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ONCOLOGY: RESEARCH ARTICLE

A retrospective analysis of recurrent pediatric ependymoma reveals extremely poor survival and ineffectiveness of current treatments across central nervous system locations and molecular subgroups

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Abstract

Background: Relapse occurs in 50% of pediatric ependymoma cases and has poor prognosis. Few studies have investigated the clinical progress of relapsed disease, and treatment lacks a standardized approach.

Methods and materials: We analyzed 302 pediatric ependymoma cases. Tumor, demographic, and treatment variables were investigated for association with relapse risk, time to recurrence, and survival after relapse. DNA methylation profiling was performed for 135/302 cases, and predominant subgroups were EPN_PFA ($n = 95$) and EPN_RELA ($n = 24$). Chromosome 1q status was ascertained for 185/302 cases by fluorescent in-situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), and DNA methylation profiles.

Results: Sixty-two percent of cases relapsed, with a median of two recurrences with no difference between posterior fossa and supratentorial locations (66% vs 55% relapse rate). One hundred seventeen (38%) cases relapsed within two years and five (2%) beyond 10 years. The late relapses were clinically heterogeneous. Tumor grade and treatment affected risk and time to relapse variably across subgroups. After relapse, surgery and irradiation delayed disease progression with a minimal impact on survival across the entire cohort. In the EPN_PFA and EPN_RELA groups, 1q gain was independently associated with relapse risk (subhazard ratio [SHR] 4.307, $P = 0.027$ and SHR 1.982, $P = 0.010$, respectively) while EPN_PFA had increased relapse risk compared with EPN_RELA (SHR = 0.394, $P = 0.018$).

Abbreviations: CCLG, Children' Cancer and Leukaemia Group; CNS, central nervous system; CSI, craniospinal irradiation; DNA, deoxyribonucleic acid; DNET, dysembryonic neuroepithelial tumor; EFS, event-free survival; EPN, ependymoma; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescent in-situ hybridization; GTR, gross total resection; MLPA, multiplex ligation-dependent probe amplification; OS, overall survival; PF, posterior fossa; PFA, posterior fossa A; PFB, posterior fossa B; RELA, V-rel avian reticuloendotheliosis viral oncogene homolog A; SHR, subhazard ratio; SIOP, International Society of Paediatric Oncology; ST, supratentorial; STR, subtotal resection; WHO, World Health Organization; YAP, yes-associated protein.

Conclusions: Recurrent pediatric ependymoma is an aggressive disease with poor outcomes, for which current treatments are inadequate. We report that chromosome 1q gain increases relapse risk in common molecular subgroups in children but a deeper understanding of the underlying biology at relapse and novel therapeutic approaches are urgently needed.

KEYWORDS
ependymoma, neuro-oncology, pediatric, radiotherapy, relapse

1 | INTRODUCTION

Ependymoma is the second commonest malignant pediatric brain tumor and is associated with poor outcomes.[1] Relapse occurs in 50%, with a five-year overall survival rate of approximately 25%.[2]

Therapy consists of surgery followed by radiotherapy, with chemotherapy for young children and those with a subtotal resection (STR).[3] Gross total resection (GTR) and irradiation have been associated with better outcomes,[4-12] but the benefits of chemotherapy are less clear.[3,6,12-17] The strongest reported risk factor for relapse is STR.[10,18,19]

Therapeutic approaches at relapse lack a standardized approach,[20] but further surgery and reirradiation have been proposed to improve prognosis.[2,21] Studies suggest that reirradiation of recurrent ependymoma is safe and feasible.[21-23] However, there are risks of brainstem radio-necrosis and late effects. Evidence that chemotherapy is beneficial at recurrence is limited to a few small studies.[24-26] Novel therapies have been investigated at relapse but have been largely limited to phase 1 studies.[27-29] Despite the lack of treatment consensus, there are no current multicenter randomized trials for recurrent ependymoma.

Molecular classification of ependymoma has advanced, with RELA-fusion positive (EPN_RELA) supratentorial (ST) ependymoma introduced to the WHO classification of CNS tumors.[30] Studies describe nine molecular ependymoma subgroups based on DNA methylation and gene expression.[31-33] These subgroups are now being integrated with known molecular characteristics to enhance disease understanding, in particular, associations have been identified between chromosome 1q gain and the aggressive posterior fossa A (EPN_PFA) tumor.[12,31]

Well-annotated studies of relapsed ependymoma have been small and often comprised of mixed adult and childhood cohorts.[2,26,34-37] We assembled a large cohort of 302 children with ependymoma to better understand recurrence. DNA methylation profiling enabled us to place this in the context of molecular subgrouping.[31]

We highlight that recurrent pediatric ependymoma is aggressive with poor outcomes for the predominant pediatric subgroups. Current interventions are associated with reduced relapse risk and increased disease-free survival time, but do not prevent recurrence. Interventions at relapse have minimal impact on long-term survival, underscoring an urgency for new therapies.

2 | METHODS

2.1 | Patient cohort

Three hundred two patients (< 18 years) were included. One hundred seventy patients were part of two international trials (SIOP 1992,[3] 1999[38]). Two hundred fifty-four patients were treated in the UK, 16 in the USA, 18 in Holland, 7 in Dublin, 4 in Denmark, 2 in Spain, and 1 in Sweden. Clinical information and tumor specimens were obtained via the Children's Cancer and Leukaemia Group. Ethical approval was obtained from the UK-wide Multicentre Research Ethics Committee (MREC11/EM/0076). Consent for inclusion in biological studies was obtained in line with national regulations.

