Second series by the Italian Association of Pediatric Hematology and Oncology of children and adolescents with intracranial ependymoma: an integrated molecular and clinical characterization with a long-term follow-up

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Abstract

Background. A prospective 2002–2014 study stratified 160 patients by resection extent and histological grade, reporting results in 2016. We re-analyzed the series after a median of 119 months, adding retrospectively patients' molecular features.

Methods. Follow-up of all patients was updated. DNA copy number analysis and gene-fusion detection could be completed for 94/160 patients, methylation classification for 68.

Results. Progression-free survival (PFS) and overall survival (OS) at 5/10 years were 66/58%, and 80/73%. Ten patients had late relapses (range 66–126 mo), surviving after relapse no longer than those relapsing earlier (0–5 y). On multivariable analysis a better PFS was associated with grade II tumor and complete surgery at diagnosis and/or at radiotherapy; female sex and complete resection showed a positive association with OS. Posterior fossa (PF) tumors scoring ≥ 0.80 on DNA methylation analysis were classified as PFA ($n = 41$) and PFB ($n = 9$). PFB patients had better PFS and OS. Eighteen/32 supratentorial tumors were classified as RELA, and 3 as other molecular entities (anaplastic PXA, LGG MYB, HGNET). RELA had no prognostic impact. Patients with 1q gain or cyclin-dependent kinase inhibitor 2A (CDKN2A) loss had worse outcomes, included significantly more patients >3 years old ($P = 0.050$) and cases of dissemination at relapse ($P = 0.007$).

Conclusions. Previously described prognostic factors were confirmed at 10-year follow-up. Late relapses occurred in 6.2% of patients. Specific molecular features may affect outcome: PFB patients had a very good prognosis; 1q gain and CDKN2A loss were associated with dissemination. To draw reliable conclusions, modern ependymoma trials need to combine diagnostics with molecular risk stratification and long-term follow-up.

Key Point

1. Integrated clinical and molecular diagnosis are needed in ependymoma.
2. Late relapses are not rare and do not have good prognosis.
3. Risk stratification needs to be confirmed with long-term follow-up.

Importance of the Study

In the present manuscript, we provide updated 10-year follow-up data on a series of 160 ependymoma patients enrolled in the protocol sponsored by the Italian Association of Pediatric Hematology and Oncology between 2002 and 2014 as well as DNA copy number analysis and gene fusion detection for 94/160 available tumor samples and methylation classification for 68. Even within the limitation of an

incomplete series of samples, our results significantly inform ongoing and future ependymoma trials on the need to integrate diagnostics with molecular risk stratification and long-term follow-up. In fact, our previously described prognostic factors were confirmed at 10-year follow-up and specific molecular features (PFB subgroup, 1q gain, and CDKN2A loss) affected outcome.

Ependymoma (EPD) accounts for about 10% of central nervous system (CNS) tumors in children and adolescents. It originates usually intracranially, from ventricular surfaces and brain parenchyma, and more rarely in the spinal cord.¹ Even after one or more surgical procedures, adjuvant radiotherapy (RT), and sometimes also chemotherapy,² progression-free survival (PFS) and overall survival (OS) are still unsatisfactory in the long term. Relapses generally occur in fact within 2 years after the initial diagnosis, but may also pose a threat to patients' lives even many years later.^{3,4}

We conducted the present follow-up study with the dual aim of: clarifying late outcomes of our treatment strategy, published with a median follow-up of around 5 years⁵; and integrating the role of methylation profiles (in those cases for which we were able to perform molecular subgrouping), and their influence on tumor presentation, response to treatment, relapse, and death. The median follow-up of the original series enrolled in the second prospective protocol from the intracranial ependymoma was 10 years at the time of this report.

In recent years, molecular profiling of ependymomas revealed the existence of multiple molecular subgroups of this disease with distinct genetic and epigenetic features.⁶ Intriguing preliminary evidence pointed to patients' clinical condition and prognosis being associated with particular features and subgroups, such as: posterior fossa (PF)A and PFB infratentorial tumors⁷; v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA) supratentorial tumors^{8,9}; gain/amplification of chromosome 1q¹⁰; and deletion of the

cyclin-dependent kinase inhibitor 2A (CDKN2A) locus.^{1,11} While these biomarkers still warrant prospective validation, retrospective analysis of series of patients enrolled in clinical trials may shed light on their relevance. In the present paper, we retrospectively analyze the subgroup allocation of 95/160 patients enrolled in the second prospective protocol from the Italian Association of Pediatric Hematology and Oncology for intracranial ependymoma for whom tumor tissue was available.

