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Chantel Cacciotti
McMaster Children's Hospital, chantel.cacciotti@lhsc.on.ca

Adam Fleming
McMaster Children's Hospital

Vijay Ramaswamy
Hospital for Sick Children University of Toronto

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INVITED REVIEW

Advances in the molecular classification of pediatric brain tumors: a guide to the galaxy

Chantel Cacciotti[1,2], Adam Fleming[1] and Vijay Ramaswamy[3,4,5]*

[1] *Division of Pediatric Hematology/Oncology, McMaster Children's Hospital, Hamilton, ON, Canada*
[2] *Dana Farber/Boston Children's Cancer and Blood Disorder Center, Boston, MA, USA*
[3] *Division of Haematology/Oncology, Department of Pediatrics, University of Toronto and The Hospital for Sick Children, Toronto, ON, Canada*
[4] *Programme in Developmental and Stem Cell Biology, Arthur and Sonia Labatt Brain Tumor Research Centre, Hospital for Sick Children, Toronto, ON, Canada*
[5] *Department of Medical Biophysics and Pediatrics, University of Toronto, Toronto, ON, Canada*

**Correspondence to: V Ramaswamy, The Hospital for Sick Children, 555 University Ave, Toronto, ON, M5G 1X8, Canada.*
E-mail: vijay.ramaswamy@sickkids.ca

Abstract

Central nervous system (CNS) tumors are the most common solid tumor in pediatrics, accounting for approximately 25% of all childhood cancers, and the second most common pediatric malignancy after leukemia. CNS tumors can be associated with significant morbidity, even those classified as low grade. Mortality from CNS tumors is disproportionately high compared to other childhood malignancies, although surgery, radiation, and chemotherapy have improved outcomes in these patients over the last few decades. Current therapeutic strategies lead to a high risk of side effects, especially in young children. Pediatric brain tumor survivors have unique sequelae compared to age-matched patients who survived other malignancies. They are at greater risk of significant impairment in cognitive, neurological, endocrine, social, and emotional domains, depending on the location and type of the CNS tumor. Next-generation genomics have shed light on the broad molecular heterogeneity of pediatric brain tumors and have identified important genes and signaling pathways that serve to drive tumor proliferation. This insight has impacted the research field by providing potential therapeutic targets for these diseases. In this review, we highlight recent progress in understanding the molecular basis of common pediatric brain tumors, specifically low-grade glioma, high-grade glioma, ependymoma, embryonal tumors, and atypical teratoid/rhabdoid tumor (ATRT).

Keywords: medulloblastoma; ependymoma; high-grade glioma; low-grade glioma; primitive neuro-ectodermal tumor (PNET); atypical teratoid/rhabdoid tumor (ATRT)

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Introduction

Treatments for pediatric malignancies have changed vastly over the last several decades due to the ability to analyze tissue on genome-wide scales and no longer relying on morphology alone for diagnosis [1]. Despite this, CNS tumors remain the leading cause of cancer-related morbidity and mortality in children. This is in part due to the high risk of side effects and therapeutic resistance. The movement towards risk stratification with integrated genomic analysis provided insight into the understanding and treatment of CNS tumors with a targeted therapeutic approach [2]. In this review, we highlight recent advances in genetics, epigenetics, and potential therapeutic approaches for common pediatric brain tumors, low-grade glioma, high-grade glioma, ependymoma, medulloblastoma, rare embryonal tumors, and atypical teratoid/rhabdoid tumor (ATRT).

Low-grade glioma (LGG)

Pediatric low-grade gliomas (pLGGs) are typically classified as WHO grade I or II, and include pilocytic astrocytoma (PA), subependymal giant cell astrocytoma (SEGA), pilomyxoid astrocytoma (PMA), pleomorphic xanthoastrocytoma (PXA), low-grade fibrillary astrocytoma or diffuse astrocytoma [3–6]. However, this differentiation of morphological entities has limited implications for prognosis, classification, and treatment. The majority of pLGGs are driven by alterations in the MAP kinase (MAPK) pathway, permitting more personalized treatment as MAPK pathway inhibitors have emerged as novel and effective therapies. The pLGGs are characterized by numerous gene mutations; the most common alteration results in constitutive activation of the Ras/MAPK signaling pathway. In neurofibromatosis type 1 (NF-1) patients, inactivation of the Ras-GTPase

activating protein neurofibromin leads to Ras activation, resulting in optic pathway tumors. In non-NF-1 patients, the most common alteration is fusion of the BRAF protein with the KIAA1549 protein, resulting in loss of BRAF regulation and activation of MAPK [7,8]. In addition, some pLGGs have *BRAF* activating point mutations, such as *BRAF* V600E [9]. The MAPK and P13K/mTOR pathways may be activated in these tumors as well through intragenetic duplication of the tyrosine kinase domain of fibroblast growth factor receptor 1 (FGFR1) [10–12].

BRAF mutations and MAPK pathway activation in pLGGs have been the driving factor for research and novel therapeutic approaches in these patients, especially in recurrent or refractory situations [1,3,9,13–15]. Selumetinib is a non-ATP competitive inhibitor of MEK-1/2 that demonstrates tumor regression and prolonged event-free survival in xenograft models. It has also shown promise in adult studies with *BRAF* abnormalities and more recently in recurrent, refractory or progressive childhood LGG with *BRAF* aberrations and NF-1-associated pLGG. Sustained responses were noted in 36% of LGG patients with a common *BRAF* aberration (KIAA1549–BRAF fusion or *BRAF* V600E mutation), with a median follow-up of 36–40 months, and partial response (PR) was documented in 40% of NF-1 LGG patients with a median follow-up of 48–60 months [16,17].

BRAF V600E is a potential highly targetable mutation in pLGGs as it is found in approximately 15–20% of tumors. Patients with *BRAF* V600E mutations tend to exhibit poorer outcomes after chemotherapy and/or radiation therapy, with 10-year progression-free survival (PFS) of 27% and 60% for *BRAF* V600E and wild-type pLGGs, respectively [18]. Dabrafenib is a potent and selective inhibitor of the V600 mutant form of the *BRAF* kinase and serves as a potential therapeutic target in pLGG [18].

