosin, **f** iFISH). **a** Microvascular proliferation characterized mainly by endothelial cell hyperplasia in a tumor with high cell density. **b** Tumor cells invading cerebral parenchyma and perivascular spaces in subcortical white matter and eliciting a marked astrocytosis. **c** Clearly demarcated regions of high and low cell density in this 'biphasic' posterior fossa ependymoma, a phenotype sometimes combined with a 'cerebriform' architecture and the presence of clefts (**d**). **e** Syncytial pattern of uniform cells, many with cytoplasmic clearing, divided by a network of branching capillaries in this 'vascular' supratentorial ependymoma. **f** Gain of 1q demonstrated by probes to *EXO1* on 1q (*red* fluorochrome) and 1p (*green* fluorochrome)

survival, $P = 0.008$, log-rank; overall survival, $P = 0.003$, log-rank).

Tumor cell invasion could be assessed in few posterior fossa ependymomas (9 %), which was insufficient for meaningful survival analysis. However, mitotic count and cell density both showed (Supp. Figure 3; Table 2) a significant association with progression-free survival ($P = 0.01$ and $P = 0.004$, respectively, log-rank) and overall survival ($P = 0.013$ and $P = 0.009$, respectively, log-rank), while necrosis, microvascular proliferation, and 'biphasic-cerebriform' morphology were not associated with outcome (Table 2). Five children had posterior fossa ependymomas with very high (>40/10 hpfs) mitotic counts, and four quickly went on to develop progressive disease.

For tumors at all anatomic sites, concordance on histopathological interpretation among the three microscopists differed according to categorical variable, being greater for cell density than microvascular proliferation or necrosis (Supp. Table 1). In a regression analysis of tumor mitotic counts, correlations among any pair of reviewers were strongly positive and highly significant (0.87, 0.82, 0.79, $P < 0.0001$, Spearman's rank correlation; Supp. Figure 4). This analysis and a comparison of calls on other variables between combinations of two observers suggested that

Table 2 Hazard ratios for PFS and OS in a Cox proportional hazards univariate regression model

Posterior fossa ependymomas ($n = 119$)				
Variable	Progression-free survival		Overall survival	
	HR	*P* value	HR	*P* value
Age at diagnosis	0.92	0.13	0.95	0.42
Sex (male:female)	1.76	0.062	2.02	0.073
Extent of surgical resection (STR:GTR)	3.13	<0.0001	3.85	<0.0001
Cell density (high:low)	2.95	0.004	3.74	0.009
Microvascular proliferation (present:absent)	1.42	0.29	1.86	0.17
Necrosis (present:absent)	1.51	0.22	1.37	0.45
Mitotic count (≥4/10 hpfs: <4/10 hpfs)	2.13	0.01	2.46	0.013
Chromosome 1q (gain:balance)	2.51	0.008	2.35	0.042
Chromosome 6 (loss:balance)	1.85	0.12	1.34	0.57

PFS progression-free survival, *OS* overall survival, *HR* hazard ratio, *STR* sub-total resection, *GTR* gross total resection, *MVP* microvascular proliferation, *hpfs* high-power fields

Table 3 Frequency of copy number abnormalities by anatomic site

	Supratentorial	Posterior fossa
Gain of 1q (*EXO1*)	0	13
Loss of 6q (*LATS1*)	0	2.5
Monosomy 6	3.7	7.6
Loss of 9p (*CDKN2A*)	7.4	0
Polysomy 9	7.4	0

The values are given in %

concordance was associated to only a minor degree with experience of microscopy or familiarity with ependymomas (Supp. Table 1).

Cytogenetic abnormalities

Gain of 1q was observed at diagnosis in 16 posterior fossa ependymomas (13 %), and hemizygous deletion at the *LATS1* locus (6q25) was found in three posterior fossa ependymomas; neither of these copy number abnormalities was detected in a supratentorial tumor (Table 3). In contrast, homozygous deletion at the *CDKN2A* locus (9p21) was detected in two supratentorial ependymomas, but in no posterior fossa tumor. Two of three tumors with *LATS1* deletion also had gain of 1q. Monosomy 6 inferred from an iFISH profile of one signal from both 6p and 6q probes was detected in another 11 ependymomas, 9 of which (82 %) presented in the posterior fossa.