Patients and Methods

Treatment

The second Italian prospective study on ependymoma stratified patients enrolled between 2002 and 2014 by extent of surgical resection (complete = no evidence of disease [NED]; incomplete = evidence of disease [ED]), and histological grade (World Health Organization [WHO] grade II vs III), as previously reported.⁵

All histological samples were centrally reviewed up front before administration of any treatments.¹²

Patients with no residual disease after surgery had different treatments based on WHO histological grade. Grade II/NED patients received focal RT up to 59.4 Gy. Grade III/NED patients also had 4 courses of vincristine, etoposide, cyclophosphamide (VEC) chemotherapy after RT. Incompletely resected (ED) patients received up to 4

VEC chemotherapy courses after surgery, then underwent second-look surgery whenever feasible, as well as having RT 59.4 Gy + 8 Gy boost on tumor residue still measurable in 3 dimensions.

On completion of the trial, all patients still alive remained in follow-up. We retrieved their updated clinical data and retrospective molecular data in accordance with the observational protocol approved by the ethical committee of the coordinating center (INT 24/20).

Molecular Classification

Using the Illumina Human Methylation 450 Bead Chip, DNA methylation data were generated for 95/160 patients whose formalin-fixed paraffin-embedded tissue samples obtained from their primary tumors were available. Data were generated at the Genomics and Proteomics Core Facility of the German Cancer Research Center in Heidelberg. Both methylation subgroups and genome-wide copy number imbalances were examined, as described elsewhere.¹³ Briefly, the Conumee package was used to generate copy number variation (CNV) plots. Status of 1q was ascertained by visual inspection of these plots. A sample was scored to have a CDKN2A loss in cases where probes covering the CDKN2A locus were lost and a score of -0.4 in the copy number plots was reached. Similarly, a chromosome 1q gain was scored if more than 75% of the loci from 1q21.1–32.1 displayed an amplification >0.4 in the CNV plots. Methylation subgroup classifier scores ≥ 0.80 were obtained for 68/95 tumors. Classifier scores <0.8 (27/95 samples) were not considered in the subsequent analyses in order to compare molecularly clean cohorts.

RELA and Yes-associated protein (YAP) gene fusion analyses were performed for supratentorial (ST) tumors using RNA retrotranscription, real-time PCR, and Sanger sequencing, as previously reported.¹⁴

Statistical Analyses

All patients enrolled and treated within the above-mentioned protocol were included in our analyses. The main endpoints of the study were OS and PFS for the whole case series. We also assessed the crude cumulative incidence (CCI) of local relapse (LR) and distant metastasis (DM). The OS was computed as the times elapsing from the dates of the first diagnostic radiological exam and the first relapse to the date of death due to any cause, and censoring at the time of the latest follow-up for patients still alive. PFS was computed as the interval between the date of the first diagnostic radiological exam and the date when progression (local or distant, whichever occurred first) was detected, censoring at the latest follow-up for patients remaining in first complete remission. OS and PFS curves were estimated using the Kaplan–Meier method and compared with the log-rank test. The CCI of LR and DM were estimated in a competing risks framework: local progression concurrent with distant progression was classified as distant progression, and the cumulative incidence curves were estimated and compared using Gray's test.

To select the most informative variables associated with OS and PFS among the characteristics of patients and their tumors—such as patient's sex and age, tumor site and grade, need for a ventriculoperitoneal (VP) shunt, residual tumor after first surgery, residual tumor after second-look surgery (before RT), interval between surgery and adjuvant treatment, symptoms and treatment at recurrence—we used the “component-wise gradient boosting,” as implemented in the “mboost” library in R v2.9-2. This is a machine learning method for optimizing prediction accuracy and selecting variables during the fitting process. Multivariable Cox models including the selected variables were run to investigate their joint prognostic effects on OS and PFS.

