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Relevance of Molecular Groups in Children with Newly Diagnosed Atypical Teratoid Rhabdoid Tumor: Results from Prospective St. Jude Multi-Institutional Trials

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Abstract

Purpose: Report relevance of molecular groups to clinico-pathologic features, germline *SMARCB1/SMARCA4* alterations (GLA), and survival of children with atypical teratoid rhabdoid tumor (ATRT) treated in two multi-institutional clinical trials.

Patients and Methods: Seventy-four participants with newly diagnosed ATRT were treated in two trials: infants (SJYC07: age<3 years; *n*=52) and children (SJMB03: age 3–21 years; *n*=22), using surgery, conventional chemotherapy (infants), or dose-dense chemotherapy with autologous stem cell rescue (children), and age- and risk-adapted radiation therapy [focal (infants) and craniospinal (CSI) (children)]. Molecular groups ATRT-MYC (MYC), ATRT-SHH (SHH), and ATRT-TYR (TYR) were determined from tumor DNA methylation profiles.

Results: Twenty-four participants (32%) were alive at time of analysis at a median follow-up of 8.4 years (range, 3.1–14.1 years). Methylation profiling classified 64 ATRTs as TYR (*n*=21), SHH (n=30), and MYC (*n*=13), SHH group being associated with metastatic disease. Among infants, TYR group had the best overall survival (OS) (*P*=0.02). However, outcomes did not differ by molecular groups among infants with non-metastatic (M0) disease. Children with M0 disease and <1.5 cm^2 residual tumor had a 5-year progression-free survival (PFS) of 72.7±12.7% and OS of 81.8±11%. Infants with M0 disease had a 5-year PFS of 39.1±11.5% and OS of 51.8±12%. Those with metastases fared poorly [5-year OS 25±12.5% (children) and 0% (infants)]. *SMARCB1* GLAs were not associated with PFS.

Conclusion: Among infants, those with ATRT-TYR had the best OS. ATRT-SHH was associated with metastases and consequently with inferior outcomes. Children with non-metastatic ATRT benefit from post-operative CSI and adjuvant chemotherapy.

Keywords

Atypical teratoid rhabdoid tumor; ATRT molecular groups; germline *SMARCB1* alterations

Introduction

Atypical teratoid rhabdoid tumor (ATRT) is a rare, aggressive central nervous system malignancy with an annual incidence of ~75 cases in the US in children ≤19 years old.[1] More than two thirds of affected children are <3 years old at diagnosis, with mortality rates approaching 70%.[1–7] Although survival has improved with the use of multi-modality therapies, outcomes remain suboptimal, with younger age at diagnosis and presence of metastases associated with the worst outcomes.[2,3,8,9] Hallmark somatic inactivating alterations of *SMARCB1* on chromosome 22q11.2, resulting in loss of its protein product INI-1 in the tumor, occur in more than 95% of patients with ATRT, with remaining patients having mutations in *SMARCA4* located on chromosome 19p13.2. Presence of heterozygous germline alterations (GLA) in either gene results in the rhabdoid tumor predisposition syndrome, but its role in prognosis remains controversial.[10–15]

Remarkably, despite very aggressive behavior, ATRT does not exhibit recurrent genetic alterations besides those in *SMARCB1* or *SMARCA4*.[16] Two international collaborative studies reported 3 molecular groups of ATRT based on tumor DNA methylation and transcriptome findings, suggesting that distinct molecular mechanisms drive oncogenesis.[17,18] There is broad consensus on specific clinicopathological characteristics of the 3 groups between the 2 studies,[19] but limited information from prospective studies regarding the clinical relevance of these groups.[20]

We report outcomes for participants with newly diagnosed ATRT treated in 2 prospective risk-adapted multi-institutional trials: those <3 years old at diagnosis, hereon called “infants” (St. Jude Young Children 07 [SJYC07]; NCT00602667) and those ≥3 years old at diagnosis, hereon called “children” (St. Jude Medulloblastoma 03 [SJMB03]; NCT00085202). Our analyses incorporates molecular grouping (ATRT-MYC, ATRT-SHH, and ATRT-TYR)[17] and aim to determine the clinico-pathologic features and prognostic significance of the 3 molecular groups. Additionally, we investigated the presence of germline *SMARCB1/SMARCA4* alterations in the study cohort and the impact of these alterations on treatment outcomes.

Patients and Methods

Study Design

SJYC07 and SJMB03 were non-randomized, phase II, risk-adapted, multi-institutional clinical trials approved by the institutional review boards of St. Jude Children’s Research Hospital and 8 participating hospitals. Participants with histological diagnosis of ATRT reported by a pathologist at the enrolling institution and centrally confirmed by study neuropathologists (DWE; BAO) who did not previously receive anti-cancer therapy were enrolled in the SJYC07 (infants) and in SJMB03 (children) studies. Written informed consent was obtained from parents or legal guardians and from participants 14–17 years old. Additionally, assent was obtained from participants 7–13 years old. Patients with other brain tumor diagnoses including medulloblastoma, supratentorial primitive neuroectodermal tumor (PNET), pineoblastoma, ependymoma, high-grade glioma, and choroid plexus carcinoma (SJYC07), and medulloblastoma, PNET, and PNET variants (SJMB03) were also

eligible for enrolling in these trials. Both studies were conducted in accordance with the World Medical Association Declaration of Helsinki principles and ethical guidelines.

Treatment Plan

Study participants underwent maximal safe surgical resection at diagnosis. Extent of resection was determined by the operating surgeon and post-operative magnetic resonance imaging (MRI), as previously described.[21] Metastatic staging included brain and spine MRI and lumbar puncture for cerebrospinal fluid (CSF) analysis (unless medically contraindicated) before study enrollment.