The standard of care for upfront therapy of newly diagnosed pLGG is surgery, with complete resection usually resulting in little to no risk of progression. Chemotherapy is reserved for those with symptoms or radiographic progression and results in a modest PFS of 30–50% [19,20]. Currently planned treatment protocols aim to biologically stratify patients to receive MEK inhibitors for tumors with *BRAF* fusions and either MEK, BRAF or combined inhibitors upfront for those with *BRAF* V600E.

Subependymal giant cell astrocytoma (SEGA) is a WHO grade I pLGG that is commonly associated with tuberous sclerosis. The majority of patients with SEGA contain inactivating mutations in *TSC1*or *TSC2* encoding hamartin or tuberin, respectively, which serve as negative regulators of mTOR and cell growth [21]. Recent advances and treatment with an mTOR inhibitor (such as everolimus, a rapamycin analogue) have shown to be effective in reducing growth of tumors as well as decreasing seizure frequency in these patients, especially in those cases where surgical resection carries substantial morbidity [22–26].

High-grade glioma (HGG)

Pediatric high-grade gliomas (pHGGs) are histologically identical to adult HGG and are graded according to the WHO classification of CNS tumors. pHGGs include glioblastoma [glioblastoma multiforme (GBM) (WHO grade IV)], anaplastic astrocytoma (WHO grade III), gliomatosis cerebri, and diffuse intrinsic pontine gliomas (DIPGs). GBM is classified as grade IV and characteristically displays high mitotic activity, extensive neovascularization, and intratumoral necrosis [6]. This disease confers a long-term survival rate of less than 10% and remains one of the few incurable pediatric cancers.

Hemispheric pHGG

pHGGs are unique in comparison to their adult counterparts, as less than 5% are *IDH1/2* mutated tumors in pediatrics. The same holds true for 1p/19q deletion, commonly found in adult oligodendroglioma and rarely seen in children. In adult neuro-oncology, HGGs lacking either aberration display a similar aggressive clinical course to GBM, even without the morphological features such as necrosis and microvascular proliferation that are commonly required for diagnostic purposes [27]. The overall genomic landscape of pHGG is quite disparate from adult HGG, including mutations in histone variants and targetable fusions including NTRK, ALK, and ROS. Similar to adult IDH1 wild-type HGGs, the most commonly altered pathway in cell cycle regulation (noted in 83% of pediatric GBM samples in one study) involves mutations in *TP53* or *PPM1D*, or homozygous deletion of *CDKN2A* or *CDKN2B*. Other genetic changes resulting in activated receptor tyrosine kinase (RTK) PI3K–MAPK signaling, with activating mutations in RTKs (such as EGFR) or downstream proteins such as NRAS, KRAS, BRAF, and PIK3CA, and gene amplification of *EGFR*, *PDGFR/KIT* or *MET* have been identified in pHGGs [28]. *PDGFRA* or *MET* amplification and *TP53* mutations are found in both pediatric and adult HGGs [1,29–31]. *H3F3A* mutations, including *G34* mutation, with loss of ATRX and ALT have been observed in 20% of pHGGs [32].

IDH1 mutations are rare in younger children and become more frequent in late adolescence and in those with cancer predisposition syndromes. However, unlike the adult *IDH1*-mutant HGGs that harbor R132H mutations, non-R132H *IDH1* mutations are found in 66% of pediatric hemispheric glioma patients [33]. These pediatric *IDH1* variants were associated with germline *TP53* mutations in 43% (3/7) of patients in one study, two with *IDH1* R123G and one with *IDH1* R132C variant. As such, the presence of these rare *IDH1* variants in pHGGs should prompt further investigations into the possibility of Li–Fraumeni syndrome [33].

Mutations resulting in changes at G34 (restricted to H3.3) are mainly found in hemispheric tumors such as GBM-like tumors or primitive neuro-ectodermal tumor (PNET) in adolescents. These mutations typically co-

occur with mutations in alpha thalassemia/mental retardation syndrome X-linked (ATRX), which is believed to promote alternative telomere lengthening [34].

BRAF V600 mutations have been identified in approximately 5% of pHGGs, with most harboring concurrent homozygous loss of *CDKN2A/B*, and morphologically are described as anaplastic pleomorphic xanthoastrocytomas (PXA-like). Although there are isolated reports of *BRAF* V600E mutant pHGGs responding to BRAF inhibitors, it is unclear if *BRAF* mutant HGGs harboring loss of *CDKN2A* will respond to targeted agents [18,35,36]. *NTRK1*, *NTRK2* or *NTRK3* gene fusions have been identified in various pediatric and adult cancers including pHGG. Larotrectinib, a selective TRK kinase inhibitor, shows promise in tumors containing *TRK* fusions in several pan-cancer studies including pediatric brain and solid tumors [37,38].

K27M mutant diffuse midline glioma and DIPG

DIPG and other histone H3 K27M-mutated diffuse midline gliomas are aggressive and universally fatal pediatric tumors. DIPG tumors are challenging to treat as surgical interventions are not routinely offered, radiation therapy offers temporary effects, and no chemotherapeutic agents have shown promise. As surgical approaches (including biopsy) are not standard of care in pediatric DIPG, the diagnosis tends to be made on radiological findings and clinical presentation. Recent advances in next-generation sequencing have identified two key molecular alterations in pHGGs: recurrent mutations in *H3F3A* and *HIST1H3B*, coding the histone variants H3.3 and H3.1. These mutations result in amino acid substitutions at position K27 (K27 mutant) or G34 (G34R or G34V) and are present in about 80% of midline GBMs or DIPGs in younger children [33,39,40]. H3K27 alterations are associated with distinct oncogenic changes and serve as potential therapeutic targets. In addition, H3.1 and H3.3 K27M tumors differ in age at diagnosis, with H3.1 mutant tumors found earlier and with a better prognosis compared with H3.3 mutant tumors [39].

Infant high-grade gliomas

In contrast to childhood and adolescent HGG, infant HGGs have more favorable outcomes [41]. Recent literature supports classification of infant gliomas by underlying molecular alterations, specifically fusions [42,43]. Hemispheric RTK-driven tumors, including ALK, ROS1, NTRK, and MET fusions, exhibit an intermediate clinical outcome with 5-year overall survival (OS) of 53.8%, 25.0%, and 42.9% for ALK, ROS1, and NTRK fused tumors, respectively. Hemispheric Ras/MAPK-driven tumors have the best long-term survival (10-year OS 93.3%) of these groups, with the suggestion that they require minimal clinical intervention post-surgery. The last group is midline Ras/MAPK-driven tumors with relatively poor outcomes after chemotherapy, with a 5-year PFS of 23.4% [42].