Differences between the study groups' demographic and clinical-pathological characteristics were assessed using standardized mean differences (SMDs).¹⁵ SMDs ≥ 0.3 were considered indicative of a relevant between-group imbalance. The association between pairs of variables was assessed using Fisher's exact test. The median follow-up was estimated with the reverse Kaplan–Meier method using OS data.¹⁶

The statistical analyses were conducted using the SAS and R software.¹⁷

Results

Clinical Results

We analyzed all clinical data at a median follow-up of 119 months after diagnosis (interquartile range, 91–141 mo), and the newly generated DNA methylation profiles, gathering information to ascertain the RELA and YAP fusion transcripts. The clinical features of all 160 patients enrolled are summarized in [Supplementary Table 1](#).⁵

Patients were a median 4 years old, and 62.5% were male. Patients' tumors involved the PF in 70.0% of cases. Tumors were classified as WHO grade III in 52.5% of cases. Residual tumor persisted after first surgery in 31.2% of patients, and after second surgery in 25.0%. A VP shunt was needed in 37.5% of cases.

The PFS was 66.2% (95% CI: 59.2–73.9%) at 5 years, and 58.5 (95% CI: 51.0–67.2%) at 10 years. The OS at 5 and 10 years was 79.9% (95% CI: 73.9–86.4%) and 73.6% (95% CI: 66.8–81.2%), respectively ([Figure 1A, B](#)).

Based on the surgical outcomes at the time of starting adjuvant treatment, the 5- and 10-year PFS were 72.6% (95% CI: 64.8–81.5%) and 66.8% (95% CI: 58.2–76.6%), respectively, for NED patients, and 52.0% (95% CI: 39.8–67.9%) and 41.6% (95% CI: 29.4–58.8%) for ED patients ($P = 0.008$). The 5- and 10-year OS were 87.2% (95% CI: 81.2–93.7%) and 82.9% (95% CI: 76.0–90.5%) for NED patients, and 64.0% (95% CI: 52.0–78.8%) and 54% (95% CI: 41.3–70.7%) for ED patients ($P < 0.001$) ([Figure 1C, D](#)).

Histological grade (on centralized review) determined a difference in both PFS and OS. The 5- and 10-year PFS were 74.9% (95% CI: 65.7–85.3%) and 66.9% (95% CI: 56.3–79.4%), respectively, for patients with WHO grade II tumors, and 58.3% (95% CI: 48.7–69.9%) and 50.9% (95% CI: 40.9–63.3%) for those with WHO grade III tumors ($P = 0.031$). The 5- and 10-year OS were 86.8% (95% CI: 79.5–94.8%) and 80.8%

Fig. 1 Kaplan–Meier curves for overall survival (A, C, and E) and progression-free survival (B, D, and F) overall (A and B), according to surgical results at the time of starting adjuvant treatment (C and D), and according to histological grade (E and F).

(95% CI: 71.6–91.2%) for the former patients, and 73.8% (95% CI: 65.0–83.8%) and 67.3% (95% CI: 57.8–78.3%) for the latter ($P = 0.051$) (Figure 1E, F).

Incidence of Events and Associations with Clinical Variables

By the time of this report, 64 patients had experienced an event: 41 had a local relapse, and 23 had a distant relapse (with a local component in 4 cases).

A higher incidence of local relapse was statistically associated with male sex, residual disease at RT, and need for a VP shunt. A higher incidence of disseminated relapse was associated with residual disease and histological grade III. Molecular subgroups showed no correlations with the site of relapse (Table 1).

The median disease-free interval for patients who experienced disease progression was 21 months (range, 2–126 mo) overall, and 24 and 18 months for local and distant relapses, respectively.

Table 1. Five- and 10-year crude cumulative incidence (CCI) estimates of local relapse (LR) and distant metastasis (DM)