Infants (SJYC07) with no evidence of CNS dissemination (M0 disease) were enrolled in the intermediate-risk arm (IR), whereas those with evidence of metastases (M+ disease) were enrolled in the high-risk arm (HR) of SJYC07 (Fig. 1A and B). Participants with M0 disease whose resection at diagnosis was less than gross total resection (GTR) were considered for second-look resection after 2 or 4 cycles of induction chemotherapy to achieve GTR before starting focal radiation therapy (RT). Those with IR disease but <1 year old when completing induction chemotherapy received additional chemotherapy to delay RT until they were 1 year old, as previously described.[21]

Children (SJMB03) were risk stratified as average risk (AR: M0 disease and <1.5 cm^2 residual tumor at primary site; prescribed 23.4 Gy CSI) or high risk (HR: M1–M3 disease or ≥1.5 cm^2 residual tumor at primary site; prescribed 36–39.6 Gy CSI), followed by consolidation chemotherapy (Fig. 1C). The SJMB03 trial was built on the predecessor St. Jude study SJMB96, the results of which have been published previously.[9,22]

Participants had to have normal organ function, performance score of at least 30, and begin treatment within 31 days of definitive surgery. Treatment was to be continued until completion of therapy, progressive or relapsed disease, unacceptable toxicity, or parental withdrawal of consent. Toxicities were graded per the Common Terminology Criteria for Adverse Events, version 3.0.

Tumor DNA Methylation, Molecular Grouping, and Germline Analysis

Tumor genomic DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue samples using the Maxwell RSC DNA FFPE kit (Promega, Madison, WI). Genome-wide DNA methylation data were generated using Illumina Infinium MethylationEPIC BeadChip arrays (Illumina, San Diego, CA) according to manufacturer's instructions. Data files were uploaded to the DKFZ Molecular Neuropathology 2.0 classifier version 11b4 (https://www.molecularneuropathology.org/mnp), which provided copy number variants and molecular groups.[23] Tumors were classified as ATRT-MYC (MYC), ATRT-SHH (SHH), and ATRT-TYR (TYR), as described previously.[17] Calibration scores of >0.9 were used as a cutoff for classifying these tumors as ATRT and for group classification on the classifier. For tumors with calibration scores <0.9 but still predicted to be ATRT by histology, additional t-distributed stochastic neighbor embedding (t-SNE) cluster analysis with a reference cohort was performed to classify them into different groups.[23] To perform the clustering, a pairwise distance matrix was generated based on the Pearson Correlation from the samples with the most variably methylated probes (s.d. > 0.228). This matrix was then supplied to RTSNE

package for Barnes-Hut t-SNE.[24] Subset of Capper dataset containing only ATRT samples was then used to further refine the clustering.

Peripheral blood DNA was used to detect the presence of germline *SMARCB1/SMARCA4* variants (Supplementary Information, germline methodology).

Statistical Analysis

ATRT was a secondary cohort for both SJYC07 and SJMB03. Herein, we describe progression-free survival (PFS) and overall survival (OS) of the ATRT cohort, consistent with secondary objectives of the trials. Additionally, the secondary biological objectives for molecular grouping of ATRT by tumor DNA methylation analysis and their association to outcomes are presented.

SJYC07 was activated in November 2007 and closed to accrual in May 2017. SJMB03 opened in June 2003 and closed to accrual in March 2013. All eligible participants for whom therapy was initiated were included in outcome analyses. All participants with adequate tumor for DNA methylation profiling were included in biological analyses. PFS was defined as time from diagnosis to date of relapse or progressive disease (PD) or death from any cause, or to date of last follow-up for patients without events. OS was defined as time from date of diagnosis to date of death from any cause or to date of last follow-up for survivors. Outcome distributions were estimated using Kaplan–Meier analysis and reported as ±one standard error (SE), where SEs were obtained by the Peto and Pike method. Fisher's exact tests and exact chi square tests examined associations among categorical variables. Exact Wilcoxon rank sum tests and Kruskal–Wallis tests examined associations between age at diagnosis and protocol-defined risk groups as well as with methylation groups. Differences in outcome distributions were examined by exact log rank tests. Cox regression was used to estimate associations with multiple predictors of outcome. Cumulative incidence of local and distant failure was estimated by methods of Kalbfleisch and Prentice.[25] Local failure and distant failure were defined as the time from diagnosis to local disease recurrence/progression or metastatic progression, respectively. Gray's test was used to compare cumulative incidence curves.

RESULTS

The trials included 52 infants and 22 children with ATRT (Table 1). A diagnostic lumbar puncture could not be obtained due to medical contraindications in 13 infants who had no imaging evidence of metastases (disease status coded as MX). All SJMB03 participants received CSI. Eleven (all M0) were treated in the AR arm and 11 (3 M0 with residual tumor, 8 M+) in the HR arm. Supplementary Fig S1 shows treatment details for both studies.

Tumor DNA methylation data were available for 67/74 (91%) patients. Of them, 56 were classified as SHH, TYR, or MYC using the DKFZ classifier with a calibration score of >0.9. Eight other samples with classifier scores for groups between 0.23 and 0.89 were additionally grouped by cluster analysis to a reference cohort. Three samples could not be further classified molecularly and are not included in methylation group–based analyses presented here. These 3 samples, like all other samples, were centrally reviewed and

confirmed histologically to be ATRT. The most common group was SHH (30/64; 47%), followed by TYR (21/64; 33%) and MYC (13/64; 20%). MYC patients were older than SHH and TYR patients (P=0.019), a higher proportion of SHH patients were M+ (P=0.016) and had GLA (p=0.057), whereas most TYR patients were M0 and had infratentorial primary tumors. Copy number alterations (CNAs) of the *SMARCB1* locus for groups were classified manually into different categories, and heatmaps demonstrating CNAs were generated (Fig. 2). Broad 22q chromosomal losses spanning across the *SMARCB1* locus was observed predominantly in the TYR group whereas more focal losses were observed in the MYC group (Fig. 2B).

At the time of analysis, 24 of 74 (32%) patients were alive at a median follow-up of 8.4 years (range, 3.1–14.1 years) from diagnosis. First events included disease progression or death from disease in 53 patients, second malignancy in 1 patient (paraspinal desmoid tumor), and metachronous rhabdoid tumor in 2 patients (kidney and pelvis soft tissues) with a GLA in *SMARCB1*. Since treatments on the 2 trials differed markedly, we investigated outcomes by protocol.