Biallelic mismatch repair deficiency high-grade gliomas

Biallelic mismatched repair deficiency (bMMRD) is a childhood cancer syndrome that often results in GBM with high mutation rates. Immune checkpoint inhibition has shown success and favorable toxicity profiles in tumors containing high mutation rates such as bMMRD. Nivolumab, a human IgG4 anti-PD-1 monoclonal antibody, inhibits the PD-1 receptor, permitting enhanced T-cell immunity. PD-L1 is expressed on the surface of glioblastoma and can induce T-cell apoptosis by binding to PD-1. One study showed a durable and profound radiological response in two pediatric bMMRD siblings with recurrent multifocal GBM treated with single-agent nivolumab [44].

Treatment of pHGG

Current treatment approaches include maximal surgical resection (with the aim of maintaining functional integrity), radiation therapy, and a variety of chemotherapeutic options (Figure 1). Agreement exists regarding improved patient outcome with total tumor resection. The Children's Cancer Group study CCG945 combined temozolomide with CCNU and demonstrated an improved 5-year PFS in patients with $>90\%$ resection ($35 \pm 7\%$ compared with $17 \pm 4\%$) [45]. However, the value of adjuvant chemotherapy specifically temozolomide with or without the addition of bevacizumab remains unclear, without a clear survival benefit compared to radiation alone [46,47]. Radiation provides a benefit and is standard treatment in patients greater than 3 years of age. To date, no targeted therapy or chemotherapy has provided a survival benefit for HGG patients, either alone or in combination with other modalities [29,48]. Current novel agents targeting key pathways are underway for pHGG, some of which include crizotinib for MET fusion tumors, nivolumab for hypermutant gliomas, dabrafenib and trametinib for *BRAF* V600 mutations, GD2/panobinostat for K27M mutations, veliparib (a PARP inhibitor) with temozolomide for G34V mutations, and larotrectinib for tumors with an NTRK fusion (Table 1). The future stratification of HGG will involve genome and RNA sequencing to identify somatic events that will further guide treatment.

Ependymoma

Ependymomas are the third most common CNS tumor in children, accounting for 6–12% of pediatric brain tumors. They can arise throughout the entire neuraxis, including supratentorial, infratentorial or spinal cord (Figure 2). In children, approximately 70% of ependymomas arise in the posterior fossa [49]. The histological grading (grade II versus III) plays little role in risk stratification, due to inter-observer variability and a lack of markers to objectively distinguish histologic grading

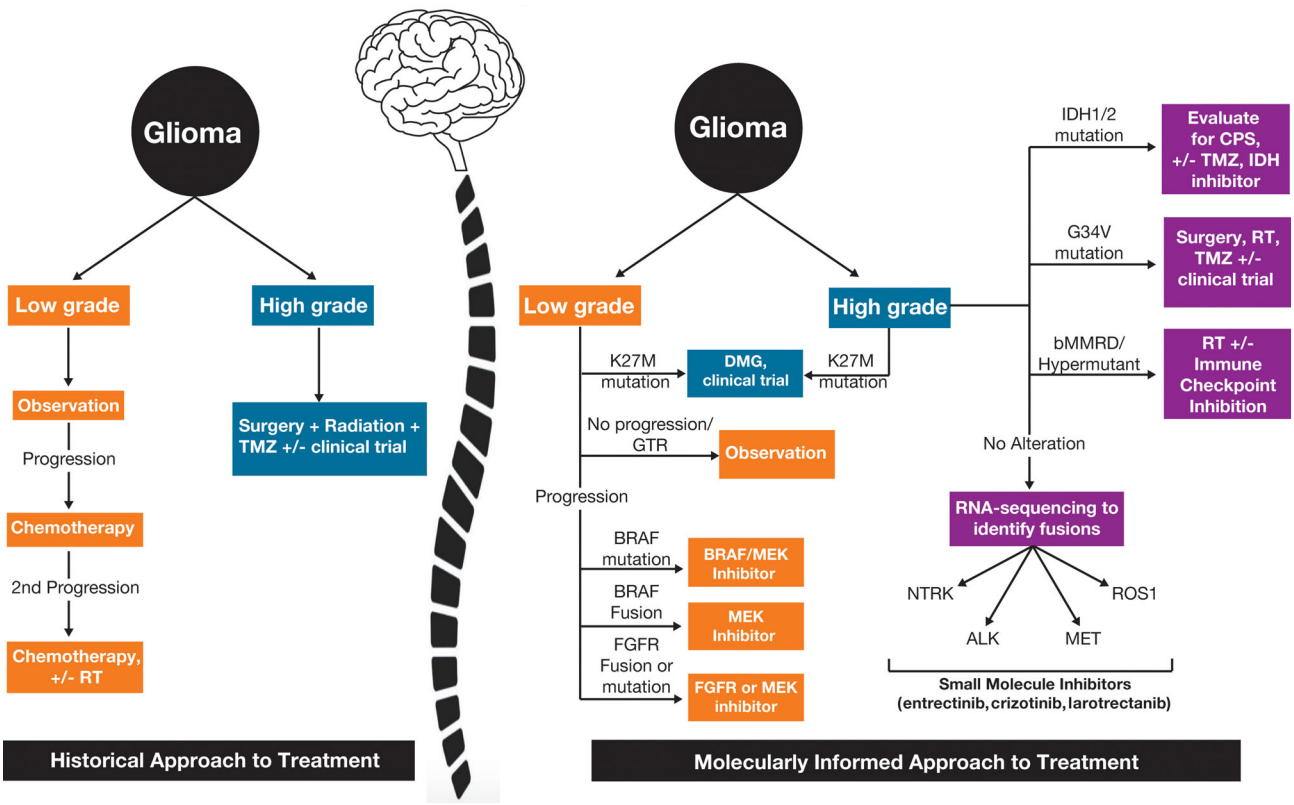

Figure 1. Historical and current treatment approaches to high-grade and low-grade gliomas. mut: mutation; RT: radiation therapy; TMZ: temozolomide; GTR: gross total resection; bMMRD: biallelic mismatch repair deficiency; DMG: diffuse midline glioma; IDH: isocitrate dehydrogenase.