Patients with grade II or III ependymoma diagnosed after 1990 were included. Diagnosis was confirmed by central pathology review in Nottingham. Fifteen cases were centrally reviewed at the University of Colorado, Denver. Nine cases received only local review. DNA methylation profiling was available for 135 primary cases.

Recurrence was defined as return of a tumor following GTR or clinical or radiological progression following STR. Imaging was centrally reviewed at primary diagnosis but reviewed locally at relapse.

Treatment was stratified by age at presentation. Children under three years were treated with surgery followed by chemotherapy, following the SIOP 92 protocol.[3] Focal radiotherapy was considered at recurrence. Children over three years received involved field radiotherapy within four weeks of surgery if GTR achieved, and involved field radiotherapy within three weeks of completion of chemotherapy if STR achieved, following the SIOP 99 study protocol.[38] Craniospinal irradiation (CSI) was not employed routinely at diagnosis. Approaches at recurrence varied by locality but involved resection followed by chemotherapy and/or focal radiotherapy or CSI. Proton beam radiotherapy was not used.

2.2 | DNA methylation profiling

DNA was extracted using the AllPrep DNA/RNA FFPE kit (Qiagen, Germany) for FFPE tissue or QIAmp DNA mini kit (Qiagen, Germany) for frozen tissue. Profiling was performed on 1000 ng bisulphite-converted DNA using Infinium HumanMethylation450 BeadChip arrays (Illumina, San Diego, USA) at UCL Genomics (UK). Ependymoma subgroups were assigned as previously described.[31] Copy-number variation was calculated using the Conumee R package.

2.3 | Statistical analysis

Cases were analyzed as a combined cohort to allow comparison with previous studies, before subgroup analysis of tumors by intracranial compartment (PF and ST) and major subgroups (EPN_PFA and EPN_RELA).

Differences in time to relapse were tested using the Wilcoxon rank-sum test. Overall and event-free survival (OS and EFS) analyses were performed using a supremum log-rank test. Kaplan-Meier curves and Cox proportional hazards models were generated using R packages "Survival" and "Survminer."[39] OS was defined as the time between date of diagnosis and death, and EFS as the time between date of diagnosis and recurrence or death. Surviving patients were censored at last follow-up.

Fine and Gray[40] competing risks regression was used to test risk of recurrence using STATA (Statacorp, Texas, USA). Death before first recurrence was included as a competing risk.

2.4 | Chromosome 1q FISH

Gain of chromosome 1q was determined using dual color interphase FISH as previously described, using commercial 1q25 and 1p36 probes (Abbot Molecular, Illinois, USA).[41]

2.5 | Multiplex ligation-dependent probe amplification (MLPA)

1q25.1 copy number was determined using probes for LHX4 (6 exons) (http://www.mrc-holland.com). MLPA was performed using the Salsa MLPA kit (MRC-Holland, Amsterdam, Holland).

3 | RESULTS

3.1 | Group and treatment characteristics

Three hundred two children with ependymoma, diagnosed between 1990 and 2015, were included (Table 1). Median OS was 170 months (range, 0-260). 114 (38%) patients died, while 184 (62%) were alive at most recent follow-up (median 97 months, range, 8-260). Median age at diagnosis was 39 months (range, 0-225).There were 216 (72%) PF, 73 (24%) ST, and 11 (4%) spinal cases (Table 1).

The GTR rate across the whole cohort was 52%. At primary diagnosis, in addition to surgery, 117/136 (86%) children under three years, received chemotherapy alone. Fifteen of 136 (11%) children received focal radiotherapy with or without chemotherapy. Four of 136 (3%) children received no treatment after surgery.

One hundred sixteen of 145 (80%) children age over 3 received either focal radiotherapy (79/116, 68%) or chemotherapy followed by radiotherapy (37/116, 32%), dependent on the extent of tumor resection. Twenty-two of 145 (15%) children age over 3 received chemotherapy only. Seven of 145 (5%) received no treatment after surgery. In all age groups, the children who received no treatment after surgery had spinal tumors ($n = 4$), died following surgery ($n = 2$), or had GTR with decision for no further treatment ($n = 5$).

At first recurrence, in addition to surgery, 99/144 (69%) patients with data received radiotherapy of any type. The remainder (45/144, 31%) received chemotherapy. Of those with data on radiotherapy type at first recurrence, 26/37 (70%) received focal treatment while 11/37 (30%) received CSI.

The majority of patients in the recurrent cohort received their first radiotherapy at recurrence (70/99, 71%) and a smaller proportion (25/99, 25%) received reirradiation. Reirradiation status was unknown for 4/99 (4%).

DNA methylation profiles were generated for 135 primary tumors plus 92 relapses from 67 patients. Clinical associations for primary tumors agreed with previous reports (Supporting Information Figure S1).[31] The cohort contained predominantly EPN_PFA ($n = 95$) and EPN_RELA ($n = 24$) cases.

Four patients had nonependymoma profiles at recurrence, two likely due to normal brain contamination. Of the remaining two, one was classified as spinal subependymoma and the other glioblastoma. The primary tumor was excluded for both.

3.2 | Relapse pattern

Of the combined cohort of 302 patients, 186 (62%) relapsed with a five-year cumulative incidence of 57%.One hundred forty-three of 216 (66%) PF tumors relapsed compared with 40/73 (55%) ST tumors. In PF and ST groups, there was over 90% long-term OS for patients without relapse (Supporting Information Figure S2). The number of recurrences ranged from one to six (median two) for PF tumors and one to eight (median one) for ST tumors. For both locations the risk of further relapse remained approximately 50% each time (Figure 1A and D). Subsequent relapses occurred more rapidly in the PF ($P < 0.001$) (Figure 1B), but not the ST, cohort ($P = 0.430$) (Figure 1E).

