Purpose of review
Brain tumors are the most common solid tumors and leading cause of cancer-related death in children. The advent of large-scale genomics has resulted in a plethora of profiling studies that have mapped the genetic and epigenetic landscapes of pediatric brain tumors, ringing in a new era of precision diagnostics and targeted therapies. In this review, we highlight the most recent findings, focusing on studies published after 2015, and discuss how new evidence is changing the care of children with brain tumors.

Recent findings
Genome-wide and epigenome-wide profiling data have revealed distinct tumor entities within, virtually, all pediatric brain tumor groups including medulloblastoma; ependymoma; high-grade and low-grade gliomas; atypical teratoid/rhabdoid tumors; and other embryonal tumors, previously called CNS primitive neuroectodermal tumors. Whenever integrated with clinical information, many molecular alterations emerge as powerful prognostic markers and should thus be used to stratify patients and tailor therapies.

Summary
Optimal integration of this newly emerging knowledge in a timely and meaningful way into clinical care is a remarkable task and a matter of active debate. The historical morphology-based classification of tumors is being replaced by a genetic-based classification, and the first generation of molecularly informed clinical trials is underway.

Keywords
atypical teratoid/rhabdoid tumor, ependymoma, high-grade glioma, low-grade glioma, medulloblastoma

INTRODUCTION
For decades, the treatment of pediatric brain tumors has been dictated by their appearance under the microscope, however, morphology alone often failed to explain the heterogeneity in clinical behavior. Brain tumors remain the leading cause of cancer-related death in children 0–14 years of age [1] and better treatments have been long awaited. For survivors, cure often comes at a high cost, with devastating and permanent neurological sequelae that have a profound impact on quality of life.

Driven by increasingly detailed molecular studies, the field of pediatric neuro-oncology is undergoing a major transformation with the ultimate goal of improving survival, functional outcome and quality of life for children with brain tumors. Despite the breadth of available molecular data, functional characterization of targets in preclinical studies and prospective validation in clinical trials are still lacking and, as such, changes in clinical practice have occurred at a slow pace. In order to lay a common ground for the application of molecular knowledge, over the last 2 years, several expert consensus statements, which define recommendations on how to translate this knowledge into targeted clinical trials for children with brain tumors, have been developed [2*,3*,4–5].

Acknowledging that this is a rapidly evolving field – with much still unknown – this review highlights the impact of recent findings on the classification and treatment of pediatric brain tumors.

*The Division of Haematology/Oncology, The Hospital for Sick Children,
†The Arthur and Sonia Labatt Brain Tumour Research Centre and the Developmental & Stem Cell Biology Program, The Hospital for Sick Children and "Department of Surgery and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada
Correspondence to Michael D. Taylor, MD, PhD, FRCSC, The Arthur and Sonia Labatt Brain Tumour Research Centre and the Developmental & Stem Cell Biology Program, The Hospital for Sick Children, 555 University Avenue, Rm 1504, Toronto, ONT M5G 1X8, Canada.
Tel: +1 416 813 7564; fax: +1 416 813 6169; e-mail: mdtaylor@sickkids.ca

Curr Opin Pediatr 2017, 29:000–000
DOI:10.1097/MOP.0000000000000562
MEDULLOBLASTOMA

Perhaps the most extensively studied pediatric brain tumor, medulloblastoma is the paradigm of how molecular studies shaped tumor classification and are changing patient management [6–11]. Once broadly described as a small round blue cell tumor with highly malignant features, the 2016 edition of the World Health Organization (WHO) classification of central nervous system (CNS) tumors describes, for the first time, four genetically defined medulloblastoma variants: the subgroups WNT, SHH, group 3, and group 4; SHH is further subdivided into p53-wild type and p53-mutated, however, they are still considered one subgroup [12]. The differences in biological and clinical behavior between subgroups and their impact in outcome is such that, nowadays and moving forward, it will no longer be acceptable to treat children with medulloblastoma in clinical trials without taking this information into account [3*].