[50]. Over the past 10 years, it has become clear that spinal, supratentorial, and posterior fossa ependymomas are different diseases (despite looking the same under a microscope) and should be considered separate entities. This idea is strengthened by integrated molecular analysis, which classifies ependymomas into nine subgroups. These have distinct age distributions, locations, and biology, suggesting that they are truly different diseases [51]. In the pediatric population, supratentorial ependymoma (ST-EPN) subgroups ST-EPN-RELA and ST-EPN-YAP1 play a dominant role, as well as posterior fossa ependymoma (PF-EPN) subgroups PF-EPN-A and PF-EPN-B [52].

Many ST-EPNs contain fusion of *RELA* with the uncharacterized gene *C11orf95*. The C11orf95–RELA fusion protein serves to drive aberrant NF-κB transcription and results in tumor formation in preclinical models [53,54]. This may serve as a potential therapeutic target. *YAP1*-fused ST-EPNs are rare, but appear enriched in infants with a superior prognosis and may represent the rare group that can be treated with surgery alone or surgery followed by chemotherapy [54]. The failure to identify a *RELA* or *YAP1* fusion should prompt a more detailed investigation using DNA methylation-based classifiers to rule out a morphological mimic [2,55].

PF-EPN-A do not exhibit the DNA rearrangements that are seen in supratentorial tumors; instead, they have been characterized by lack of DNA copy number abnormalities and absence of recurrent DNA mutations, with the exception of *EZHIP/CXorf67* mutations in 20% [56–58]. Additional studies have shown that there is significant heterogeneity within PF-EPN-A and PF-EPN-B, with nine subtypes of PF-EPN-A and five subtypes of PF-EPN-B [59,60]. PF-EPN-A are characterized by a poor prognosis group enriched for chromosome 1q gain, termed PFA1c, and loss of chromosome 13q may represent a marker of poor prognosis in PF-EPN-B. Interestingly, gain of chromosome 1q seems to have no prognostic value in PF-EPN-B [60]. PF-EPN-B occur in older children and adults and are characterized by multiple arm-level copy number aberrations. There are profound differences in prognosis between PF-EPN-A and PF-EPN-B, whereby PF-EPN-B have an excellent outcome [61,62]. Upfront gross total resection and focal radiation are required to treat PF-EPN-A, with survival rates still less than 50%; a proportion of PF-EPN-B could potentially be treated with gross total resection alone [61]. The role of surgery and radiation in ST-EPN is unclear, although those patients with *YAP1* fusions may have a more favorable prognosis [54].

The management of ependymoma, particularly PF-EPN, has changed over the last three decades, leading to significant improvements in patient outcomes [62]. The role of radiation was evaluated in the Children's Oncology Group ACNS0121 study, showing that outcomes are excellent in children as young as 1 year of age with complete surgical resection and focal radiotherapy. This study showed prospectively that PF-EPN-A

Table 1 Common mutations/alterations in pediatric CNS tumors with potential therapeutic targets

Tumor	Alteration/pathway involved	Specific gene mutation/alteration	Targeted treatment
Low-grade gliomas			
pLGG	MAPK pathway- Activation of RAS/MAP signaling pathway	*BRAF* V600E point mutation	Dabrafenib +/− trametinib (BRAF/MEK inhibitor)
		BRAF–KIAA1549 fusion	Selumetinib/trametinib (MEK1/2 inhibitor)
	MAPK pathway and PI3K/mTOR pathway	*FGFR1*	AZD4547 (FGFR1,2,3 inhibitor) Selumetinib/trametinib (MEK1/2 inhibitor)
pLGG-NF1	Inactivation of RAS-GTPase resulting in RAS activation	*NF1*	Selumetinib/trametinib (MEK1/2 inhibitor)
SEGA	mTOR	*TSC1* or *TSC2* inactivating mutation	Everolimus (mTOR inhibitor)
High-grade gliomas			
HGG	Receptor tyrosine kinases	*NTRK1/2/3* gene fusions	Entrectinib (NTRK, ALK, ROS1 inhibitor)
		MET amplification/fusion	
		ALK fusions	Crizotinib/foretinib (MET inhibitor)
		ROS1 fusions	
		PDGFRA amplification	Larotrectinib (NTRK inhibitor)
	Ras/MAPK signaling	*BRAF* V600E	Dabrafenib +/− trametinib (BRAF/MEK inhibitor)
	bMMRD	*POLE* and others	Immune checkpoint inhibitors (Nivolumab/pembrolizumab)
	Metabolic	*IDH1/2*	Ivosidenib (IDH1 inhibitor) Enasidenib (IDH2 inhibitor)
	Cell cycle	*CDKN2A/B* deletion	Ribociclib/palbociclib (CDK4/6 inhibitor)
	Other	Somatic and germline *TP53*	
DMG (thalamic and pontine)		Histone 3.1 and 3.3 mutation	GD2 CAR T-cell Panabinostat (HDAC inhibitor)
Ependymoma			
Supratentorial		*C11orf95–RELA* fusion, *YAP1* fusion	None
PF-EPN-A/B		Somatic SNV: *EZHIP* CNV: 1q gain	None
PF-EPN-B		Multiple arm-level CNV	None
Medulloblastoma			
WNT		Somatic: *CTNNB1* mutations Germline: *APC* mutations CNV: Monosomy 6	Therapy de-escalation
SHH		Somatic: *PTCH*, *SMO*, *SUFU*, *TP53*, *U1*, *TERT* Germline: *PTCH*, *SUFU*, *TP53* CNV: *MYCN*, 9q deletion, 2p gain, *PTEN* loss	SMO antagonist (vismodegib)
Group 3		SNV: KBTBD4 CNV: isochromosome 17q, *MYC* amplification, *GFI1* activation, *PVT1–MYC* fusions	Gemcitabine, pemetrexed (SJMB12) Praxisertib (CHK1 inhibitor SJELIOT) Ribociclib/palbociclib (CDK4/6 inhibitors – SJDAWN) Panobinostat (HDAC inhibitor)
Group 4		SNV: *KDM6A* CNV: *SNCAIP* duplication, isochromosome 17q, *MYCN* amplification, *CDK6* amplification	Gemcitabine, pemetrexed (SJMB12) Praxisertib (CHK1 inhibitor SJELIOT) Ribociclib/palbociclib (CDK4/6 inhibitors – SJDAWN)
Other			
ATRT		Germline and somatic *SMARCB1* (*hSNF5/INI1*) mutations	Tazemetostat (EZH2 inhibitor)
ETMR		CNV: *C19MC* amplification SNV: *DICER1* mutation	None

ATRT, atypical teratoid/rhabdoid tumor; ch, chromosome; DIPG, diffuse intrinsic pontine glioma; DMG, diffuse midline glioma; EPN, ependymoma; ETMR, embryonal tumor with multilayered rosettes; GBM, glioblastoma multiforme; HGG, high-grade glioma; NF1, neurofibromatosis type 1; PF, posterior fossa; pLGG, pediatric low-grade glioma; SEGA, subependymal giant cell astrocytoma.