Median time to first relapse for the combined cohort was 18 months (range, 0-165), 19 months for PF tumors (0-165), and 16.5 months for ST tumors (1-124). Sixty-nine (38%) patients relapsed beyond two years and five (3%) after 10 years. The patients who relapsed late were heterogeneous. Four had PF, and one a ST, tumor. There were two EPN_PFA, one EPN_PFB and two with unknown subgroups. Three of five were alive at follow-up. Three received chemotherapy and radiotherapy and two chemotherapy alone. Three of five achieved GTR. Only the ST case had 1q gain.

The total number of relapses was associated with OS in the PF cohort ($P = 0.041$), survival being better for patients with only one recurrence (Figure 1C), but not in the ST cohort (Figure 1F).

A higher proportion of EPN_PFA and EPN_YAP cases relapsed than other subgroups (Figure 2A). However, EPN_RELA patients who recurred experienced most relapses (mean: EPN_PFA 2, EPN_PFB 1, EPN_RELA 3, EPN_YAP 1). Relapse rate was low in EPN_PFB and EPN_SPINE (Figure 2B). Two of nine EPN_PFB cases relapsed but both were alive 10 years after diagnosis. Four of five EPN_YAP cases relapsed; two were disease free at 10 years, one relapsed a second time but was alive after five years and one died after three months.

TABLE 1 Primary tumor cohort

Parameter		Number of cases			
		PF[a]	ST[b]	[c]SP	Unknown
Age at diagnosis (years)	0-5	151	29	0	1
	5-10	40	23	4	1
	10-15	16	14	6	0
	15-18	8	5	1	0
	Unknown	1	2	0	0
Gender	Male	124	36	7	2
	Female	88	37	4	0
	Unknown	4	0	0	0
Grade	WHO II	131	29	11	2
	WHO III	84	44	0	0
	Unknown	1	0	0	0
Extent of resection	Gross total	101	35	6	2
	Subtotal	93	34	4	0
	Unknown	22	4	1	0
Focal radiotherapy at primary diagnosis	Yes	85	39	7	0
	No	123	30	4	1
	Unknown	8	4	0	1
Chemotherapy at primary diagnosis	Yes	143	41	3	1
	No	59	27	8	1
	Unknown	14	5	0	0
Treatment timing at primary diagnosis	CT[d] only	114	25	0	1
	Focal RT[e] only	56	26	4	0
	CT followed by focal RT	28	14	3	0
	None	4	3	4	0
	Unknown	14	5	0	1
Chromosome 1q gain	No gain	104	37	3	0
	Gain	29	12	0	0
	Unknown	83	24	8	2
Molecular subgroup	EPN_PFA	92	3	0	0
	EPN_PFB	9	0	0	0
	EPN_RELA	0	24	0	0
	EPN_YAP	0	5	0	0
	EPN_SPINE	0	0	2	0

[a]PF, posterior fossa.
[b]ST, supratentorial.
[c]SP, spinal.
[d]CT, chemotherapy.
[e]RT, radiotherapy.

Methylation patterns were determined for 40 primary tumors and paired first recurrences. Thirty-nine (98%) remained in the same subgroup at relapse. One EPN_PFA switched to a DNET, which could have represented normal brain contamination. All profiled second relapses ($n = 11$) remained in the same subgroup.

3.3 | Factors associated with relapse risk

In multivariate analysis of the combined cohort, factors associated with increased risk of relapse were younger age, STR, no radiotherapy at diagnosis, and chromosome 1q gain (Supporting Information Tables S1 and S2).

In univariate analysis of PF tumors, greater risk of relapse was associated with younger age, no radiotherapy, receipt of chemotherapy, and chromosome 1q gain, but in multivariate analysis only 1q gain remained significant (subhazard ratio [SHR] 3.042, $P < 0.001$). For ST tumors, STR and 1q gain were associated with relapse risk in univariate and multivariate analyses (SHR0.320,$P = 0.004$ and SHR2.770, $P = 0.004$) (Table 2).