The impact of molecular classification is already changing every aspect of medulloblastoma treatment. An international retrospective analysis of a large cohort of children with medulloblastoma found that the impact of extent of resection is minimal whenever subgroup affiliation is considered [13]. Maximum safe surgical resection remains the standard of care but this review challenges the neurosurgical dogma of the value of total resection for treatment of medulloblastoma and cautions against removing small residual tumors, given the lack of survival benefit and added risks of a poor neurological outcome. Craniospinal irradiation (CSI) is a cornerstone of treatment (in children older than 3–5 years) but the long-term side effects are numerous and often devastating. Not surprisingly, the first generation of subgroup-tailored clinical trials prioritizes reduction/de-intensification of treatment for children with tumors showing favorable biology, such as localized WNT tumors, mostly through reduction of CSI dose (clinical trials.gov identifiers: NCT01878617, NCT02066220 and NCT02724579) or using a chemotherapy-only approach (NCT02212574). Similarly, group 4 tumors with chromosome 11 loss have an excellent outcome, and future studies will reduce therapy for these patients [3*].

Building upon the current knowledge and attempting to refine the current classification, multiple international collaborative studies, have very recently, further dissected the heterogeneity within medulloblastoma subgroups [14*–16*]. The degree of overlap between the subtypes proposed by each study varies because of differences in study design and methodological approach. Overall, these studies show that there are distinct clusters of patients within each subgroup. Group 3 and group 4 tumors – entered in the WHO classification as provisional entities [12] – are heterogeneous but represent distinct subgroups and have minimal overlap [14*,15*]. In line with previous studies [3*,10], a subset of patients with group 4 tumors with chromosome 11 loss with an excellent outcome was identified [3*,10,16*] and should be prioritized for de-escalation of therapy, whereas metastatic group 3 tumors have an aggressive course and poor outcome [14*,16*]. Using an integrated clustering approach applied to DNA methylation and gene expression datasets, Cavalli et al. [14*] defined 12 subtypes of medulloblastoma (see Fig. 1) – 2 WNT, 4 SHH, 3 group 3, and 3 group 4 – and correlated them with survival information, revealing new biologically and clinically relevant patient clusters, especially for SHH and group 3 tumors. Interestingly, amongst SHH tumors TP53 mutations were highly prognostic and enriched in SHHα but not prognostic in non-SHHα, suggesting that TP53-mutated SHH tumors (included in the new WHO classification as a separate entity of very-high risk tumors, and prioritized for introduction of novel therapies) may not have a universally dismal outcome. Infant SHH medulloblastoma segregated into two SHH subtypes, SHHβ (poor outcome, metastatic with higher copy number alterations) and SHHγ (good outcome). Currently infants are stratified for treatment based on the presence of histological features consistent with ‘desmoplasia’ or MBEN (medulloblastoma with extensive nodularity) but the most recent infant study of the Children’s Oncology Group (ACNS1221, clinical trials.gov ID NCT02017964) closed prematurely because of an unexpectedly high number of treatment failures. SHHγ subtype was indeed enriched for MBEN tumors but included other histologies as well and subtype affiliation

**KEY POINTS**

- Molecular studies led to the identification of distinct tumor subgroups in virtually all pediatric brain tumors and as such a new molecular classification of brain tumors is emerging.
- Tumor precision diagnostics is rapidly becoming part of routine diagnostics and changing patient care.
- Collaborative, multiinstitutional clinical trials will be key to prospectively validate biomarkers and treatments assigned based on tumor molecular profile.
- A strategy is needed to ensure inclusion of children with rare tumor entities in molecularly informed clinical trials.
was more prognostic than morphology-based classification [14]. Amongst group 3 subtypes, group 3γ had the worst outcome, including but not limited to MYC amplified tumors, thus expanding the identification of the group of patients at higher risk for treatment failure beyond MYC status [14].

Northcott et al. reanalyzed and expanded previous genomic, epigenomic and transcriptomic medulloblastoma datasets. Though there is no information on clinical outcome or prognostic implications, novel driver events were identified through this analysis – such as KBTBD4 insertions and activation of PRDM6 through enhancer hijacking. These findings should facilitate the development of both preclinical models, that are currently lacking for group 3 and 4 tumors, as well as novel therapies for medulloblastoma patients [15].

A crucial aspect of clinical practice in the molecular era is to ensure that sufficient and appropriate material is collected for molecular studies. Though the transcriptome of medulloblastoma is spatially homogenous – allowing robust subgrouping from a single sample – genetic driver events are seldom clonal and spatial intratumoral heterogeneity could be a barrier to effective assignment of genetic events [17].