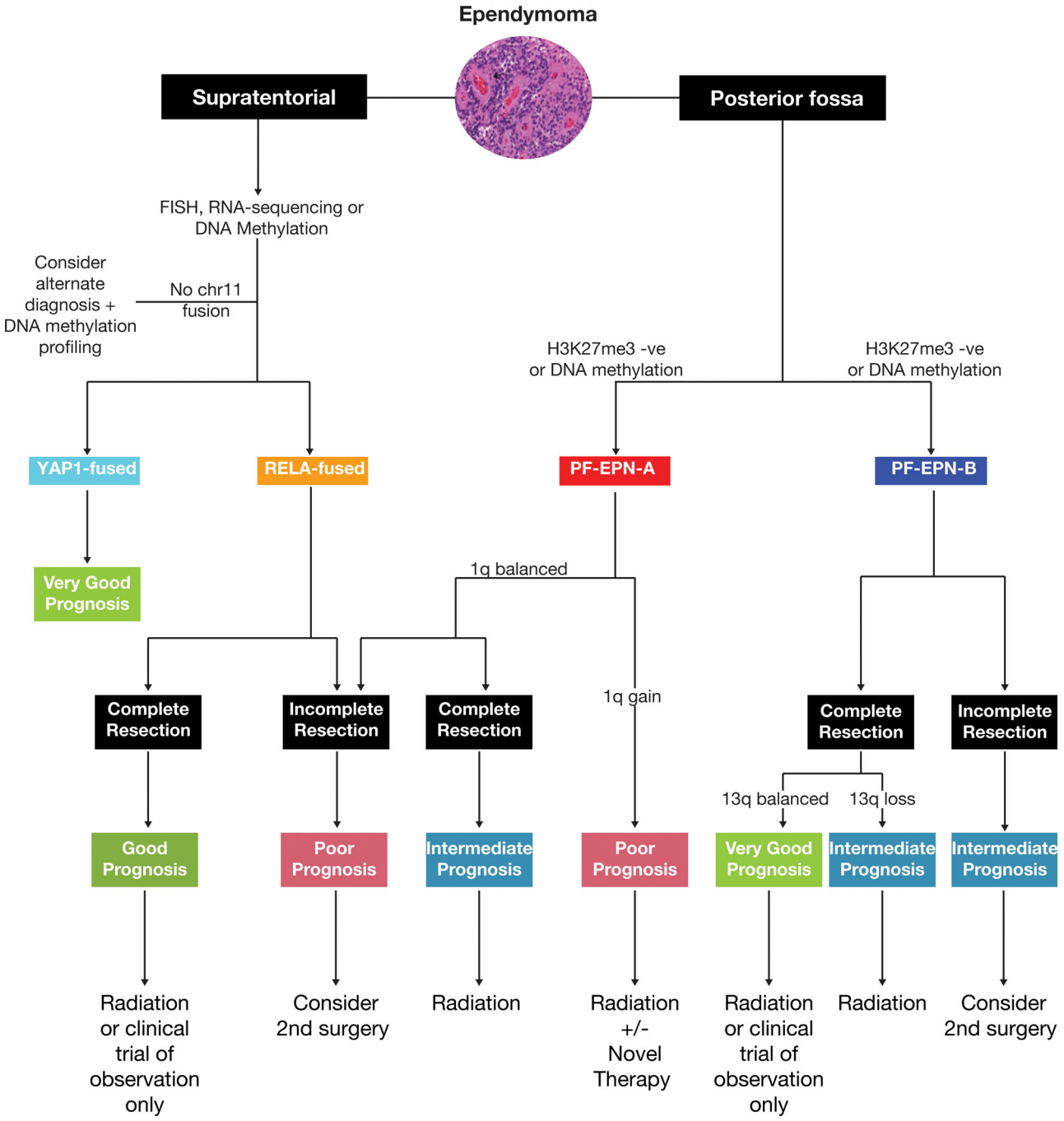

Figure 2. Molecular-based classification of ependymoma. FISH: fluorescence *in situ* hybridization.

with 1q gain have dismal outcomes despite complete resection. Incomplete surgical resection resulted in poor outcomes in both PF-EPN and ST-EPN [63]. Considering the significance of achieving a complete resection safely, treatment at a high volume pediatric neurosurgical center is highly recommended for all patients. The role of chemotherapy in ependymoma is yet to be defined; some studies have shown no survival advantage [64,65], whereas other studies suggest a role for chemotherapy to delay radiation. Objective responses are rarely observed [66–68]. Recurrent ependymomas have a dismal prognosis; surgery and radiation may provide benefit in palliation, with some data suggesting that craniospinal irradiation may be of value [69,70]. As such, in the era of molecular diagnostics, complete resection followed by upfront involved field radiation represents the highest likelihood of treatment success [59]. Treatment outside surgery and radiation has been disappointing.

Medulloblastoma

Medulloblastoma accounts for 15–20% of pediatric brain tumors and can occur at any age from infancy to

adulthood; it is most commonly seen in children between 3 and 9 years of age [71]. The term medulloblastoma was historically used to describe all small round blue cell tumors (SRBC) of the cerebellum. Despite prior knowledge about histological variations such as classic and nodular desmoplastic, most patients have traditionally been treated with a similar approach [72]. The finding of *SMARCB1* mutations (*hSNF5*/INI1) in ATRT provided evidence that not all SRBC tumors were the same entity [73]. Further studies have differentiated medulloblastoma from other types of CNS SRBC tumors, such as embryonal tumor with multilayered rosettes (ETMR), and separated them from embryonal tumors that were formerly classified as CNS-PNET. These are now distinguishable from medulloblastomas [74,75] through novel techniques, such as demonstrating recurrent amplified fusion between embryonal gene *THY1* and a primitive specific microRNA cluster on chromosome 19 (*C19MC*) for ETMR [76] (Figure 3).