There was a clear relationship between gain of 1q at diagnosis and poor outcome, with *P* values (log-rank test) of 0.003 for progression-free survival and 0.028 for overall survival (Fig. 2; Table 2). Of three children with posterior fossa ependymomas incorporating a hemizygous deletion of *LATS1*, two have died of disease, and one is alive with disease 47 months after diagnosis. Loss at the *LATS1* 6q25 locus, whether by focal hemizygous deletion or monosomy 6, was not significantly associated with an adverse outcome among children with posterior fossa tumors (progression-free survival, $P = 0.12$, log-rank; overall survival, $P = 0.57$, log-rank). Of the children with supratentorial tumors characterized by *CDKN2A* deletion, one has died of disease and the other is alive and disease-free 14 months after diagnosis.

Stratification models of disease risk

Because distinct histopathologic features were outcome indicators in tumors from above and below the tentorium and cytogenetic abnormalities were also site-specific, evaluation of these variables in models of disease risk alongside clinical and genetic variables was necessarily performed separately for ependymomas in each anatomic compartment. This approach proved statistically invalid for the limited number of supratentorial tumors ($n = 27$), so multivariable survival analysis focused upon posterior fossa ependymomas. Extent of surgical resection, tumor cell density, and gain of 1q were identified as independent outcome indicators (Table 4; Supp. Table 2), and these variables were used in stratification models of disease risk.

In one model (Fig. 3a, b), low-risk disease was defined as a totally resected ependymoma with low cell density and no gain of 1q, and high-risk disease as a subtotally resected tumor with high cell density or gain of 1q. Remaining tumors were classified as intermediate-risk disease, and separation between survival curves was highly significant ($P < 0.0001$, log-rank). In this categorization, progression-free survival distributions were also significantly different when high-risk and intermediate-risk groups and low-risk and intermediate-risk groups were compared ($P < 0.0001$, log-rank; $P = 0.018$, log-rank, respectively). In a second model provided to show the stark difference in outcomes between just two groups (Fig. 3c, d), high-risk disease was defined as in the first model, and the two other categories were combined as standard risk ($P < 0.0001$, log-rank). We also explored a categorization that used mitotic count in place of cell density, both these variables being highly associated. This also yielded significant separation of progression-free survival curves ($P < 0.0001$, log-rank; Supp Fig. 5).

A final model sought to test the principles behind a stratification scheme published on a large series of