SJYC07 Outcomes

Participants with intermediate risk disease (n=34) disease had a 5-year PFS of 31.4±9.2% and OS of 43.9±9.5%, whereas the 5-year PFS and OS for high risk participants (n=18) were 0% (Fig. 3A and B).Participants with M0 disease (n=23) had a 5-year PFS of 39.1±11.5% and OS of 51.8±12% (Fig. 3C and D). Univariate analysis showed the best survival for infants in the TYR group (5-year OS estimates 58.8±11.9%, P=0.023) (Fig. 4A and B). Since patients in the TYR group were more likely to be non-metastatic at presentation (Fig. 2A and Supplementary Table S1), we performed the same comparison by risk group and found no difference in outcome by methylation group in M0 patients (P=0.74 for PFS and 0.73 for OS; Fig. 4C and D). Additionally, outcomes did not differ by age at diagnosis, sex, primary location of tumor, and extent of best resection before RT for those with IR disease (Supplementary Table S2 and Supplementary Fig. S2A–D).

SJMB03 Outcomes

Children with average risk disease (n=11) had a 5-year PFS of 72.7±12.7% and OS of 81.8±11%, whereas those with high risk disease (n=11) had a 5-year PFS and OS of 18.2±9.5% (Fig. 3A and B). Children with M+ disease (n=8) had a 5-year OS of 25.0±12.5%. There was no difference in outcomes by sex or tumor location. In univariate analyses, outcomes of those with average risk disease were better than those with high risk disease (Supplementary Table S3).

Outcomes for MYC, SHH, and TYR groups by study and risk stratification are shown in Supplementary Fig S3A–C.

Germline Alterations and Outcomes

Thirty percent (16/53) of study participants who completed germline testing were positive for heterozygous GLA in either *SMARCB1* (15/16) or *SMARCA4* (1/16) [2/17 (12%) SJMB03 participants with positive results vs. 14/36 (39%) of SJYC07 participants] (Fig. 5

and Supplementary Table S4). Median age at diagnosis for patients with a positive GLA was significantly lower at 0.7 years (range, 0.0–6.9) versus those without GLA having a median age of 2.3 years (range, 0.0–12.1) (P=0.003). There was a significant association between metastatic status and GLA. More M+ patients had GLA (8/17, 47%) compared to M0 patients (5/29;17%), MX excluded (P=0.044) None of the MYC participants were positive for GLA compared to 11/24 (46%) SHH and 4/13 (31%) TYR participants (P=0.057) (Fig. 2). There was no evidence of GLA being associated with outcomes in SJYC07 participants (Supplementary Fig. S4A and B).

Treatment Failures

Fifty of 74 study participants had PD or relapse. Median time to treatment failure for infants (n=39) was 5.7 months (range, 1.6–79.4 months). Three of 39 infants had PD more than 2 years from diagnosis, with 1 of them having tissue-confirmed PD after 7 years from diagnosis. Disease progression was observed during pre-maintenance phase in 18/23 infants who failed on the intermediate risk arm and all 16 infants who failed on the high-risk arm. None of the infants developed PD during the maintenance phase of chemotherapy. The median time from completion of maintenance therapy to PD for the remaining 5/23 infants who failed was 12.7 months (range, 0–67.2). Four of these 5 infants had ATRT-TYR and one ATRT-SHH (Supplementary Table S5). Only 5 patients are alive after PD at a median follow-up of 3.9 years (range, 0.2–8.1 years) since progression. Pattern of failure for those in the IR stratum (n=23) was local (n=9), distant (n=8), or combined (n=6). However, failure was predominantly distant (n=7) vs local (n=3) vs combined (n=2) for 12 of 23 patients who had PD after completing focal RT.

Median time to PD for children (n=11) was 9.2 months (range, 3.5–52.0 months). Two participants experienced treatment failures beyond 2 years, at 50.3 and 52 months from diagnosis. Tissue confirmation was not available for these 2 patients to determine whether this was true PD, metachronous tumor, or a second malignancy. Neither patient harbored germline *SMARCB1/SMARCA4* alterations. All patients with PD died at a median of 2.0 months after PD (range, 0.4–54.1 months). Supplementary Fig. S5 shows the cumulative incidence of local and distant failures by protocol.

Toxicity

Febrile neutropenia was the most common adverse event (Supplementary Table S6). There were no cases of RT-induced necrosis in either trial. An infant with M+ disease developed febrile neutropenia and respiratory syncytial virus pneumonitis that required high-frequency ventilation and died after the family decided to withdraw support due to his medical condition and poor prognosis of ATRT.

DISCUSSION

This study reports survival data for infants and children with newly diagnosed ATRT treated in SJMB03 and SJYC07 trials. We observed very good outcomes in children ≥3 years old with M0 disease by using immediate post-operative CSI and adjuvant chemotherapy. However, patients with metastatic ATRT had a dismal prognosis. We also report clinical

characteristics and outcomes of 3 epigenetic groups of ATRT, which is the largest prospectively treated cohort reported to date. Our study, very importantly, reports new findings while confirming some of the clinico-pathological characteristics of 3 epigenetic groups reported previously in retrospective studies.[17] As reported previously, we found a predominance of SHH and TYR groups in younger children and the predominance of MYC group in older children. We also confirmed the pattern of somatic *SMARCB1* CNA, with the TYR group showing a predominance of broad losses and the MYC group a predominance of focal losses. The majority of children with M+ disease were in the SHH group, whereas those in the TYR group primarily presented with localized posterior fossa tumors. The TYR group had a better OS, but for infants with M0 disease, group affiliation was not associated with outcome, in contrast to that reported in a recent study.[26] We observed that infants with TYR group have an indolent progression and longer survival compared to those with SHH or MYC group (Figure 4 and Supplementary Table S5). Also, it is significant to note that TYR group overwhelmingly presents with localized disease. The biological basis, if any, of this relative indolent behavior remains to be elucidated.