Not only have other SRBCs been identified and reclassified, studies have shown that distinct subtypes of medulloblastoma exist with unique characteristics specific to each category (Figure 3). Unbiased genomic analyses have revealed that medulloblastoma actually comprises at least four distinct molecular variants. These are often termed WNT (wingless), SHH (Sonic Hedgehog), group 3, and group 4 [77]. These subgroup classifications enhance or replace our reliance on histopathological classification, as outlined in the 2016 revised WHO classification [6]. The four groups have different cells of origin, with WNT tumors arising from the brainstem, SHH from the external granular layer of the cerebellum, and group 4 tumors arising from unipolar brush cells [78–80]. The histological variants retain some degree of prognostic significance but suffer from extreme inter-observer variability and lack of biological insight, making way for molecular classification that harbors more robust biological and prognostic insights. Recently, it has been shown that multiple subtypes exist within each subgroup, with characteristically unique demographics, structural alterations, epigenomics, transcriptomes, and outcomes.

WNT tumors are typically seen in older children and teenagers; this group compromises approximately 10% of tumors, which have an excellent prognosis with a greater than 95% 10-year event-free survival [81,82]. The most common somatic mutations in the WNT subgroup occur in exon 3 of *CTNNB1* encoding β-catenin and can be identified by direct sequencing or nuclear immunopositivity for β-catenin. The 10% of WNT tumors without *CTNNB1* mutations usually harbor germline mutations in the adenomatous polyposis coli (*APC*) gene [82–85]. Approximately 80% have a deletion of one copy of chromosome 6 (monosomy 6) [86]. WNT tumors arise from the developing lower rhombic lip of the brainstem rather than the cerebellum and frequently invade the lateral recess [80,87,88].

The SHH group affects patients from infancy to adulthood and accounts for approximately 30% of medulloblastomas [89]. SHH signaling is linked to binding to the receptor Patched1 (PTCH1), leading to derepression of smoothened (SMO) activity and activation of GLI1 transcription factors [90]. Mutations in the pathway commonly occur in PTCH1, but alterations in SMO

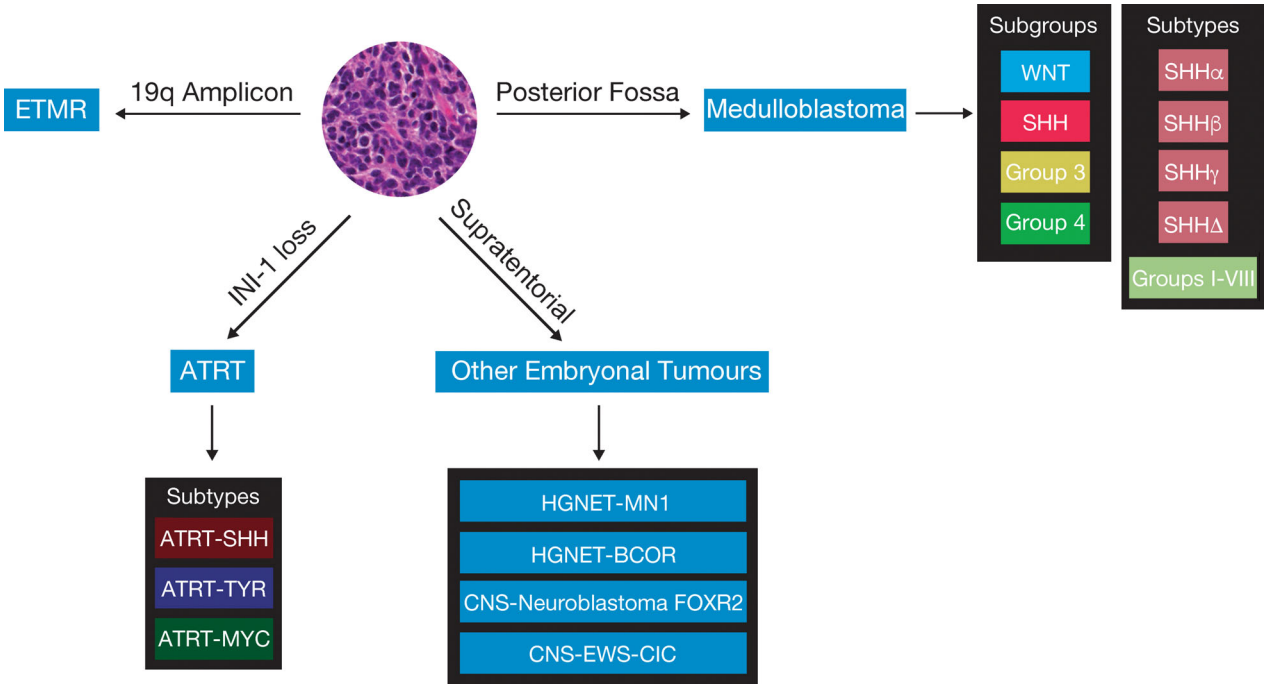

Figure 3. Molecular-based classification of small round blue cell tumors. ETMR: embryonal tumor with multilayered rosettes; ATRT: atypical teratoid/rhabdoid tumor.

and suppressor of fused (SUFU) have also been described, as well as deletions of 9q in this group [77,91]. In infants, there are two predominant types of SHH medulloblastoma, termed SHHβ/SHH-I and SHHγ/SHH-II, with stark differences in outcome. SHHβ/SHH-I are enriched for metastatic disease, *SUFU* mutations, PTEN loss, and 2p gain, and without intensified therapy have dismal outcomes. SHHγ/SHH-II have excellent outcomes regardless of therapy; they have a bland genome and may be suitable candidates for de-escalation of therapy. In older children, SHHα predominates and those with *TP53* mutations, particularly germline, have highly unstable genomes and dismal outcomes, and comprise very-high-risk disease not predicted to respond to SMO antagonists such as vismodegib. In adults, the predominant form of medulloblastoma is SHH, and tumors are characterized by frequent *PTCH* and *SMO* mutations, with *TERT* promoter mutations present in the majority of cases. In 50–60% of cases, SHH recurs locally in the tumor bed. SHH tumors arise from the external granule layer and almost always are found within the cerebellum itself, not the fourth ventricle [78,79,92]. Suzuki *et al* reported recurrent hotspot mutations (r.3A>G) of U1 spliceosomal small nuclear RNA (snRNA) in 50% of SHH medulloblastomas [93]. They were found in 97% of adults (SHHδ) and 25% of adolescents (SHHα) but are absent in infants. SHHα patients are thought to represent a high-risk group and the splicing mediated by mutant U1snRNA, which inactivates tumor suppressor PTCH1 and activates oncoproteins GLI2 and CCND2, may represent a target for therapy [93].