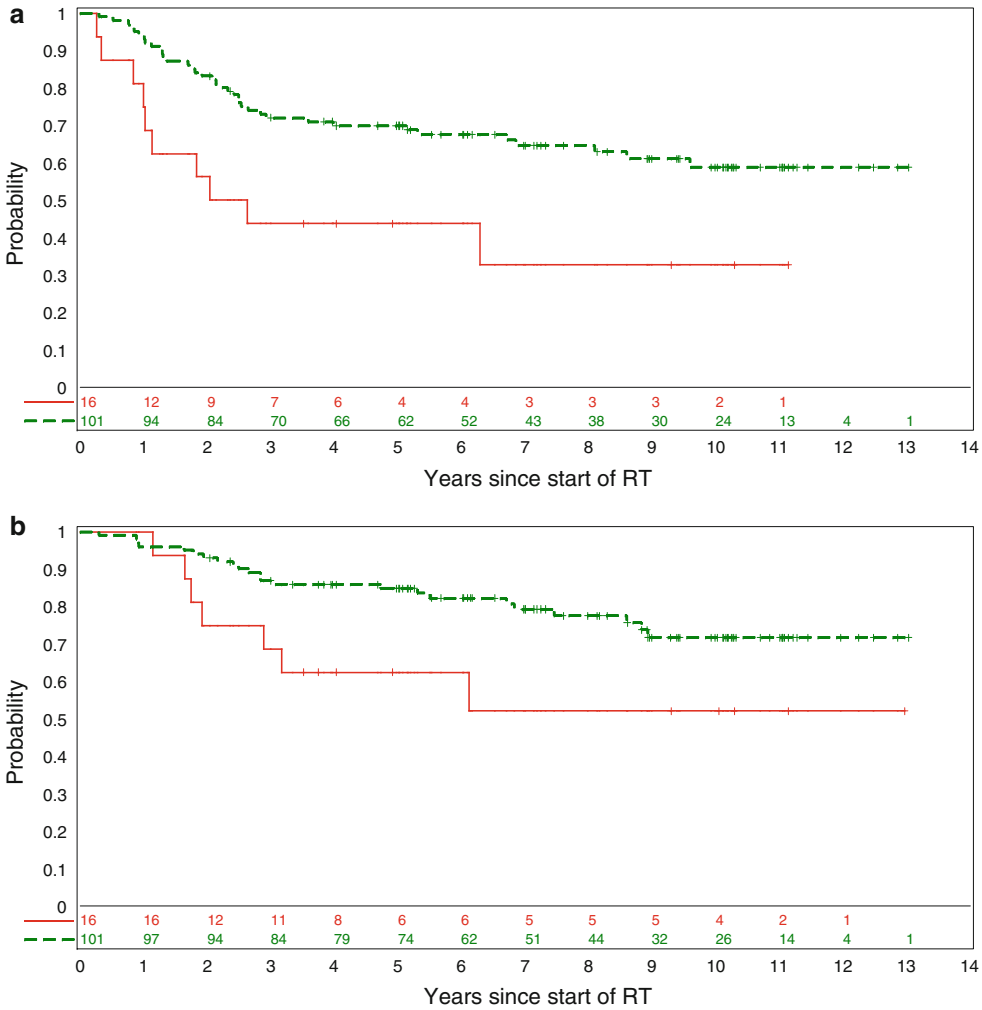


Fig. 2 Posterior fossa ependymoma. Progression-free survival (**a**) and overall survival (**b**)—survival curves split by 1q status; gain (*red*) and no gain (*green*)

Table 4 Hazard ratios for PFS and OS in a Cox proportional hazards multivariate regression model

Variable	Progression-free survival		Overall survival	
	HR	*P* value	HR	*P* value
Posterior fossa ependymomas ($n = 119$)				
Extent of surgical resection (STR:GTR)	4.76	<0.0001	5.00	<0.0001
Cell density (high:low)	3.04	0.008	3.82	0.021
Mitotic count (≥4/10 hpfs: <4/10 hpfs)	1.28	0.45	1.17	0.71
Chromosome 1q (gain:balance)	3.04	0.006	2.33	0.086

PFS progression-free survival, *OS* overall survival, *HR* hazard ratio, *STR* sub-total resection, *GTR* gross total resection, *hpfs* high-power fields

ependymomas [15]. Derived from cytogenetic data across tumors from all anatomic sites and generating three groups: (1) ependymomas with polysomy 9 and/or loss of chromosome 6 (monosomy 6 or hemizygous deletion of *LATS1*); (2) ependymomas with a balanced profile on chromosomes 1, 6, and 9; and (3) ependymomas with gain of 1q and/or homozygous deletion of *CDKN2A*, this categorization produced survival curves that were not quite statistically different in our dataset ($P = 0.08$, log-rank; Supp. Figure 6).

Discussion

Ependymoma is a heterogeneous disease, and this is reflected by its histopathological and histogenetic diversity [11, 14, 30, 33]. Our understanding of the biology behind this heterogeneity remains poor and has yet to be translated into refined therapeutic strategies. The WHO classification of nervous system tumors includes distinct pathological variants of ependymoma, but established clinical associations are limited to the grade I myxopapillary variant and subependymoma; the classic (grade II) tumor and its variants (clear cell, tanycytic, and papillary) or the