Treating children with ATRT has historically been challenging, with dismal outcomes reported by several studies.[3,5,27,28] The contribution of RT in improving survival is controversial.[2,5,9,29] Although a multi-center study reported a 2-year PFS of 58% with multi-modality therapy, the majority of children <3 years old in that study had a follow-up period of <2 years from diagnosis, whereas all surviving children >3 years old received RT, including some receiving CSI.[8] The European Rhabdoid registry study EU-RHAB reported a 6-year OS of 45% despite the use of multi-modality therapy, including intrathecal chemotherapy, whereas the Children's Oncology Group (COG) study ACNS0333 reported a 2-year OS of 48% when using high-dose chemotherapy (HDCT) and RT, which included 4 therapy-related deaths.[2,20,26,30,31]

In our study, use of trimodality therapy in infants with M0 disease was associated with a 5-year PFS of 39.1±11.5% and OS of 51.8±12%. Interestingly, those with MX disease had inferior survival, suggesting that some of these participants may have had metastatic disease in the form of positive CSF, which was missed due to the absence of a LP. Hence, we recommend that every effort be made to obtain a diagnostic LP. If this is not possible, these children should be considered as having high-risk disease and treated accordingly. We have taken this approach in our ongoing ATRT trial SJATRT (NCT02114229). Outcomes were better for children ≥3 years old with M0 disease with non-bulky (<1.5 cm^2) residual tumor treated with adjuvant CSI and consolidation chemotherapy, with a 5-year PFS of 72.7±12.7% and OS of 81.8±11%, thus confirming our previous report of a single institution series showing excellent outcomes for this group of children.[9] Use of CSI at a young age is associated with neurocognitive decline, with the additional risk of endocrine dysfunction and skeletal growth retardation.[32] However, given the aggressiveness of ATRT and its resistance to multi-modality therapies, optimizing treatment at initial diagnosis is vital to the survival of these children. This warrants an informed discussion between the treating physician and family at the time of initial diagnosis to balance improved survival and possible long-term CSI-associated adverse outcomes. Children with metastases at diagnosis continue to fare poorly despite receiving CSI. Despite better outcomes reported compared to our results with the use of HDCT in conjunction with RT for those with M+ disease in COG ACNS0333,

outcomes remain suboptimal, highlighting an urgent need to develop novel therapies for this group of patients.[20]

The association between germline predisposition to rhabdoid tumors and prognosis is controversial. Although a study noted a higher risk of death in patients with a germline predisposition, other studies have not confirmed this observation.[2,14,15] We found that germline predisposition was not associated with adverse prognosis in the SJYC07 cohort. This association could not be tested in the SJMB03 cohort, since only 2 participants harbored the alterations. We also found an association between metastatic status and GLA, with M+ patients more likely to harbor a GLA, and a marginal association between group affiliation and germline predisposition, with a higher incidence in the SHH group and none of the participants in the MYC group having a GLA in either *SMARCB1* or *SMARCA4*.

Weaknesses of our study include the single-arm phase II design, relatively small sample sizes, and reliance on comparison to historical controls. As ATRT is an extremely rare condition, a large randomized study would be difficult to complete in a reasonable time frame. Also, given the rarity of this cancer, multi-variate analysis could not be conducted due to the small number of patients and events within each molecular group.

In conclusion, our results demonstrate that the TYR group is associated with non-metastatic disease and superior survival in infants, whereas the SHH group is associated with metastatic disease and extremely poor outcomes in the presence of metastases. No association with outcomes was detected for molecular group affiliation in infants with M0 disease. Presence of germline *SMARCB1* alterations was not associated with inferior survival. The use of maximal safe surgical resection, post-operative CSI, followed by adjuvant consolidation chemotherapy in children ≥3 years old with M0 disease, yields very high survival and should be considered as a treatment option by practicing oncologists for this highly aggressive, often fatal pediatric CNS malignancy. A subset of children <3 years old with M0 ATRT achieved long-term survival with the SJYC07 therapy and RT without using HDCT. Future clinical trials should use this information for risk stratification of patients and further refine it by combining biologically driven and possibly group-specific targeted therapies with conventional trimodality therapies to improve outcomes for children with this highly aggressive brain tumor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational relevance

ATRT-MYC, ATRT-SHH, and ATRT-TYR are three molecular groups of atypical teratoid rhabdoid tumor (ATRT). However, group-specific outcomes remain to be clearly and unequivocally defined in prospective studies. In this study, reporting outcomes of children with newly diagnosed ATRT from two prospective multi-institutional clinical trials, we demonstrate that the ATRT-TYR group is associated predominantly with non-metastatic disease and superior overall survival, whereas the ATRT-SHH group is associated with metastatic disease and extremely poor outcomes in the presence of metastases. Metastatic disease is also associated with germline alterations in *SMARCB1*. Presence of metastases is a high-risk clinical feature associated with treatment failure. Future trials should use molecular grouping in addition to clinical features to risk stratify patients and optimize therapy for this highly malignant pediatric cancer.