Group 3 medulloblastomas account for 25% of cases; arise exclusively in children; frequently metastasize; and have a poor prognosis, with approximately 50% OS at 5 years. These tumors are characterized by an increased frequency of copy number alterations, including loss of chromosome 17p and gain of 17q to generate an isochromosome 17q and *MYC* amplification in 20% [94]. Given the poor prognosis of this group of tumors, there is considerable interest in improved treatment options. Applying combined DNA methylation and gene expression, three subtypes of group 3 have been identified: 3α, 3β, and 3γ, where group 3γ tumors have a poor survival and are enriched for *MYC* amplification. Similar groups have been identified using DNA methylation, with a poor prognosis group enriched for *MYC* and rarely *MYCN* [83,95,96]. Cross-species high-throughput screening identified medications such as gemcitabine, pemetrexed, and panobinostat as potential agents, which are being tested prospectively in the SJMB12 clinical trial (NCT018788617) [97]. Group 3 tumors, particularly those that are irradiated, recur almost exclusively with metastatic dissemination [98].

Group 4 medulloblastomas are the most common but a poorly understood subgroup; they account for 35% of cases. These tumors also have a high degree of chromosome copy number aberrations, including 80% harboring isochromosome 17q (i17q). They are characterized by amplification of *MYCN* in a small subset, and somatic nucleotide variants in the histone demethylase *KDM6A*. Applying integrated clustering, three group 4 subtypes have been identified. Group 4α are enriched for *CDK6* amplification, 7q gain, 8p loss, and i17q; group 4β are enriched for tandem duplications in *SNCAIP*, and i17q represents the only copy number aberration; and group 4γ are enriched for *MYCN* amplification, 7q gain, 8p loss, and i17q. In addition, tandem duplication of the Parkinson's gene *SNCAIP* on chromosome 5 is identified in a subset of group 4 medulloblastomas [99,100]. Similar groups using DNA methylation have been described. The cells of origin for group 4 medulloblastoma are unipolar brush cells and they frequently present with a lack of gadolinium enhancement on MRI [78,79].

A meta-analysis of three subtyping efforts suggests that additional heterogeneity exists at the level of DNA methylation across group 3 and 4 medulloblastomas, with eight groups being described [95]. The clinical relevance of this new classification is still a work-in-progress; however, there seem to be subgroups with an excellent prognosis beyond just group 4 with chromosome 11 loss and 7q gain. Group 3 and 4 tumors show in-frame insertions in KBTBD4, a BTB-BACK-Kelch domain ubiquitin ligase adaptor that facilitates ubiquitination of target substrates and may serve as a target for future therapy [83]. In addition, group 3 and 4 tumors demonstrate genomic structural variants resulting in activation of growth factor independent 1 family proto-oncogenes, *GFI1* and *GFI1B*, termed 'enhancer hijacking' [83,101]. Approximately one third of group 3 medulloblastomas demonstrate somatic genomic rearrangements in association with mutually exclusive GFI1 or GFI1B activation and 5–10% of group 4 medulloblastomas harbor structural variants associated with GFI1/GFI1B activation, making these poorly understood subgroups more identifiable and possibly serving as a targeted therapy approach [101].

The current management of medulloblastoma in older children (greater than 4 or 5 years of age) includes maximal safe surgical resection followed by chemotherapy and radiation. Those classified as 'average risk' are patients with total (or near-total) resection and no evidence of metastatic disease at the time of diagnosis. These patients are treated with adjuvant craniospinal radiation as well as a boost to the tumor bed and chemotherapy. Average-risk medulloblastoma patients have a greater than 80% 5-year event-free survival [102]. Significant toxicity associated with surgical resection, radiation, and chemotherapy in the form of cerebellar mutism, neurocognitive deficits, hearing loss, and endocrine abnormalities are known in these patients [103]. Recent clinical trials are focused on reducing the dose of craniospinal radiation in an effort to minimize long-term effects in these patients [104]. Medulloblastoma risk stratification consensus utilizing currently available biomarkers has been established: low risk (> 90% survival), average (standard) risk (75–90% survival), high risk (50–75% survival), and very high risk (< 50% survival) [105]. The WNT subgroup and non-

metastatic group 4 tumors with whole chromosome 11 loss or whole chromosome 17 gain are recognized as low-risk tumors that may qualify for reduced therapy. High-risk disease is common in patients with metastatic SHH or group 4 tumors, and in *MYCN*-amplified SHH. Very-high-risk patients include *MYC*-amplified group 3 patients with metastatic disease or SHH tumors with *TP53* mutations [105]. Infant and younger children pose a challenge as radiation therapy tends to be avoided or delayed in patients under the age of 3; instead, these patients are treated with surgery and intensive chemotherapy as upfront modalities [104,106,107]. In infants, SHH tumors have an excellent prognosis, while group 3 tumors have a dismal prognosis when applying radiation sparing strategies [107].

Identifying therapeutic targets utilizing molecular characterization may improve patient care and aim to reduce toxicities associated with therapy. WNT tumor patients have an excellent prognosis, which may suggest a de-intensifying strategy [108], whereas *MYC* amplification including the *PVT1–MYC* fusion in group 3 has been a difficult area to target. Some early clinical trials have shown efficacy of the SMO inhibitor vismodegib in SHH medulloblastoma phase 1 trials, although *TP53* mutant SHH and downstream activation such as *SUFU* mutations predominate in the majority of relapses, making this approach unlikely to work. Vismodegib was found to inhibit SMO and represses the SHH pathway in one of three patients with recurrent SHH medulloblastoma, and was not shown to have an effect in other medulloblastoma subtypes [109]. Given the paucity of information on group 3 and 4 medulloblastomas, no specific treatments have been developed to date, although the near ubiquitous metastatic pattern of relapse suggests that specific treatment of the metastatic compartment will be required for cure.