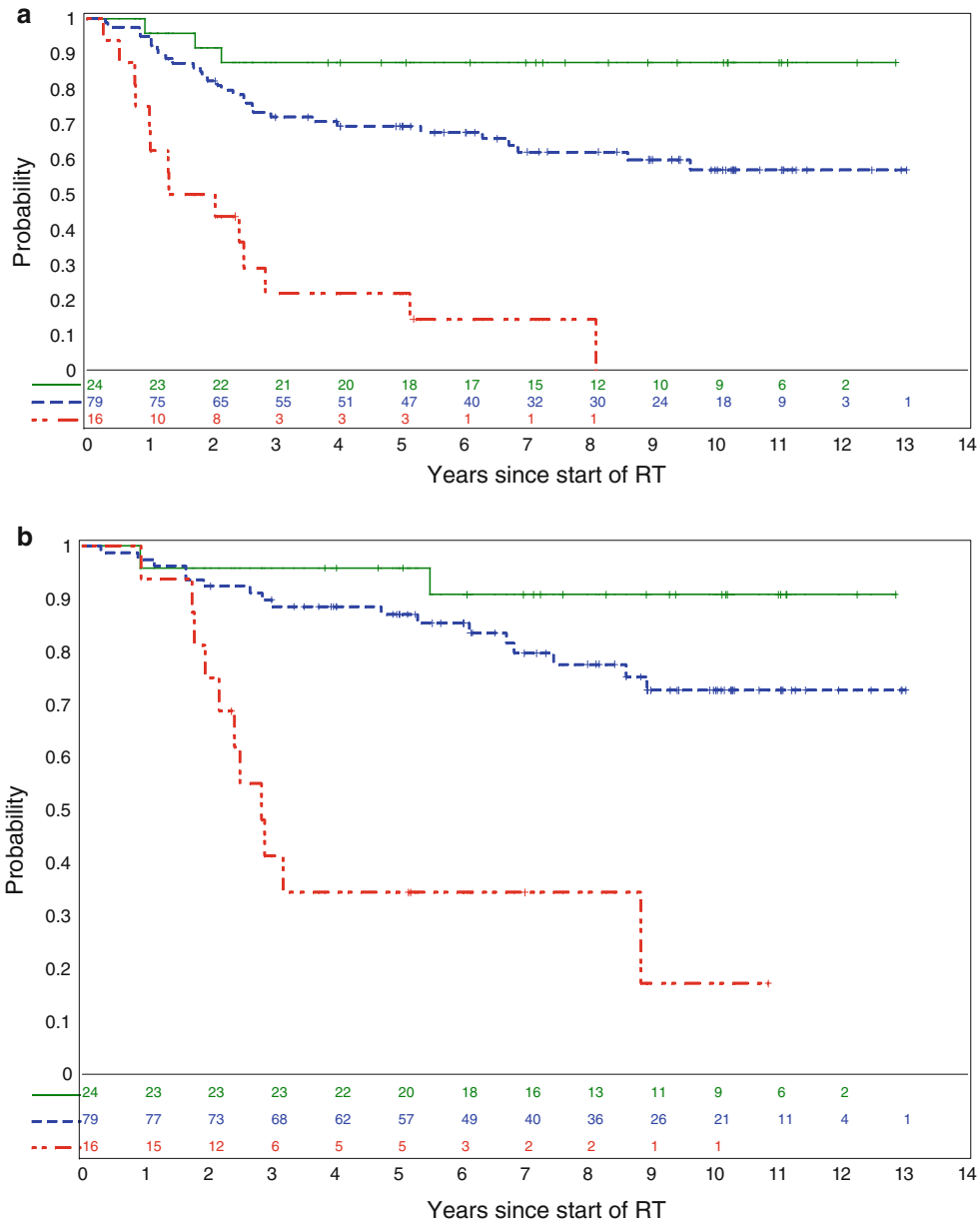


Fig. 3 Models of disease risk for posterior fossa ependymoma. Model 1 (three-tier): progression-free survival (**a**) and overall survival (**b**), low risk (*green*) = totally resected tumors with low cell density and no 1q gain; high risk (*red*) = subtotally resected tumors with high cell density or 1q gain. Remaining tumors (*blue*) were classified as intermediate risk. Model 2 (two-tier): progression-free survival (**c**) and overall survival (**d**), high risk (*blue*) = subtotally resected tumors with high cell density or 1q gain. Remaining tumors (*green*) were classified as standard risk. Both models produce highly significant ($P < 0.0001$) differences in survival curves