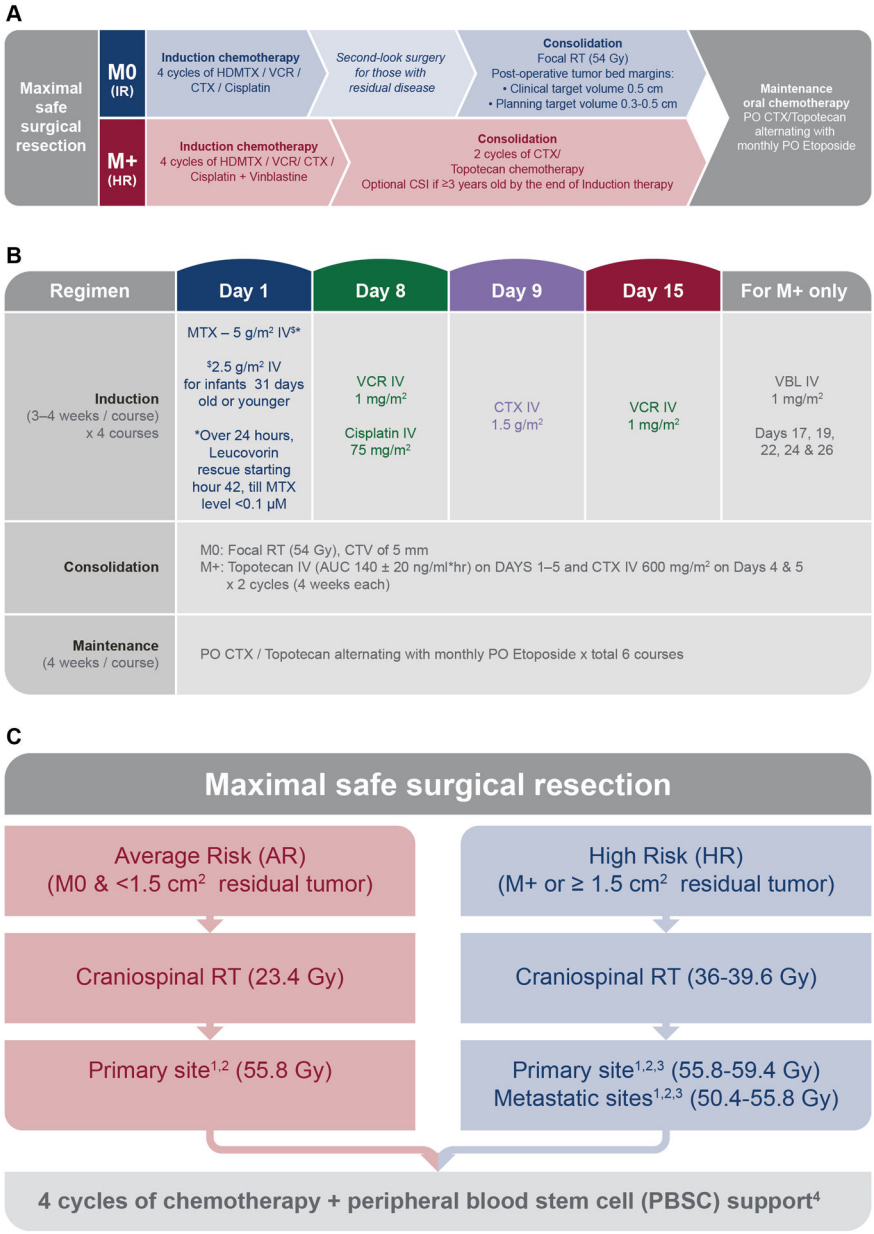
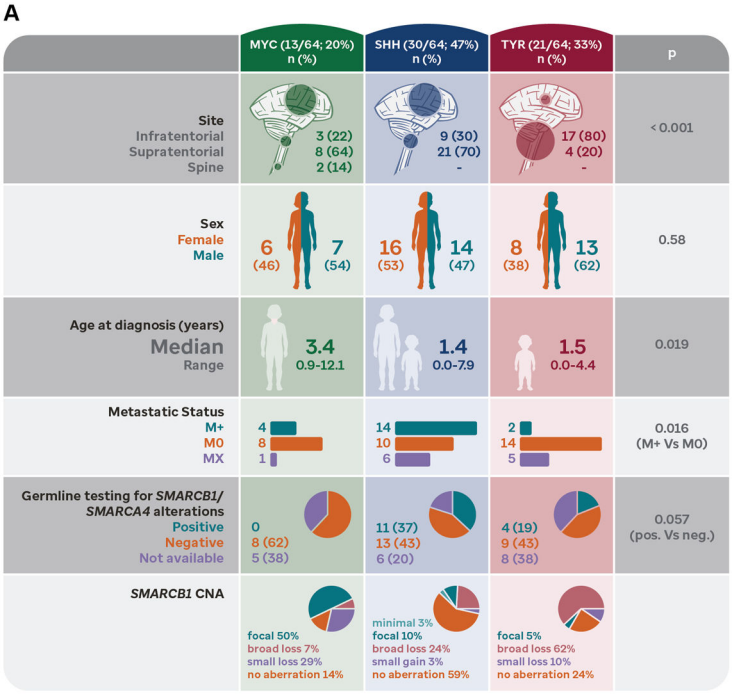


Figure 1. SJYC07 and SJMB03 treatment schema

Schematics of the study design for SJYC07 and SJMB03. M0, non-metastatic disease; M+, metastatic disease; IR, intermediate risk; HR, high risk; IV, intravenous; HDMTX, high-dose methotrexate; VCR, vincristine; CTX, cyclophosphamide; VBL, vinblastine; RT, radiation therapy. (A) Risk-adapted treatment schema for SJYC07 participants 0 to <3 years old at diagnosis. (B) SJYC07 chemotherapy regimen. Maintenance therapy: Cycle A: Days 1–10–cyclophosphamide (30 mg/m^2 PO daily) and topotecan (0.8 mg/m^2 PO daily). Days 11–21–cyclophosphamide (30 mg/m^2 PO daily). Days 21–28–rest. Cycle B: Days 1–28–etoposide (50 mg/m^2 PO daily). Maintenance cycles repeated for a total of 24 weeks: A1-B1-A2-B2-A3-B3. (C) Risk-adapted treatment schema for SJMB03 participants (3–21 years old at diagnosis). ANC, absolute neutrophil count; PBSC, peripheral blood stem cell;

[1]Cumulative doses. [2]Clinical target volume margin 1.0 cm, and planning target volume margin 0.3–0.5 cm. [3]Primary site dose >55.8 Gy was optional for tumors ≥1.5 cm^2 and doses to metastatic sites ≥0.5 cm were at the investigator's discretion. [4]Cisplatin (day −4)–75 mg/m^2 IV, vincristine (days −4 and +6) 1.0 mg/m^2 (max dose 2 mg) IV, cyclophosphamide (days −3 and −2) – 2 g/m^2 IV, PBSC (2×10^6 CD 34^+ cells/kg) – day 0, filgrastim (day + 1) – 5 μg/kg/day SQ/IV daily, 24 h after the infusion of PBSC until ANC > 2000/μL 2 days after nadir.