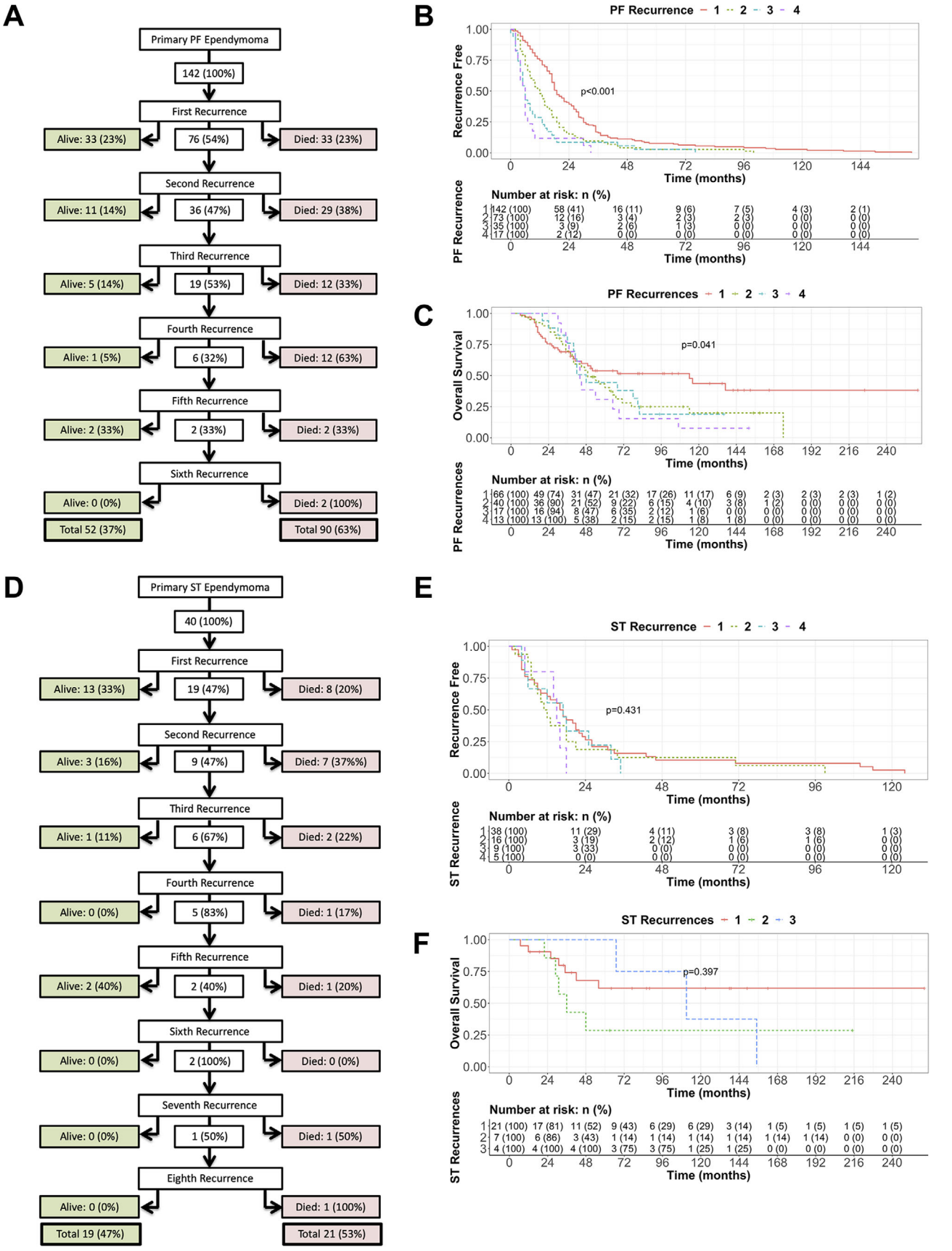

FIGURE 1 (**A**) Pattern of relapsed posterior fossa ependymoma. The probability of further relapse remained at approximately 50%. One case was not included where the total number of relapses was unknown. (**B**) The interval between each recurrence significantly shortened for each subsequent recurrence in the PF cohort ($P < 0.001$). Each survival curve represents the time for each numbered recurrence to occur. (**C**) Patients

EPN_PFA cases had a higher risk of relapse than EPN_RELA cases (SHR = 0.394, $P = 0.018$). For EPN_PFA tumors only grade (SHR 1.791, $P = 0.027$) and 1q gain (SHR 1.982, $P = 0.010$) were associated with relapse risk while for EPN_RELA only 1q gain (SHR 4.307, $P = 0.027$) was associated (Table 3).

3.4 | Factors associated with time to first relapse

The 186 relapsed patients were analyzed for factors associated with time to first relapse (Supporting Information Table S3).

In univariate analysis of the combined cohort, faster relapse was seen for grade III tumors (16 vs 22 months, $P = 0.001$), STR (17 vs 20 months, $P = 0.018$), receipt of chemotherapy (17 vs 24 months, $P = 0.007$) and lack of radiotherapy (23 vs 17 months, $P = 0.002$) (Supporting Information Figure S3A-C, E). Significantly delayed radiotherapy (beyond four months after surgery) did not have an impact ($P = 0.173$). Combined GTR and radiotherapy delayed relapse compared with no radiotherapy (31 vs 18 months, $P = 0.008$) (Supporting Information Figure S3D). In multivariate analysis of the combined cohort, grade III (HR 1.572, $P = 0.030$) and STR (HR 0.642, $P = 0.048$) remained associated with faster relapse (Supporting Information Table S4).

In univariate analysis of PF tumors ($n = 142$), faster relapse was seen for grade III (17 vs 22 months, $P = 0.002$), STR (18 vs 22 months, $P = 0.008$), receipt of chemotherapy (18 vs 28 months, $P = 0.008$), male gender (18 vs 25 months, $P = 0.039$), and lack of radiotherapy (17 vs 27 months, $P = 0.001$) (Supporting Information Figure S4). In multivariate analysis, grade III (HR 1.586, $P = 0.018$) and STR (HR 0.617, $P = 0.015$) remained associated with faster relapse (Supporting Information Table S4).

with PF ependymoma who relapsed only once had an improved OS compared with those who relapsed more than once ($P = 0.041$). Analysis only included patients with up to four relapses, with cases suffering higher numbers too low. (**D**) Pattern of relapsed supratentorial ependymoma. The probability of further relapse remained at approximately 50%. (**E**) The interval between each recurrence did not significantly change for each subsequent recurrence in the PF cohort ($P = 0.430$), although numbers for this analysis were small. Each survival curve represents the time for each numbered recurrence to occur. (**F**) Patients with ST ependymoma who relapsed only once had no difference in OS compared with those who relapsed more than once ($P = 0.397$). Analysis only included patients with up to three relapses, with cases suffering higher numbers too low