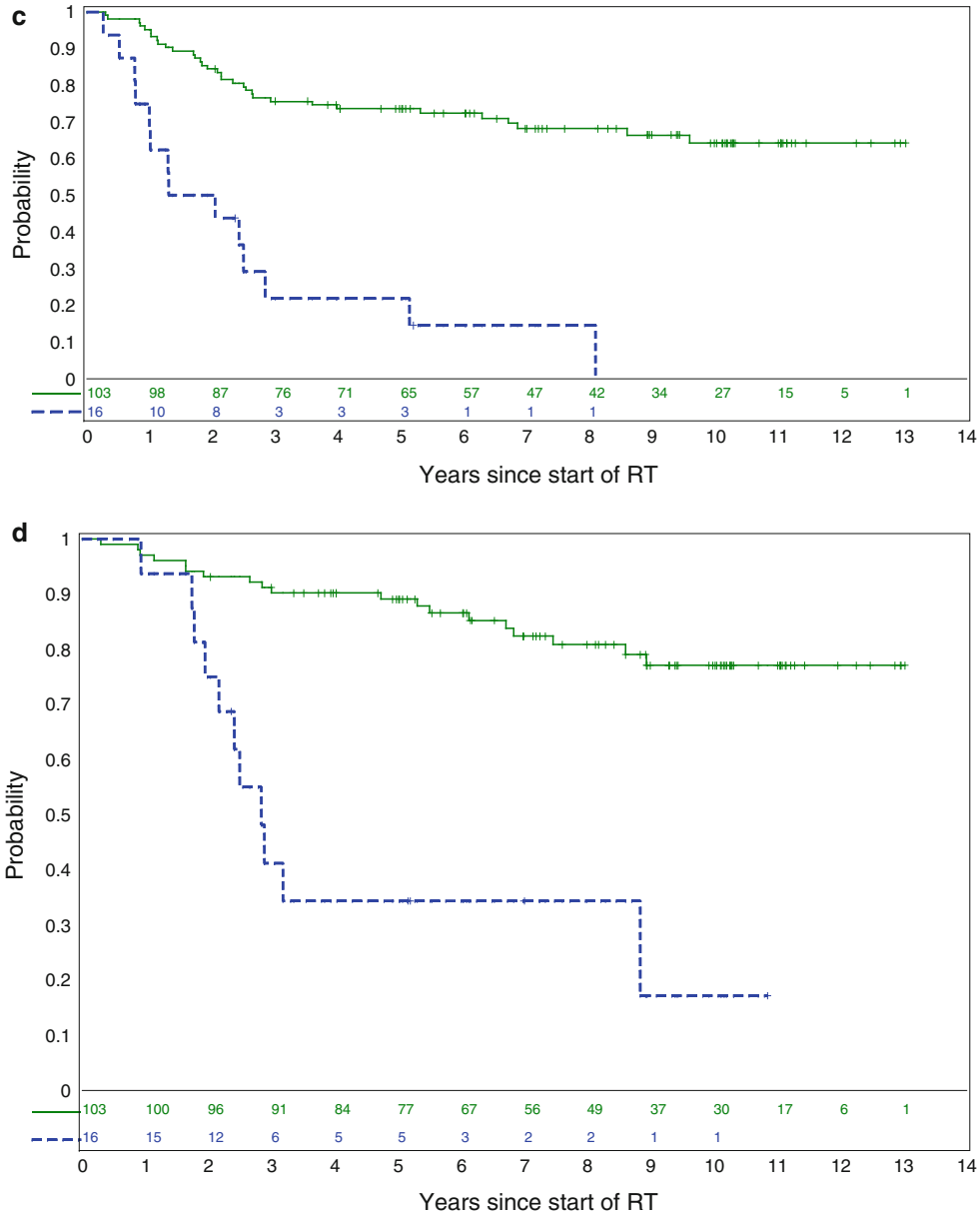
anaplastic (grade III) tumor have not been linked unequivocally to clinical phenotype or outcome [8, 11].

In particular, the pathological grading of pediatric intracranial ependymomas continues to provoke debate [2, 3, 31]. From the pathologist's perspective, considerable histological variability exists among and within tumors, making grading difficult. Such difficulty is reflected by studies that report ratios of grade II to grade III tumors that range between 17:1 and 1:7, a striking discordance that likely represents intratumoral heterogeneity, the uneven application of criteria for anaplasia by review pathologists, and idiosyncratic patient cohorts [8]. Whether children with grade II and those with grade III ependymomas have significantly different outcomes remains unclear, and there is dissent among articles with a focus on histopathological features as independent prognostic or predictive factors [8–10, 29].

In the present study, we chose to examine associations between outcome and specific histological variables, rather than tumor grade, in a large series of patients treated uniformly on the SJCRH RT1 trial. The therapeutic strategy, which involved resecting as much tumor as possible prior to conformal radiotherapy while seeking to avoid significant neurological deficit, has produced the best survival data for ependymoma reported in the literature to date and thus represents the optimal setting in which to determine the role of histopathological evaluation.

Ependymomas uncommonly infiltrate adjacent brain; such that a rigorous approach to surgical resection, possibly involving several operations to remove residual tumor, will

Fig. 3 continued



probably be more successful with a supratentorial than posterior fossa tumor, because the latter is more likely to be apposed to vital structures. This perspective is supported by lower frequencies of sub-total resection and relapse among supratentorial tumors across our cohort, and it is notable that the only histopathological feature significantly linked to the outcome of children with supratentorial ependymomas was microscopic infiltration of adjacent brain.