A



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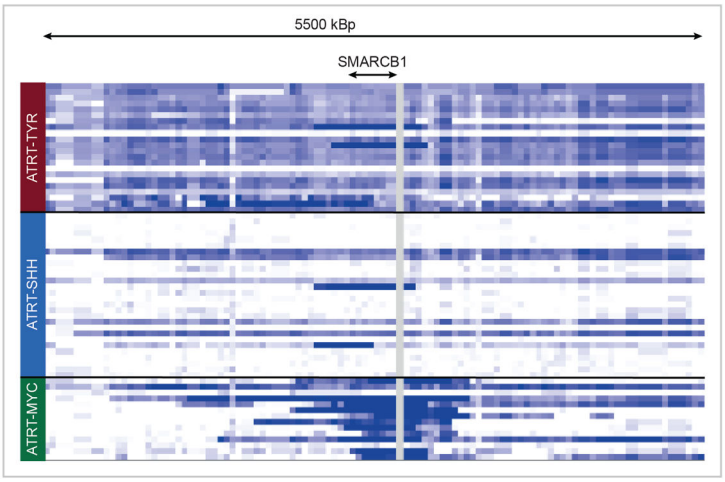

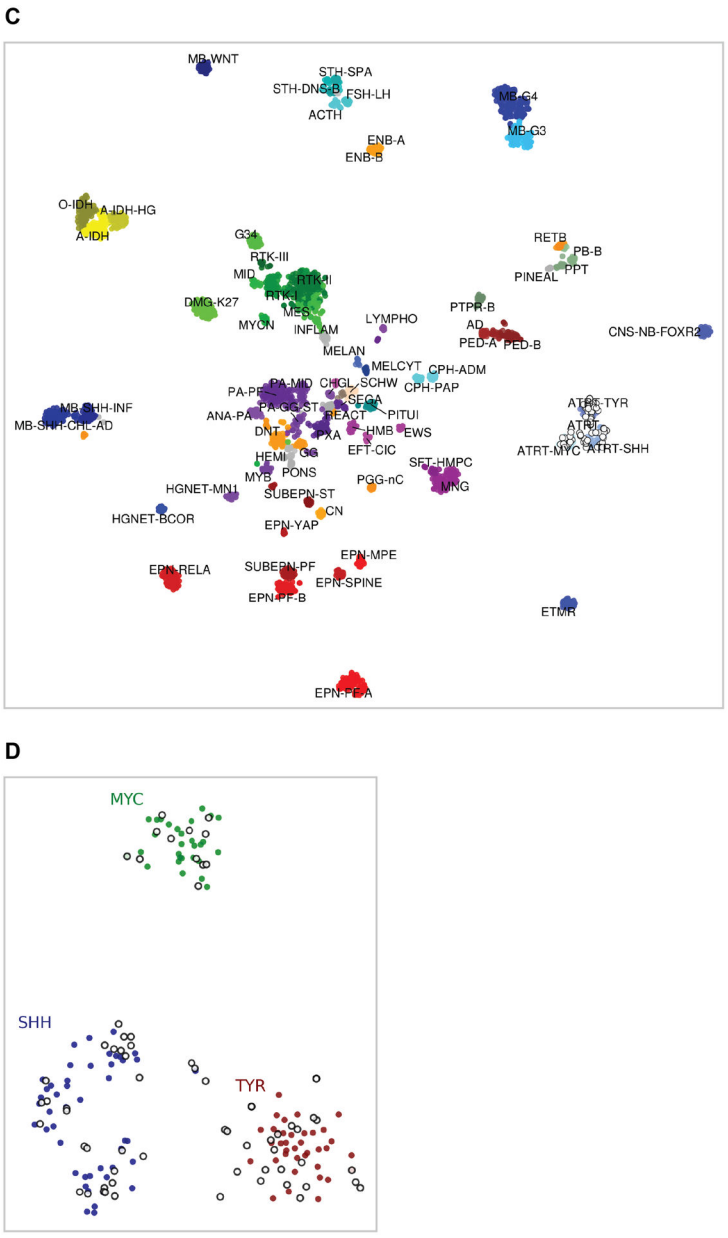

Figure 2. Clinico-pathologic correlates, copy number analysis and unsupervised clustering of ATRT molecular groups

(A) Patient characteristics by methylation subgroup (n=64). M0, non-metastatic disease; M +, metastatic disease; MX, cerebrospinal fluid not obtained at diagnosis, but no evidence of metastases in imaging; pos., positive; neg., negative; CNA, copy number alterations. CNAs of the SMARCB1 locus on chromosome 22 were classified manually into different categories. Pie charts represent the percentage of changes in the respective ATRT groups. (B) Heatmap showing copy number states of the SMARCB1 locus in the 3 ATRT groups. Losses are displayed in blue. t-SNE plot of unsupervised clustering of DNA methylation data for study ATRT samples (white circles) demonstrating clustering with (C) ATRT samples and (D) ATRT groups from the reference Capper Dataset [MYC (green), (SHH (Blue) and TYR (Maroon)]