**FIGURE 1.** Molecular studies decipher multiple distinct entities within morphologically defined brain tumor groups.
Previously irradiated medulloblastoma recurrences are incurable and, compared with primary tumors, critically understudied. Recent evidence has shown that there is significant clonal divergence at relapse and that driver genetic events emerge via selection during treatment and are often undetectable in the corresponding primary tumor sample [18**]. Novel therapies are more likely to be used at recurrence but are often assigned using molecular information from the primary tumor. Moreover, patients with group 3 and group 4 tumors present with metastatic relapse, rather than disease recurrence at the primary site [11]. These findings have major implications in clinical practice, suggesting that whenever targeted therapies are considered at relapse, a biopsy of the recurrent tumor should be attempted. In late relapses or local group 3 and group 4 relapses, an additional indication for biopsy is to establish whether it is in fact a true recurrence or a postradiation secondary high-grade glioma.

Additional challenges remain on the preclinical side and efforts are needed to create better models for drug discovery, especially for highly aggressive subtypes of medulloblastoma, and increased clinical research on understudied patient populations, such as older teenagers and young adults [19,20].

**PEDIATRIC HIGH-GRADE GLIOMAS**

Pediatric High-Grade Gliomas (pHGGs) include a heterogeneous group of malignant tumors classified as anaplastic astrocytoma (WHO Grade III) and glioblastoma (WHO Grade IV), including the Diffuse Intrinsic Pontine Glioma (DIPG), which are pHGGs located in the pons often diagnosed based solely on clinical and radiological characteristics. Though prevalent throughout the entire age spectrum, high-grade gliomas in children are different from their adult counterparts. Unlike adult HGGs, only a small fraction (<5%) of pHGGs are IDH1/2 mutated tumors, and these are found mostly in older adolescents. The new WHO 2016 classification added the diffuse midline glioma, H3 K27M-mutant, that includes some DIPG, but reflects mostly the spectrum of alterations in adult tumors, using the presence of IDH mutation to stratify high-grade lesions.

The relevant subgroups in the pediatric population are defined by key molecular alterations [4]: recurrent mutations in the genes H3F3A and HIST1H3B, coding the histone variants H3.3 and H3.1, respectively, resulting in a lysine to methionine substitution at amino acid residue 27 (K27M) or glycine to arginine or valine at position 34 G34R/V [21–24]. These mutations are mutually exclusive and have a typical anatomical pattern of distribution, marking distinct pHGG subgroups and likely reflecting different cells of origin. Whereas H3.1 K27M is restricted to the pons (DIPG), H3.3 K27M is found in pHGGs of the pons and other midline structures and H3.3 G34R/V exclusively in the cerebral hemispheres. There are additional genetic alterations characteristic of each subgroup: H3.3 K27M tumors are enriched for TP53 and FGFR1 mutations, H3.1 K27M DIPGs ACVR1 mutations and H3.3 G34R/V ATRX mutations [22,23,25,26]. An additional subgroup comprises BRAFV600E mutated tumors, often co-occurring with CDKN2A deletion, of cortical location and with better outcome [21]. In this subgroup are included secondary HGGs, lesions transformed from pediatric low-grade gliomas harboring BRAFV600E mutation and CDKN2A deletion [27].

The remaining pHGGs with wild type BRAF, histone, and IDH, account for nearly half of pHGGs and present a variety of heterogeneous genetic and epigenetic characteristics [4]. An integrative genetic analysis of pHGGs found novel MET fusions in ~10% of tumors, providing a rationale for the use of small molecule inhibitors to treat these patients. Given the aggressive behavior of these tumors, multiagent therapy will likely be necessary to overcome emergence of resistant clones [28*]. A focused analysis of H3-/IDH-wild type pHGGs found three main clusters of tumors: one with a dismal prognosis and enriched for MYCN amplification and two further subtypes characterized by PDGFRA (intermediate prognosis) and EGFR amplification (longer overall survival, median 44 months) [29].

Glioblastomas, particularly in children, may arise in the context of cancer predisposition syndromes such as Li Fraumeni Syndrome (LFS) and biallelic mismatch repair deficiency (bMMRD) [30]. A high index of suspicion and early diagnosis is crucial not only because of implication for surveillance, which improves long-term overall survival of affected individuals [31–33], but also because it may have impact on treatment. As with sporadic pHGGs, bMMRD tumors are resistant to conventional therapies, however, there are encouraging initial reports of durable responses in bMMRD patients with hypermutant GBMs treated using immune checkpoint inhibitors [34**], and clinical trials are underway.