	5-year CCI LR (95% CI)	10-year CCI LR (95% CI)	<i>P</i> value	5-year CCI DM (95% CI)	10-year CCI DM (95% CI)	<i>P</i> value
Sex			<0.001			0.307
Female	5% (2–15%)	11% (5–25%)		15% (8–28%)	20% (11–34%)	
Male	30% (22–41%)	36% (27–47%)		12% (7–21%)	12% (7–21%)	
Age, y			0.087			0.418
Under 3 y	24% (15–41%)	37% (24–56%)		18% (9–34%)	18% (9–34%)	
3 y or over	19% (13–28%)	23% (16–32%)		11% (7–19%)	14% (9–23%)	
Tumor location			0.113			0.579
Supratentorial	17% (9–32%)	17% (9–32%)		8% (3–22%)	15% (7–31%)	
Posterior fossa	23% (16–32%)	31% (23–42%)		15% (10–24%)	15% (10–24%)	
Residual disease after surgery						0.016
No residual WHO grade II	13% (6–27%)	18% (9–35%)		2% (0–15%)	2% (0–15%)	
No residual WHO grade III	18% (10–31%)	22% (13–35%)		19% (12–32%)	22% (13–35%)	
Residual, any grade	32% (21–48%)	40% (28–57%)		16% (8–30%)	19% (10–34%)	
Status before radiation therapy			0.045			0.099
NED	16% (11–24%)	23% (16–32%)		11% (7–18%)	12% (7–20%)	
ED	35% (23–54%)	38% (25–56%)		20% (11–38%)	24% (13–43%)	
WHO grade			0.585			0.029
Grade II/classic	19% (12–30%)	24% (16–37%)		7% (3–15%)	9% (4–20%)	
Grade III/anaplastic	23% (15–34%)	29% (20–41%)		19% (12–30%)	21% (13–32%)	
Ventricular shunt			0.028			0.529
No	16% (10–25%)	21% (14–32%)		13% (8–22%)	13% (8–22%)	
Yes	29% (19–43%)	35% (24–50%)		13% (7–26%)	19% (11–34%)	
Symptoms at recurrence			0.142			0.197
No	57% (44–74%)	---		27% (17–45%)	---	
Yes	40% (23–71%)	45% (27–76%)		45% (27–75%)	50% (31–81%)	
Treatment at recurrence			0.054			0.543
No	---	---		---	---	
Yes	48% (37–63%)	60% (49–75%)		35% (24–50%)	38% (27–53%)	

Of the 64 relapsing patients, 31 had an OS beyond 5 years after their diagnosis: 21 had relapsed earlier, and 10 relapsed more than 5 years after being diagnosed (Supplementary Table 2). The 4-year OS after relapsing was 78.7% (95% CI: 62.0–99.8%) and 74.1% (95% CI: 48.4–100%) for patients relapsing early and later, respectively ($P = 0.192$).

Survival Endpoints

On univariable assessment, female sex and absence of residual tumor after first surgery and/or before RT were significantly associated with a better OS and PFS. An ST origin, the absence of symptoms, and treatment on recurrence were associated with a better OS, while age over 3 years at diagnosis, histological WHO grade II, and no need for a VP shunt were associated with a better PFS (Table 2).

After completing the selection procedures on the multivariable models including the selected covariates

(Supplementary Table 3), a better PFS was significantly associated with WHO grade II tumor and complete surgery at first diagnosis and/or at RT, while female sex and complete resection showed a positive association with OS, confirming previous reports.⁵

Associations Between Clinical Variables

A significant positive association emerged between tumor origin and complete resection (64.5% in PF vs 81.2% in ST tumors, $P = 0.027$); the need for a VP shunt (46.4% in PF vs 16.7% in ST tumors, $P < 0.001$); high tumor grade (60.7% in PF vs 39.3% in ST tumors, $P = 0.009$); and age under 3 years (35.7% in PF vs 10.4% in ST tumors, $P = 0.001$). The need for a VP shunt and a higher histological grade were more frequent in patients under 3 years of age at diagnosis (51.1% vs 32.2% [$P = 0.030$], and 66.7% vs 47.0% [$P = 0.034$], respectively). No statistically significant associations were found between sex and tumor origin, need