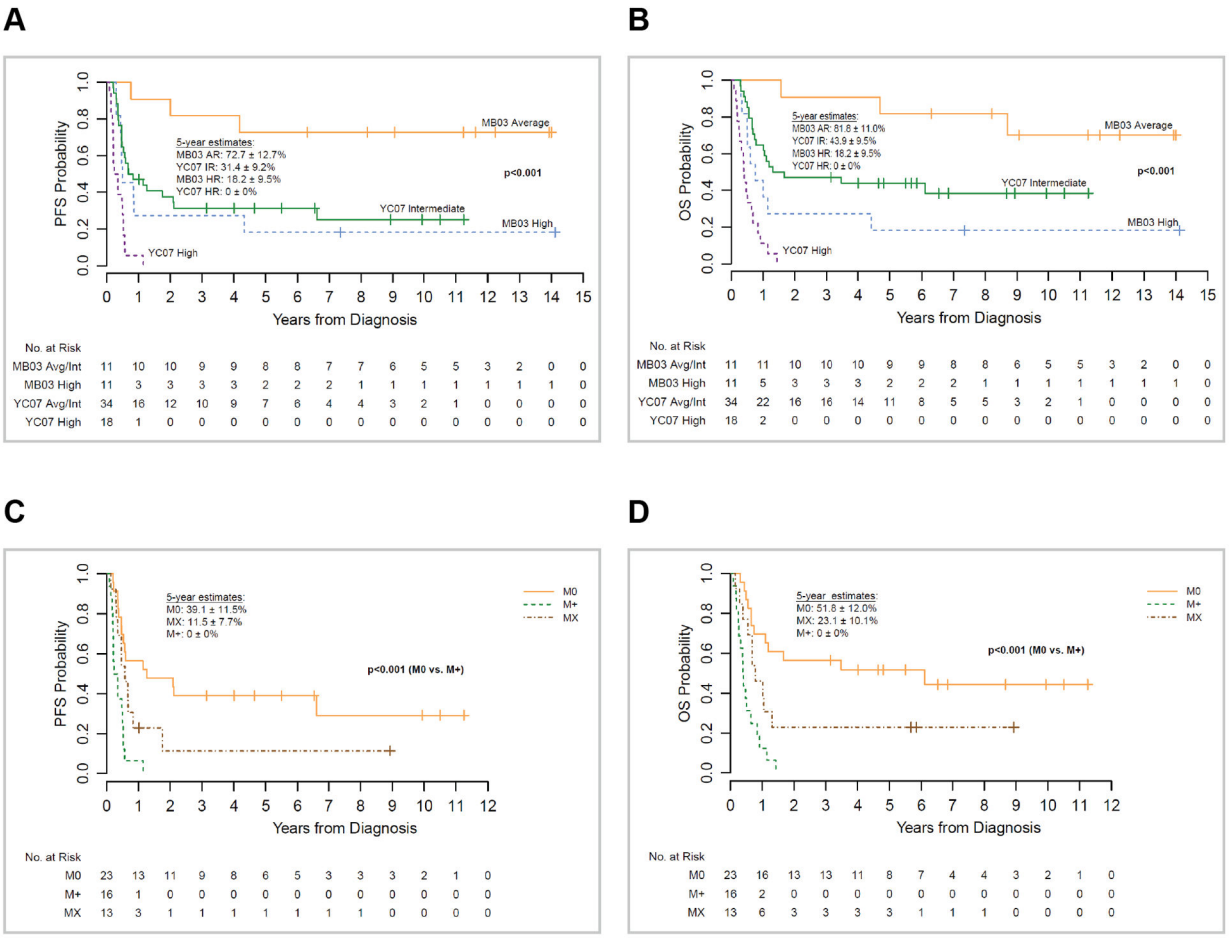

Figure 3. Kaplan–Meir survival curves for all patients and for SJYC07 participants.

(A) Five-year progression-free survival (PFS) and (B) overall survival (OS) by protocol and risk group for 74 participants. Five-year (C) PFS and (D) OS by extent of disease for SJYC07 participants (n=52) with M0, non-metastatic disease; M+, metastatic disease; and MX, CSF not obtained at diagnosis, but no evidence of metastases in imaging.

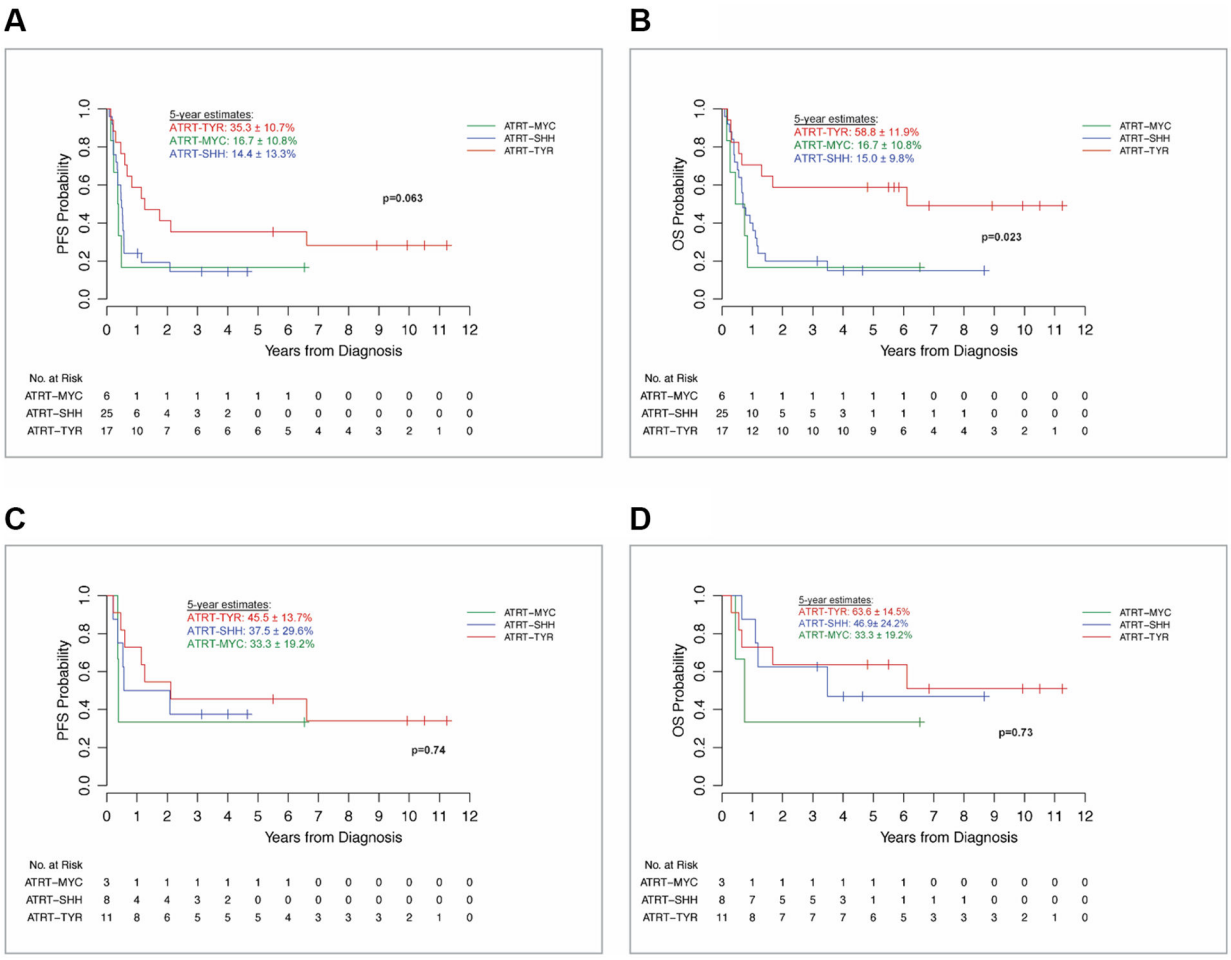

Figure 4. Kaplan–Meir survival curves by molecular groups for participants in SJYC07 trial