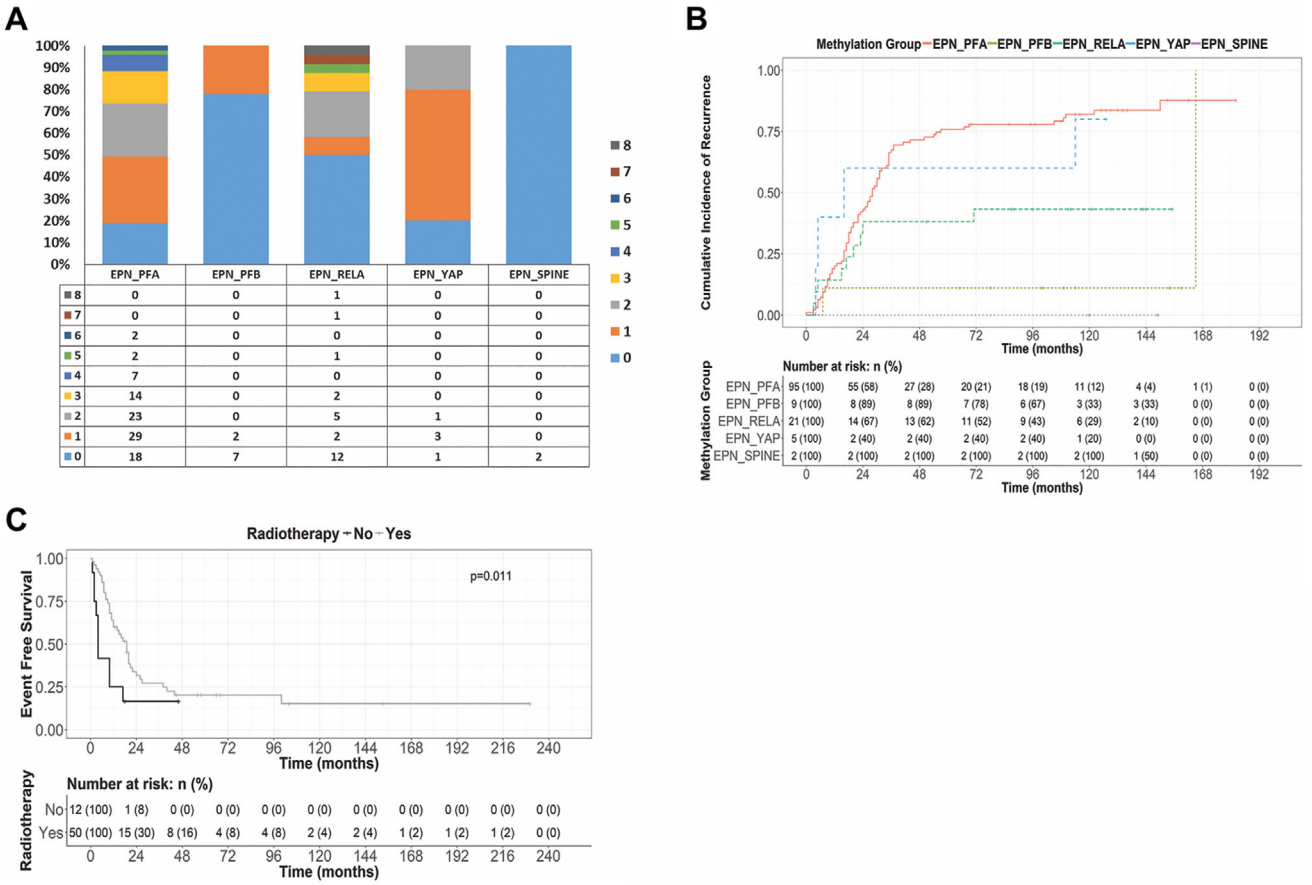

FIGURE 2 (**A**) Proportion of cases who suffered different numbers of relapses by molecular subgroup. (**B**) EPN_PFB and EPN_SPINE subgroups displayed a lower relapse incidence than the EPN_PFA, EPN_RELA and EPN_YAP subgroups. For three EPN_RELA cases, the time to relapse was not known, so was not included. (**C**) Patients with EPN_PFA tumors irradiated at first relapse had an improved EFS ($P = 0.011$)

TABLE 2 Univariate and multivariate competing risks for tumors based on location

	Univariate				Multivariate			
	SHR	95% CI	*P*	Number of cases tested	SHR	95% CI	*P*	Number of cases tested
Posterior fossa tumors								
Age (continuous)	0.996	0.992-0.999	0.022	212	0.994	0.986-1.002	0.115	120
Gender (female vs male)	0.796	0.571-1.110	0.179	209	–	–	–	–
Grade (III vs II)	1.371	0.979-1.919	0.066	212	1.407	0.870-2.277	0.164	120
Resection (GTR vs STR)	0.711	0.503-1.005	0.054	191	0.709	0.447-1.125	0.144	120
Focal radiotherapy (yes vs no)	0.674	0.483-0.934	0.020	205	0.477	0.205-1.109	0.085	120
Chemotherapy (yes vs no)	1.583	1.090-2.299	0.016	198	0.626	0.278-1.413	0.259	120
Chromosome 1q (gain vs no gain)	1.902	1.230-2.943	0.004	132	3.042	1.819-5.087	<0.001	120
Supratentorial tumors								
Age (continuous)	0.998	0.993-1.004	0.537	70	–	–	–	–
Gender (female vs male)	0.978	0.517-1.849	0.944	70	–	–	–	–
Grade (III vs II)	1.669	0.872-3.192	0.122	70	–	–	–	–
Resection (GTR vs STR)	0.362	0.180-0.726	0.004	69	0.320	0.126-0.694	0.004	46
Focal radiotherapy (yes vs no)	0.562	0.295-1.071	0.080	69	–	–	–	–
Chemotherapy (yes vs no)	1.412	0.716-2.784	0.319	68	–	–	–	–
Chromosome 1q (gain vs no gain)	2.232	1.033-4.821	0.041	47	2.770	1.382-5.552	0.004	46

In ST cases ($n = 41$), only grade III was associated with faster relapse (22 vs 15 months, $P = 0.044$) (Supporting Information Figure S5).