Table 2. Five- and 10-year Kaplan–Meier estimates for OS and PFS

	5-year OS (95% CI)	10-year OS (95% CI)	<i>P</i> value	5-year PFS (95% CI)	10-year PFS (95% CI)	<i>P</i> value
Sex			0.004			0.015
Female	90% (83–98%)	85% (76–96%)		80% (71–91%)	69% (58–83%)	
Male	74% (66–83%)	66% (58–77%)		58% (49–68%)	52% (43–64%)	
Age, y			0.126			0.039
Under 3 y	73% (62–88%)	68% (56–84%)		58% (45–74%)	45% (32–64%)	
3 y or over	83% (76–90%)	76% (68–85%)		70% (62–78%)	63% (55–73%)	
Tumor location			0.042			0.073
Supratentorial	90% (81–99%)	82% (71–95%)		75% (64–88%)	69% (56–84%)	
Posterior fossa	76% (68–84%)	70% (62–80%)		62% (54–72%)	54% (45–65%)	
Residual disease after surgery			<0.001			0.003
No residual WHO grade II	96% (90–100%)	96% (90–100%)		85% (76–96%)	80% (69–93%)	
No residual WHO grade III	81% (71–91%)	73% (63–86%)		63% (52–76%)	57% (46–71%)	
Residual, any grade	64% (52–79%)	54% (41–71%)		52% (40–68%)	42% (29–59%)	
Status before radiation therapy			<0.001			0.002
NED	87% (82–94%)	83% (76–90%)		73% (66–82%)	65% (57–75%)	
ED	58% (44–75%)	47% (33–67%)		45% (32–63%)	39% (26–58%)	
WHO grade			0.051			0.031
Grade II/classic	87% (80–95%)	81% (72–91%)		75% (66–85%)	67% (56–79%)	
Grade III/anaplastic	74% (65–84%)	67% (58–78%)		58% (49–70%)	51% (41–63%)	
Ventricular shunt			0.068			0.021
No	85% (78–92%)	79% (72–88%)		71% (63–80%)	66% (57–76%)	
Yes	72% (61–84%)	63% (51–78%)		58% (47–72%)	46% (34–62%)	
Symptoms at recurrence			0.002			0.039
No	61% (48–78%)	45% (32–63%)		54% (40–71%)	43% (29–63%)	
Yes	25% (12–53%)	15% (5–43%)		100%	80% (52–100%)	
Treatment at recurrence			<0.001			—
No	—	—		—	—	
Yes	55% (44–70%)	38% (26–54%)		—	—	

for a VP shunt, histological grade, or extent of resection, or between extent of resection and tumor grade or age (data not shown).

Analyses of Clinical and Molecular Features

There were no major differences in clinical characteristics between patients for whom molecular analyses were feasible and those for whom they were not. There was a slightly higher prevalence of histological WHO grade III and no need for a VP shunt among the former patients (Supplementary Table 4).

When patients' tumors were subgrouped by DNA methylation profiling outcome, their scores robustly classified 68/95 patients (with scores ≥ 0.8). Supplementary Table 5 shows the clinical features of the patient group whose tumors scored ≥ 0.8 compared with those scoring below 0.8. The latter group included a higher proportion of male patients, and those under 3 years of age.

Of the 68 tumors with a reliable subgrouping score after methylation profiling, 50 were located in the PF and 18 were ST. Among the PF tumors, 41 were classified as PFA, and 9 as PFB.¹⁰ Among 18 ST tumors analyzed, 15 were classified as RELA,^{8,9} while 3 scored for other molecular entities (ie, anaplastic pleomorphic xanthoastrocytoma, MYB-rearranged low-grade glioma,¹⁸ and high-grade neuroepithelial tumor with MN1 alteration [CNS HGNET-MN1]).¹⁹ Another 3 ST tumors from the original sample of 160 were classified as RELA-positive by gene fusion analysis alone. The total number of RELA fusion samples was therefore 18/32 available tissues of ST tumors, while 13/32 ST tumor samples did not score for RELA, or did not have the fusion genes. Finally, 1 ST tumor revealed the YAP1-MAMLD1 fusion gene²⁰ (Supplementary Figure 1).

We did not observed prognostic impact of RELA status for patients with ST ependymoma. PFS and OS [95% CI] at 5/10 years were 61.5% [40.0–94.6%]/61.5% [40.0–94.6%] vs 77.8% [60.8–99.6%] / 68.1% [47.5–97.5%] and 84.6% [67.1–100%]/84.6% [67.1–100%] vs 94.4% [84.4–100%]/82.6% [66.6/100%] for patients without and with RELA,

respectively (P values equal to 0.521 and 0.979 for PFS and OS, respectively).

PFA and PFB subtypes were not associated with sex, histological grade, or need for a VP shunt. The 5- and 10-year PFS were 53.7% (95% CI: 40.4–71.3%) and 43.0% (95% CI: 29.3–63.1%), respectively, for the 41 patients with PFA tumors, and 100% and 80.0% (95% CI: 51.6–100%) for the 9 patients with PFB tumors ($P = 0.039$). The 5- and 10-year OS were 70.7% (95% CI: 58.1–86.1%) and 64.5% (95% CI: 51.0–81.6%) for patients with PFA tumors, and 100% at both times for patients with PFB tumors ($P = 0.058$) (Supplementary Figure 2).