Other embryonal tumors

Other embryonal tumors of the CNS are highly aggressive, poorly differentiated tumors occurring predominately in young children. Controversy exists regarding the histogenesis of these tumors and the term PNET has been removed from the most recent nomenclature, although some rare entities such as medulloepithelioma have remained [6]. A molecularly distinct entity, 'ETMR, *C19MC*-altered tumors' has been added, encompassing embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma, and medulloepithelioma [76,110]. ETMRs harbor recurrent amplifications and fusions of a microRNA cluster on chromosome 19, frequently fused to *TTYH1*, and a small proportion of ETMRs without *C19MC* alterations harbor *DICER1* mutations [111].

A large study of histologically described PNETs [75] identified four new CNS tumor types by DNA methylation and transcription profiling: CNS neuroblastoma with FOXR2 activation (CNS NB-FOXR2), CNS Ewing sarcoma family tumor with CIC alteration (CNS EFT-CIC), CNS high-grade neuroepithelial tumor with MN1 alteration (CNS HGNET-MN1), and CNS high-grade neuroepithelial tumor with BCOR alteration (CNS HGNET-BCOR) (Figure 3). Each of these is associated with distinct histopathological and clinical features as well as genetic alterations, although the clinical relevance of these new entities is yet to be discerned. Nevertheless, the study suggests that embryonal tumors are a diagnosis of exclusion, and supratentorial tumors morphologically described as 'PNET' require extensive molecular workup to exclude ependymoma, ETMR, ATRT, and glioblastoma.

Atypical teratoid/rhabdoid tumor

Rhabdoid tumors located within the CNS are classified as atypical teratoid/rhabdoid tumors (ATRTs), an aggressive malignancy that is typically identified in young children. Rhabdoid tumors can also occur outside of the CNS, predominantly in kidneys, liver or soft tissues. ATRTs are defined by biallelic *SMARCB1* loss-of-function alterations [112]. About 35% of ATRT patients have heritable *SMARCB1* alterations predisposing them to multiple rhabdoid tumors [113], suggesting the need for genetic counseling for surveillance and implications in future pregnancies, although the value of this is yet to be known [114]. Other studies have shown that loss of *SMARCB4* is critical in ATRT development, but less frequent [115]. ATRTs encompass three epigenetic subgroups with distinct genomic profiles and *SMARCB1* genotypes [116]. ATRT-TYR tumors are more common in the infratentorial regions, ATRT-MYC tumors are commonly seen in the supratentorial area, and ATRT-SHH tumors are seen in both infra- and supra-tentorial areas. Age also differs amongst these subsets, with very young children (0–1 year) commonly identifying the TYR group and ATRT-MYC tending to occur in older children. ATRT-TYR and to a lesser degree the ATRT-SHH group are characterized by hypermethylated genomes, whereas the ATRT-MYC group is not. Studies have shown different pathway upregulation based on subgroup, which leads to potential therapeutic targets for these diseases. ATRT-TYR tumors show upregulation of melanogenesis, EZH2, DNMTs, CCND1, VEGFA, and ERBB2, with more tumors showing *SMARCB1* deletion constituting a 22q loss, whereas the ATRT-SHH group tends to exhibit upregulation in SHH pathway, EZH2, DNMTs, and CDK6, with no aberration in *SMARCB1*. The ATRT-MYC subtype upregulates MYC and HOX, EZH2, DNMTs, and ERBB2, with focal *SMARCB1* deletion predominantly [117].

ATRTs were previously considered incurable, although outcomes have improved with intensified therapy, particularly multimodal therapy as in the Children's

Oncology Group ACNS0333 study, with some suggestion that ATRT-SHH benefit from high-dose chemotherapy approaches and ATRT-TYR benefit from radiotherapy [116,118–121]. The EZH2 inhibitor tazemetostat is a rational approach to target the SWI/SNF complex and is being investigated in upcoming clinical trials; however, there is a paucity of early phase human data at the present time [122].

Conclusion

The molecular classification of pediatric CNS tumors is a rapidly evolving field and has transformed the knowledge and approach to pediatric tumors. Our current classification schemes date back to the 1920s. The rapid advances observed over the past 10 years have improved classification, identified new entities, and provided the basis for new diagnostic and treatment paradigms that are more precise and personalized. Indeed, a plethora of molecularly informed clinical trials are ongoing, including several being evaluated in front-line therapies. Unlike current therapies, particularly radiation and cytotoxic chemotherapy that result in life-long sequelae, novel targeted therapeutic agents have shown promise in being more effective and less toxic. With further advances evolving rapidly in the molecular era of neuro-oncology, the approach to pediatric brain tumors is undergoing a major revolution, with more accurate diagnosis and personalized, biologically informed treatment, which will undoubtedly result in improved outcomes including improved quality of life.

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Abbreviations

ATRT	atypical teratoid/rhabdoid tumor
ATRX	alpha thalassemia/mental retardation syndrome X-linked
bMMRD	biallelic mismatch repair deficiency
CAR	chimeric antigen receptor
CNV	copy number variation
DIPG	diffuse intrinsic pontine glioma
ETANTR	embryonal tumor with abundant neuropil and true rosettes
ETMR	embryonal tumor with multilayered rosettes
FGFR1	fibroblast growth factor receptor 1
GBM	glioblastoma multiforme
HGG	high-grade glioma
LGG	low-grade glioma
NF-1	neurofibromatosis type 1
OS	overall survival
PA	pilocytic astrocytoma
PF-EPN	posterior fossa ependymoma
PFS	progression-free survival
pHGG	pediatric high-grade glioma
pLGG	pediatric low-grade glioma
PMA	pilomyxoid astrocytoma
PNET	primitive neuro-ectodermal tumor
PR	partial response
PXA	pleomorphic xanthoastrocytoma
RTK	receptor tyrosine kinase
SEGA	subependymal giant cell astrocytoma
SNV	somatic nucleotide variation
SRBC	small round blue cell tumor
ST-RPN	supratentorial ependymoma

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