There was no difference in time to recurrence ($P = 0.142$) between EPN_PFA and EPN_RELA tumors. Grade III EPN_PFA tumors relapsed more quickly than grade II ($P = 0.020$). No other factors were significant for the molecular subgroups.

3.5 | Factors associated with survival of relapsed patients

3.5.1 | At primary diagnosis

No treatments at diagnosis were associated with OS at relapse in any cohort (Supporting Information Table S5).

3.5.2 | At relapse

In the combined cohort, patients with metastatic relapse had worse median OS (20 vs 45 months, $P = 0.022$) and EFS (12 vs 20 months, $P = 0.040$) (Figure 3A). Metastasis with or without local relapse had equally poor outcomes (Supporting Information Figure S6A and B).

Irradiation, of any type, at first relapse was associated with early improved median EFS (19 vs 8 months, $P = 0.003$) and OS (33 vs 20 months, $P = 0.006$) (Figure 3B).There was no difference between first irradiation and reirradiation. Radiotherapy type (focal or CSI) was not associated with outcome.

GTR at first relapse was associated with sustained improved EFS (25% vs 0% 10-year survival, $P = 0.013$) (Figure 3C). Patients who received neither GTR nor radiotherapy had the poorest EFS ($P = 0.009$) (Supporting Information Figure S6C). Combined GTR and radiotherapy were associated with delayed time to second relapse ($P = 0.007$) (Supporting Information Figure S6D).

In univariate analysis of the PF cohort, patients with metastases had worse median OS following relapse (20 vs 34 months, $P = 0.034$). Radiotherapy of any type was associated with improved median EFS (17 vs 4.5 months, $P = 0.001$) and OS (32 vs 9 months, $P = 0.001$), a benefit also seen for EFS in the EPN_PFA cohort (19 vs 4 months, $P = 0.011$). GTR at first recurrence was associated with improved EFS but not OS (20 vs 10 months, $P = 0.048$).

In multivariate analysis of PF tumors, improved EFS and OS were associated with radiotherapy of any type (EFS: HR 0.483, 95% CI

TABLE 3 Univariate and multivariate competing risks for tumors based on the molecular subgroup

	Univariate				Multivariate			
	SHR	95% CI	*P*	Number of cases tested	SHR	95% CI	*P*	Number of cases tested
EPN_PFA tumors								
Age (continuous)	1.003	0.998-1.007	0.207	94	–	–	–	–
Gender (female vs male)	0.936	0.601-1.458	0.770	92	–	–	–	–
Grade (III vs II)	1.612	1.020-2.545	0.041	94	1.791	1.067-3.006	0.027	86
Resection (GTR vs STR)	1.019	0.643-1.616	0.935	87	–	–	–	–
Focal radiotherapy (yes vs no)	1.158	0.747-1.797	0.512	92	–	–	–	–
Chemotherapy (yes vs no)	0.654	0.424-1.009	0.055	86	–	–	–	–
Chromosome 1q (gain vs no gain)	1.701	1.052-2.752	0.030	86	1.982	1.177-3.338	0.010	86
EPN_RELA tumors								
Age (continuous)	0.995	0.984-1.007	0.442	21	–	–	–	–
Gender (female vs male)	0.629	0.174-2.284	0.482	21	–	–	–	–
Grade (III vs II)	1.043	0.273-3.985	0.951	21	–	–	–	–
Resection (GTR vs STR)	0.548	0.152-1.972	0.357	21	–	–	–	–
Focal radiotherapy (yes vs no)	0.551	0.154-1.970	0.359	21	–	–	–	–
Chemotherapy (yes vs no)	0.851	0.217-3.330	0.816	20	–	–	–	–
Chromosome 1q (gain vs no gain)	4.307	1.178-15.757	0.027	20	–	–	–	–

0.235-0.990, $P = 0.047$, OS: HR 0.439, 95% CI 0.208-0.927, $P = 0.031$) but not GTR. Radiotherapy was also associated with improved EFS in EPN_PFA (HR 0.180, 95% CI 0.583-0.557, $P = 0.003$).

There was no improved EFS, OS, or time to recurrence in ST or EPN_RELA cohorts with any combination of surgery or radiotherapy.

4 | DISCUSSION

We present an analysis of relapsed pediatric ependymoma highlighting its dismal prognosis. The large cohort, supported by extended follow-up and molecular data, has allowed a more comprehensive assessment of outcomes than previously. The best predictor of relapse risk across all subgroups was chromosome 1q gain. Although primary surgery and irradiation reduced relapse risk variably in different intracranial locations, once a patient recurred these interventions gave, at best, short-term benefits, confirming the need for better therapies.