All 9 patients with PFB tumors were over 3 years old at diagnosis, as opposed to 17 (41.5%) of the patients with PFA tumors ($P < 0.001$). After first surgery, 8 of the 9 were NED versus 27 (65.9%) of the patients with PFA tumors ($P = 0.130$); WHO grade II was diagnosed in 43.9% of PFA tumors versus 8/9 of PFB ones ($P = 0.014$) (Supplementary Table 6).

The status of 94 of the 95 samples retrieved was ascertained in terms of 1q gain and CDKN2A loss—2 molecular events previously associated with a worse survival^{21,22} 1q gain was found in 15 patients' samples, 11 of the 67 (16%) tumors originating in the PF, and 4 of the 27 (14.8%) ST tumors; and CDKN2A loss was documented in 3/31 (9.6%) ST tumor samples, and in none of the PF tumors. We defined the group of patients with either 1q gain or CDKN2A loss as "biomarker-positive," and the patients with neither anomaly as "biomarker-negative." In 67 tumors originating in the PF, 91% of the 11 patients with biomarker-positive tumors were aged over 3 years at diagnosis versus 55% in the biomarker-negative group ($P = 0.022$), 64% of biomarker-positive tumors were WHO grade III versus 46.4% of biomarker-negative ones (P n.s) and only 1/11 patient (9%) versus 26/56 (46%) needed a shunt at diagnosis ($P = 0.017$). For these patients there was no difference in sex, presence of residual disease neither at diagnosis nor after second look, symptom manifestation or possibility for re-treatment at relapse (data not shown). Overall, the 18 biomarker-positive patients had worse 5- and 10-year PFS and OS (95% CI), although the differences were not statistically significant. The 5- and 10-year PFS were 50.0% (31.5–79.4%) and 41.7% (23.2–74.7%) for the biomarker-positive group versus 69.6% (59.9–80.8%) and 61.9 (51.3–74.8%) for the biomarker-negative group ($P = 0.071$). The 5- and 10-year OS were 66.7% (48.1–92.4%) and 61.1% (42.3–88.3%) for the former group versus 84.2% (76.3–92.8%) and 79.3% (70.4–89.3%) for the latter ($P = 0.105$). These differences were largely due to a significantly ($P = 0.007$) higher crude cumulative incidence (95% CI) of distant metastasis in the biomarker-positive group, in which the 5- and 10-year estimates were 27.8% (12.8–60.2%) and 36.1% (17.9–72.7%), as opposed to 9.3% (4.5–18.9%) at both times in the biomarker-negative group (Figure 2).

Considering 1q gain alone in the PFA subgroup,^{4,21,23,24} we found similar results. Only the incidence of distant relapses had a tendency to be lower in the biomarker-negative group, albeit without reaching statistical significance probably due to the small number of patients involved and the consequent loss of statistical power (Supplementary Figure 3).

A very similar, unfavorable OS probability (95% CI) was observed after relapse, with 5- and 10-year estimates of

26.7% (8.9–80.3%) at both times in the biomarker-positive group, and 37.8% (22.0–65.0%) and 28.4% (13.0–62.1%) in the biomarker-negative group (Figure 3).

Discussion

The feasibility of identifying the cell of origin by means of epigenetic studies in CNS tumors has broadened our understanding of the molecular subtypes of ependymal tumors that may behave like separate entities.^{1,9,25} As shown here, the long-term reassessment of patients included in a controlled clinical trial can lend more significance to the impact of these molecular subgroups, underpinning their prognostic relevance.

In our ten-year follow-up, the impact of clinical variables remained the same as previously reported, thus confirming (also in multivariable analysis) a better PFS for patients with WHO grade II tumors and complete resection, and a better OS for female patients and complete resection.⁵ The value of surgery and histological grade was likewise demonstrated in the latest ACNS0121 prospective trial too,²⁶ as well as in some other earlier trials.³

The likelihood of a complete resection was greater for ST tumors, as this tumor origin also implied a less frequent need for a VP shunt. Compounding this more favorable risk profile, histological WHO grade II was more common among ST tumors, and patients were mostly more than 3 years old. The possibility of this "favorable outcome" group being spared RT was explored in the ACNS0121 trial: 11 patients with completely-resected, WHO grade II, ST tumors were followed up without any adjuvant treatment after resection, achieving a 5-year PFS of 61.4%, and an OS of 100%.²⁶

In the series re-analyzed here, residual disease at different stages of treatment was associated not only with local relapse but also with tumor dissemination. Nearly 15% of relapses appeared after 5 years, but there were no differences between these patients and the remainder of the series as regards patient or tumor features (ie, sex, age, need for a VP shunt, surgical outcome, tumor grade, molecular subtype and chromosomal unbalances), or relapse pattern (local vs disseminated or both). Late relapses did not impact on survival afterward, which was comparable with that of patients relapsing earlier. This indirectly confirms that the tumor's clinical and biological features remained much the same over time³ and that PFS for patients with ependymoma has not plateaued even at 10 years of median follow-up.