**PEDIATRIC LOW-GRADE GLIOMAS**

Pediatric low-grade gliomas (pLGG) comprise many histologically diverse benign tumors of glial origin classified as WHO Grades I and II that have an excellent overall survival [35,36]; although, if unresectable, often have a long and protracted course.
Some pLGGs are associated with genetic predisposition syndromes, most notably neurofibromatosis type 1 (15%), with inactivating mutations in the tumor suppressor gene NF1 leading to an activation of the Ras/MAPK pathway and increased risk of pLGG.

In a short period of time, multiple studies have shown that the majority of sporadic pLGGs are also driven by activation of the Ras/MAPK pathway, typically through alterations at the level of the BRAF oncogene with BRAF gene fusions (most commonly KIAA1549:BRAF) or point mutations BRAFV600E, and to a lesser extent through FGFR fusions (FGFR1-TACC3 the most common), FGFR1 mutations, MYB/MYBL or NTRK rearrangements [37,38]. In addition, MYB-QKI fusions were recently identified alterations that are specific for angiogenic gliomas [39].

BRAF fusions are an important diagnostic hallmark, present in two thirds of pLGGs [40] and can be quickly detected in the clinic with FISH, RT-PCR or a NanoString assay [41]. Beyond the diagnostic and potential therapeutic implications, there is evidence that overall, pLGGs harboring BRAF fusions – even when accounting for extent of resection – have a better outcome than nonfused tumors [40]. BRAFV600E is another targetable alteration found in 17% of pLGGs, across a broad spectrum of locations and histologies, and that can be easily detected by routine immunohistochemistry. A landmark large international collaborative study showed that unrected BRAFV600E tumors with CDKN2A deletion have a significantly worse outcome, with a 5-year progression-free survival (PFS) of 24% [42**]. Moreover, though malignant transformation is rare in pLGGs when compared with the adult counterparts, most pediatric secondary HGGs harbor BRAFV600E mutation and CDKN2A deletion [27].

Undoubtedly, these findings have major clinical and therapeutic implications. The new edition of the WHO classification retains a morphology-based classification [12] but with overwhelming evidence that genetic events are not only more powerful biomarkers than histology but also targetable; screening for BRAF alterations is rapidly becoming part of everyday practice and informing treatment decisions. As such, molecular analysis of at least BRAF fusions and BRAFV600E should be integrated into the classification and biopsy of tumors in non-NF1 patients, in addition, including biopsy at time of progression, is strongly recommended [5]. Targeted therapies using MEK inhibitors, such as selumetinib [43,44], and BRAFV600E inhibitor dabrafenib [45] in children with progressive pLGGs show promising results and should be evaluated as upfront treatment.

**EPENDYMOMA**

Ependymomas are prevalent in children and adults and can arise in the supratentorial, infratentorial or spinal compartments of the CNS. Even prior to molecular studies, the issue of histological grading (WHO II versus III) in ependymoma was controversial because of concerns about reproducibility, making it problematic as a basis for patient stratification. Ependymomas comprise nine subgroups with distinct age distribution, location and biology [46]. Relevant in the pediatric population are the supratentorial subgroups: ST-EPN-RELA and ST-EPN-YAP1, and the posterior fossa subgroups: PF-EPN-PFA and PF-EPN-PFB (Fig. 1). The most recent edition of the WHO CNS tumors classification includes only ST-EPN-RELA but others will likely be incorporated in the future. The majority of supratentorial ependymomas (ST-EPN-RELA) have a C11ORF95-RELA gene fusion, and the remaining harbor fusions involving the YAP1 oncogene (ST-EPN-YAP1). On the basis of retrospective data, supratentorial tumors with YAP1 fusions have good prognosis but the prognostic impact of RELA fusion remains contentious and should be evaluated in prospective studies. Posterior fossa ependymomas in children belong to PF-EPN-PFA or PF-EPN-PFB subgroups. PF-EPN-PFA tumors are most common in the first decade of life (median age 3 years), have a worse outcome, high risk of recurrence, and higher incidence of metastases at recurrence [47], whereas PF-EPN-PFB tumors are predominantly diagnosed in older adolescents and adults [46,48].

Despite the new knowledge gained and the consensus that a new patient stratification scheme independent of histological grading should be instituted, alternative therapies are lacking and children with ependymoma are still being treated based on age, location, and extent of surgical resection [2*].