With posterior fossa tumors and less chance of a macroscopic or microscopic removal of tumor, other histopathological variables become relevant. In particular, our data indicate that high cell density and mitotic count are significant outcome indicators. Microvascular proliferation and necrosis, which are included among WHO criteria for high-grade gliomas, were not significantly associated with outcome. In ependymomas, regions and nodules characterized by high cell density are often where most mitotic activity can be found, and a highly significant correlation between these two variables was demonstrated across data from each of the three reviewers.

Previous studies have used measures of cell proliferation, including mitotic count, to separate ependymomas into groups with distinct survivals, although widely different cut-off values have prevented consensus on how to integrate such measures into diagnostic practice [1, 9, 23, 24, 34]. Some of these studies also explored the utility of evaluating microvascular proliferation and necrosis to grade ependymomas, with variable results. Agreement on

Table 5 Risk factors for pediatric intracranial ependymoma

	Supratentorial		Posterior fossa	
	Low risk	High risk	Low risk	High risk
Extent of surgical resection	Gross total resection	Sub-total resection	Gross total resection	Sub-total resection
Pathology	–	Infiltration of adjacent brain by tumor cells	Low cell density/mitotic count <4/10 hps	High cell density/mitotic count ≥4/10 hpfs
Cytogenetics	–	–	Gain of chromosome 9[a]	Gain of chromosome 1q

hpfs high-power fields

[a] Trend towards good outcome

the presence of these two histopathological features was less frequent among three observers in the present study, suggesting that aspects of their evaluation might be more subjective than for cell density or mitotic count. This conclusion was similarly reported in a separate study of histopathologic variables in ependymoma assessed by five neuropathologists [8]. In contrast, excellent concordance on the scoring of tumor cell density and mitotic count was achieved in this study by three observers with contrasting experience of ependymoma histopathology.

Cytogenetic abnormalities in ependymoma have also been the focus of survival studies. Current molecular cytogenetic methods to evaluate copy number abnormalities in tumors include comparative genomic hybridization and iFISH, and both have been used to study ependymoma [4, 6, 13, 15, 18, 25]. However, most studies employ iFISH, which allows the proportion of tumor cells with a particular profile of probe signals to be determined. Gain of chromosome 1q is one of the most common cytogenetic abnormalities in ependymoma (26 % in one study) and was detected in 13 % of posterior fossa tumors in the present study. It has been shown to associate independently with poor outcome in several cohorts and was a strong predictor of adverse outcome in our study [4, 15, 18, 27, 33]. Furthermore, the frequency of 1q gain appears to increase among recurrent ependymomas [18]. Copy number abnormalities at 6q25 (*LATS1*) and 9p21 (*CDKN2A*) occurred too infrequently for meaningful outcome analysis in this study, but have been linked to favorable or poor outcome in other studies [15, 22]. A recent study utilizing iFISH data in a "molecular staging" model proposed three tiers of risk defined by different combinations of cytogenetic abnormalities [15]. Our data could be fitted to this model and provide modest support for it; our cytogenetic subgroups just fail ($P = 0.08$, log-rank) to show significant separation on survival analysis.

Two recent studies focusing on the biology of ependymoma reinforce previous data to indicate that the disease is distinct at sites across the neuraxis; gene expression profiling clearly distinguishes ependymomas from supratentorial and infratentorial compartments [14, 30, 33]. While there appears to be some disagreement on the number of molecular subgroups generated in this way, a few fundamental principles emerge from these two datasets. Among posterior fossa tumors, gain of 1q tends to segregate with a molecular profile that is also characterized by young age of onset and, in the study by Witt et al., a poor outcome. This anatomical heterogeneity, which also manifests as distinct pathologic phenotypes and cytogenetic abnormalities in supratentorial and posterior fossa tumors, argues for different therapeutic approaches to ependymomas above and below the tentorium [4, 14, 28, 33]. Our data align with such proposals; outcome indicators are different for ependymomas at these two sites, and gain of 1q appears to be a robust marker of posterior fossa tumors with a poor outcome (Table 5). The recommendation for supratentorial ependymomas would be to optimize surgical resection and to regard brain invasion, alone among histopathological features, as a risk factor for recurrence. In contrast, pediatric posterior fossa ependymomas could be stratified into two or three disease-risk groups, using cytogenetic data on 1q status and two clinicopathological variables; extent of surgical resection and tumor cell density, which is strongly associated with mitotic count.

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Ethical standard This study was conducted with institutional ethics committee approval—St. Jude Children's Research Hospital XPD07-107/IRB.

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