Sixty-two percent of cases relapsed, with 57% five-year cumulative incidence and no difference between intracranial locations. This was higher than two recent studies,[42,43] although all patients were irradiated at primary diagnosis compared with 45% in our cohort. For irradiated patients, we found a similar relapse incidence to Ducassou[42] (38% vs 31.3% for local relapses) but the incidence was still higher than Merchant[43] (16.2% for local relapses). A higher rate of GTR was achieved in Merchant's cohort and in the most recently published study on ependymoma outcomes by Upadhyaya.[12] However, a similar rate to our study was seen by Ducassou.

The majority of patients who relapsed did so within two years. However, in agreement with previous studies,[34,44] a number of patients relapsed later, raising implications for follow-up duration. Very late recurrences were heterogeneous with no clear demographic associations. Consistent with previous studies,[26] patients presenting with metastatic relapses had poor prognosis. We found metastatic relapse, with or without local relapse, to have equally poor outcomes, in contrast to Messahel,[2] where combined local and distant relapses had poorer prognosis than distant only.

GTR and radiotherapy at primary diagnosis were associated with reduced risk of, and increased time to, relapse with resection having a greater impact on risk in ST tumors, and radiotherapy in PF tumors. However, in agreement with previous research,[44] neither treatment prevented recurrence with approximately half of patients receiving either intervention relapsing. GTR plus radiotherapy delayed relapse

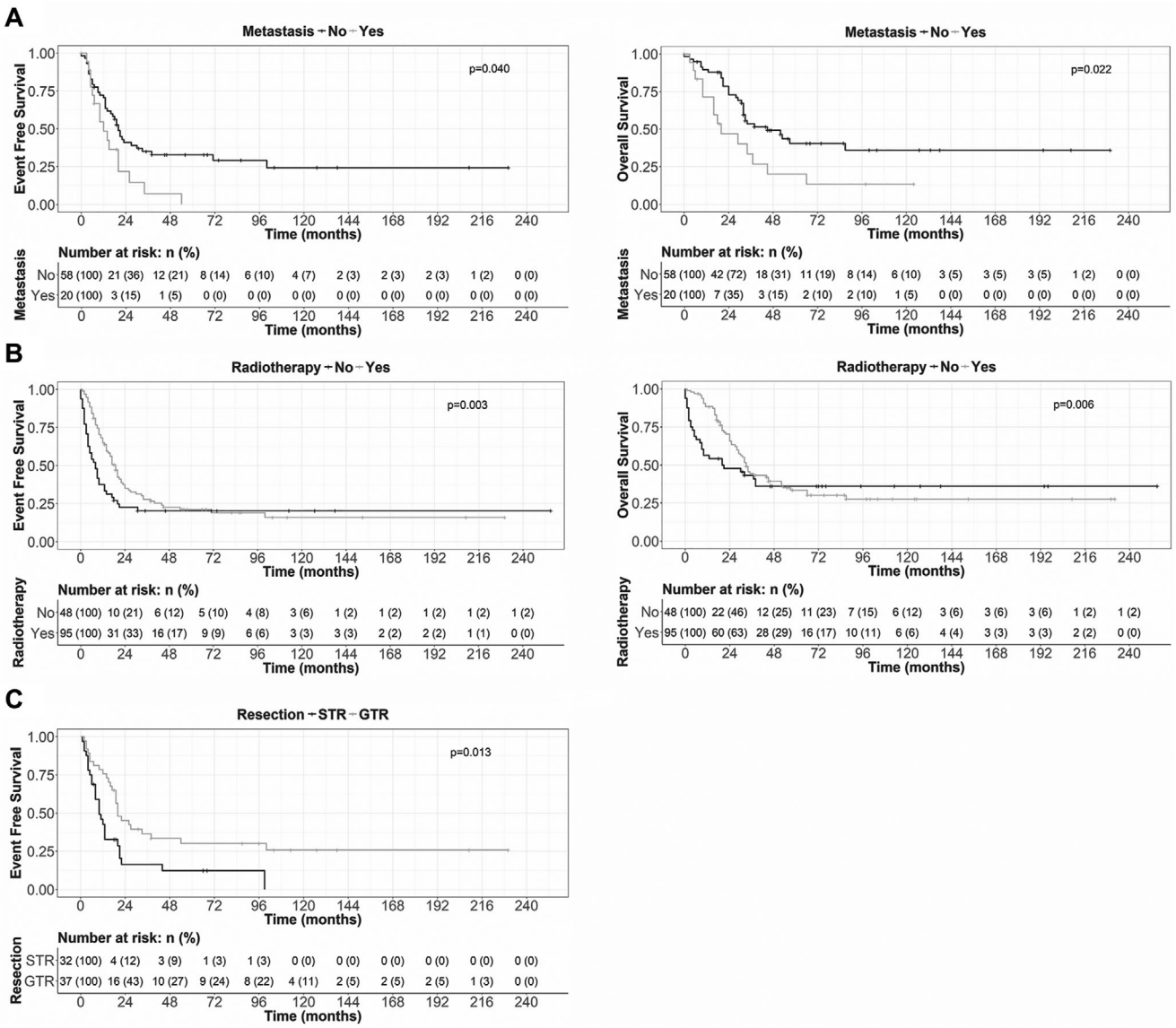

FIGURE 3 (**A**) Patients with metastatic relapses had a significantly worse EFS ($P = 0.040$) and OS ($P = 0.022$), following first relapse. (**B**) Irradiation at relapse improved EFS ($P = 0.003$) and OS ($P = 0.006$). (**C**) GTR at first relapse resulted in improved EFS ($P = 0.013$)

but did not reduce relapse risk or improve prognosis for relapsed cases, compared with either treatment alone.