**ATYPICAL TERATOID/RHABDOID TUMORS AND OTHER EMBRYONAL TUMORS**

Atypical teratoid/rhabdoid tumors (AT/RT) are rhabdoid tumors of the CNS, a group of aggressive malignancies that typically affect very young children (under the age of 3 years) and may arise in other non-CNS sites, most commonly the kidneys. The genetic landscape of AT/RTs is defined by inactivating mutations or deletions of the tumor suppressor genes SMARCB1 or, less frequently, SMARCA4. AT/RTs are no longer considered incurable but the prognosis remains overall very poor and the majority of patients succumb to their tumors within 1 year after diagnosis. AT/RTs are constituted of three epigenetic subgroups with distinct genomic profiles and SMARCB1 genotypes [49*,50,51*].
**Hematology and oncology**

About one-third of patient’s harbor germline mutations and families of children affected should be offered genetic testing and counseling to discuss surveillance and implications for future pregnancies, although the value of surveillance in carriers of this rare and aggressive disease is unknown [52].

A new entity introduced in the WHO 2016 classification was embryonal tumors with multilayered rosettes, C19MC-altered. These are aggressive tumors that predominantly affect infants and are characterized by a unique signature with amplification of a cluster of microRNAs C19MC. It includes most tumors previously described as medulloepithelioma, ependymoblastoma, and embryonal tumors with abundant neuropil and true rosettes (ETANTR) [53].

Classification of another group of rare and aggressive tumors – collectively known as CNS-primitive neuroectodermal tumors (PNET) – is undergoing major adjustments [54] and the term PNET was removed from the nomenclature [12].

**CONCLUSION**

The molecular classification of pediatric brain tumors is rapidly evolving. From creating a novel nomenclature that reflects the biology – rather than the morphology – of tumors, to the use of biomarkers to improve patient stratification and assign targeted therapies.

Future clinical trials must be molecularly informed and molecularly balanced, with mandatory collection and central review of tumors to allow prospective validation of biomarkers.

Genomic technology has facilitated the identification of novel tumor subtypes and has rapidly led to improved stratification and specific tailoring of therapy to minimize toxicity (e.g. dose reduction or elimination of radiotherapy) and/or enhance response (targeted therapies or immune-based treatment approaches). However, subdividing pediatric brain tumors from a few entities into multiple molecular variants (Fig. 1) – some of which are exceedingly rare – may critically hamper or even preclude the inclusion of children with rarer tumors in clinical trials.

The challenges ahead are plentiful but tumor precision diagnostics should become part of routine patient care; international collaborations will be essential to successfully execute clinical studies.

**Acknowledgements**

None.

**Financial support and sponsorship**

Research in the Taylor lab (M.D.T.) is financially supported by a Program Project Grant from the Terry Fox Research Institute and grants from the CureSearch Foundation, the National Institutes of Health (R01CA148699 and R01CA159859) and The Brain Tumour Foundation of Canada Impact Grant of the Canadian Cancer Society and Brain Canada with the financial assistance of Health Canada (grant #703202), The Canadian Institutes of Health Research, the Pediatric Brain Tumor Foundation, the Ontario Institute for Cancer Research through funding provided by the Government of Ontario, and a Stand Up To Cancer St. Baldrick’s Pediatric Dream Team Translational Research Grant (SU2C-AACR-DT1113); Stand Up To Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research. Both M.D.T. and A.G.S. receive support from Worldwide Cancer Research. M.D.T. is also supported by The Garron Family Chair in Childhood Cancer Research at The Hospital for Sick Children and The University of Toronto. A.G.S. holds a Garron Family Cancer Centre Clinical Research Fellowship. V.R. is supported by Meagan’s Walk, an ABTA Discovery Grant, the Brain Tumour Foundation of Canada, Garron Family Cancer Centre Pitblado Discovery Grant and a Collaborative Ependymoma Research Network basic science fellowship.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

3. Consensus statement outlining recommendations for diagnosis, clinical management and future studies of pediatric supratentorial and posterior fossa ependymoma.
4. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.
5. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.
6. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.
7. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.
8. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.
9. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.
10. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.
11. Consensus statement summarizing the clinical implications of medulloblastoma molecular variants and outlining a blueprint for molecularly informed clinical trials.

Month 2017
Review of pediatric brain tumors Guerreiro Stucklin et al.

16. Studies using different approaches to refine molecular classification and define medulloblastoma subtypes.
18. Studies using different approaches to refine molecular classification and define medulloblastoma subtypes.
20. Studies using different approaches to refine molecular classification and define medulloblastoma subtypes.
37. Large study characterizing the molecular subgroups of ATRT.