It is noteworthy that retrospective methylation array analysis was available for only a limited number of patients (95 out of 160 [59.4%]), and a robust molecular subgroup assignment was reached only in 68/95 samples (71.6%). The reason for this is the relatively stringent cutoff that we applied to accept only samples in the molecular analysis which displayed unambiguous molecular classification. The main reason that 27 samples could not be allocated to a subgroup was a high content of nonneoplastic cells in the sample. This is frequently observed when applying methylation array classification²⁷ and a common problem mainly in tumors with a high degree of stromal infiltration.

The generation of copy number profiles from methylation array data was more robust and was possible in 94/95

Fig. 2 Kaplan-Meier curves for OS (A) and PFS (B) and incidence curves for local relapse (C) and distant metastasis (D) according to molecular events.

Fig. 3 Kaplan-Meier curves for post-relapse overall survival of biomarker-positive versus biomarker-negative patients. Biomarkers included are gain of 1q and loss of 9p/CDKN2A.

cases (98.9%). The copy number analysis could thus be performed in samples in which molecular subgrouping was not possible. An additional caveat of our study refers to the way that copy number aberrations were determined:

although the scoring of numeric chromosome alterations in tumors is well established and generally accepted, an additional level of confirmation¹³ could be reached by validating the CNV alterations by using other methods (eg, multiplex ligation-dependent probe amplification). However the dearth of the available material precluded this possibility in our cohort. Future studies will need to address this issue.

Overall, the results concerning the ependymoma subgroups are to be seen in the context of a much more limited series than that for which clinical data were available.

It was previously reported that methylation profiling could identify 2 PF ependymoma subgroups, PFA and PFB, and that PFB ependymomas had a better prognosis.^{1,10} The better prognosis for patients with PFB tumors was confirmed in the present study. As expected, this molecular subgroup correlated with other favorable features, such as older age, grade II tumors, and the absence of residual tumor before RT. On the other hand, we observed one patient with a late relapse—an occurrence already mentioned in the retrospective analysis by Cavalli et al.²⁸ These results, obtained in a homogeneously treated series like ours, suggest that patients with PFB tumors still need careful follow-up in current protocols before changing adjuvant treatment.

In patients with ST tumors, RELA status had no impact on prognosis. Previous reports on mixed case series had suggested a negative prognostic role of RELA

subgroup,^{1,9,29} but our results are in line with more recent, prospective data.²⁶ Further prospective clinical trials that include upfront molecular subtyping will be needed to resolve this issue. ST ependymomas are still a “mystery,” and their heterogeneity is still not fully understood.³⁰ RELA fusion-negative ST ependymomas confirmed their heterogeneity as tumors that do not fall into any of the existing molecular subgroups, and that are unlikely to form a single category—which is why there is no homogeneous prognosis for such patients.^{31,32} The only case of YAP1-MAMLD1 ST tumor showed the same features as those already published by Andreiulo et al³³ and did not relapse.

Both 1q gain and 9p/CDKN2A loss are chromosomal features previously associated with a poor prognosis.^{21,22,34–37} In our cohort too, 1q gain or CDKN2A loss correlated with a worse prognosis and significant dissemination at relapse.

Given the features correlating with tumor dissemination, if our findings will be confirmed in ongoing prospective trials, it might be worth selecting a patient group that warrants first-line craniospinal irradiation to ensure tumor control, instead of just reserving it for tumor re-irradiation.³⁸

To the best of our knowledge, there are no other comparable prospective trials for the diagnosis and treatment of pediatric ependymoma which have been published with such a long follow-up. We believe that our clinical and biological observations will be able to facilitate the interpretation of the results of ongoing trials.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (<http://neuro-oncology.oxfordjournals.org/>).

Keywords

children | follow-up | intracranial ependymoma | molecular events | prognosis

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