Although interventions at primary diagnosis reduced relapse risk in univariate analysis, they did not confer improved OS for relapsed patients. Treatment at relapse has lacked consensus but recent guidelines have recommended the use of reirradiation and further surgery.[20] We found both GTR and irradiation improved prognosis, in agreement with previous studies.[2,21-23,36,43] However, only short-term survival benefits were seen, which were limited to combined and PF cohorts, raising questions about the best approach for patients with ST tumors.

Irradiation at relapse doubled time to further progression in combined and PF cohorts but was associated with no difference in the ST cohort. Furthermore, consistent with previous reports, we saw an improved prognosis for patients receiving any radiotherapy at relapse, whether or not they were irradiated at primary diagnosis.[26] We saw similar survival rates for reirradiated patients as Lobon et al.[22] (30% five-year OS). Higher rates for reirradiated patients were identified by Tsang et al.[23] (57% five-year OS) and Bouffet et al.[21] (80% three-year OS). However, both studies had shorter follow-up times (median; 1.8 and 3.7 vs 6.5 years).

The survival of PF patients irradiated at relapse was better than those not irradiated, suggesting it confers short-term benefit but this was not replicated in the ST group. We identified similar survival rates for patients who were not irradiated at relapse to Zacharoulis et al.[26] (five-year OS 35% vs 26%). Survival rates in both our study and Zacharoulis et al. were higher than Bouffet et al.[21] (three-year OS 7%).

Patients who received chemotherapy at primary diagnosis had higher relapse risk and shorter time to progression. This was likely due to these being higher risk cases not receiving more beneficial treatments, which was reflected in loss of significance in multivariate analysis in agreement with previous findings.[26]

Tumor grade and patient age were associated with relapse risk. There is disagreement as to the reliability of histological grading.[2,12,35,45] Our study suggests grade can correlate with outcome in large retrospective cohorts from multiple centers. However, grade did not remain a predictor of risk in the ST cohort, possibly reflecting the smaller size of this group. The additional association of patient age with relapse risk could have been influenced by variation in treatments or molecular profiles, with EPN_PFA disproportionately affecting the youngest children.[31]

Chromosome 1q gain has been associated with poorer EFS.[12,41,46,47] Our analysis demonstrated that it was also independently associated with relapse risk across location and main molecular subgroups. This builds on work by Upadhyaya who found that 1q gain is associated with inferior outcomes in EPN_PFA.[12] The lack of association with outcome in the relapsed cohort suggests it was not prognostic after relapse, in contrast to Tsang et al. who found the marker to be prognostic in PF tumors with distant relapse.[23]

Relapse patterns within molecular subgroups have not previously been investigated in detail.[31,48] We found relapse risk was higher for EPN_PFA compared with EPN_RELA cases, which agrees with studies where EPN_PFA cases displayed a poorer EFS.[31] However, EPN_RELA cases, on average, suffered more relapses. Despite these differences, relapsed patients from both subtypes had poor prognosis, suggesting that although subtypes are biologically and clinically distinct, relapse is important for both.

Despite small numbers, we identified relapsed cases in EPN_PFB and EPN_YAP subgroups. The majority of these cases demonstrated long-term survival, consistent with previously reported favorable prognosis.[31,48]

Interestingly, if EPN_PFA and EPN_RELA subgroups were considered independently, surgery and radiotherapy were not associated with relapse risk. For relapsed EPN_PFA cases, radiotherapy was associated with better EFS, although radiotherapy and GTR have previously been associated with improved EFS In EPN_PFA, the association was not seen across all cohorts analyzed.[31,48] EPN_RELA cases have only been investigated previously by Pajtler et al.[31] who found irradiation, but not GTR, was associated with improved EFS. In light of these contradictory data, further work is needed to fully understand the impact of therapy on the molecular subgroups at diagnosis and recurrence.

Extended follow-up of our cohort provided more detail on long-term outcomes of relapsed ependymoma than previous work.[2,34,37,50] Whilst treatment approaches have changed little over time, improvements in resources and support strategies may mean that this study provides an overly pessimistic outlook. In particular, reported GTR rates are higher in more recent studies.[12] However, we have demonstrated that children with GTR still do relapse, and it is undeniable that outcomes for children with relapsed ependymoma remain remarkably poor.

Inclusion of data from multiple centers provides a wider snapshot of ependymoma. However, this has the disadvantage of lack of uniformity in treatment approaches, particularly at recurrence. An additional challenge resulting from the retrospective nature of the data was that numbers with complete data for analysis of radiotherapy type at recurrence(focal vs CSI) were low. Whether reirradiation should be focal or craniospinal remains an important question that this retrospective study could not answer. A prospective, randomized study is needed to address this question.

A further limitation is the lack of central imaging review at recurrence and this should be performed in the future to standardize diagnosis. Finally, the inclusion of large numbers of young children made detailed analysis of non-EPN_PFA entities challenging. Further studies into relapse outcomes for other molecular subgroups are urgently needed.

In conclusion, recurrent pediatric ependymoma is highly aggressive with extremely poor outcomes. The data suggest relapsed cases form a subset for whom current therapies are inadequate. Relapsed patients with EPN_PFA and EPN_RELA tumors had an equally poor prognosis suggesting relapse is important across major pediatric subtypes with gain of 1q being a universal predictor of poor outcome. A deeper understanding of recurrence biology, in molecularly stratified cohorts, is also needed to identify drivers of relapse.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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