(A) Five-year PFS and (B) OS by tumor DNA methylation group for infants in SJYC07 (n=48). Five-year PFS (C) and OS (D) by tumor DNA methylation group for participants in SJYC07 with M0 disease (n=22)

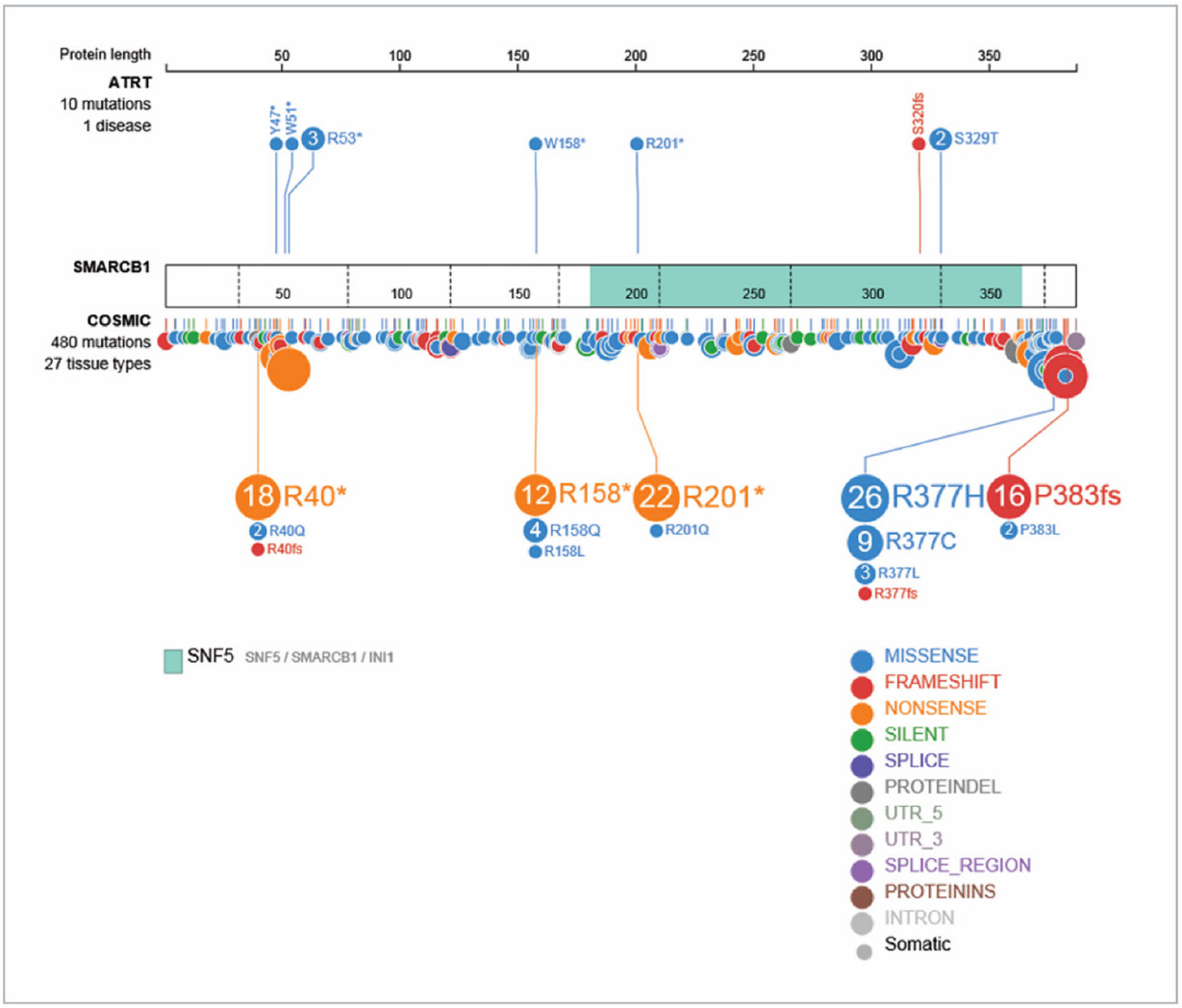

Figure 5. Germline alterations in the study cohort

Visualization of sequence mutations in SMARCB1 with ProteinPaint. SMARCB1 mutation profile in the pediatric dataset (top) and COSMIC database (bottom). The number of samples affected by each mutation is indicated by the text within each disc as well as the disc size. The color represents each class of mutations relative to the gene structure. The SNF5 domain is shown in green.

Table 1.

Patient Characteristics (*n*=74)

	Protocol	
	SJMB03 (*n*=22)	SJYC07 (*n*=52)
	***n* (%)**	***n* (%)**
Sex		
Female	9 (41)	24 (46)
Male	13 (59)	28 (54)
Race		
Asian	2 (9)	3 (6)
Black	0	6 (11.5)
Multiple	0	3 (6)
Unknown	1 (4.5)	0
White	18 (82)	39 (75)
Other	1(4.5)	1 (1.5)
Age Group at Diagnosis		
<1 year	-	24 (46)
1–<3 years	-	28 (54)
≥3 years	22 (100)	-
Age at Diagnosis (years)		
Median	5.3	1.2
Range	3.1–12.1	0.0–3.0
Germline *SMARCB1/SMARCA4* Alterations		
Positive	2 (9)	14 (27)
Negative	15(68)	22 (42)
Not available	5 (23)	16 (31)
Metastatic Status		
M0	14 (64)	23 (44)
M1	2 (9)	3 (6)
M2	1 (4.5)	7 (13.5)
M3	5 (22.5)	6 (11.5)
MX (CSF not obtained)	-	13 (25)
Risk Group		
Average risk (AR)	11 (50)	-
Intermediate risk (IR)	-	34 (65)
High risk (HR)	11 (50)	18 (35)
Primary Tumor Site		
Infratentorial	7 (32)	27 (52)
Supratentorial (includes pineal region tumors)	13 (59)	25 (48)
Spine	2 (9)	-

CSF, cerebrospinal fluid; M0, non-metastatic disease; M+, metastatic disease.