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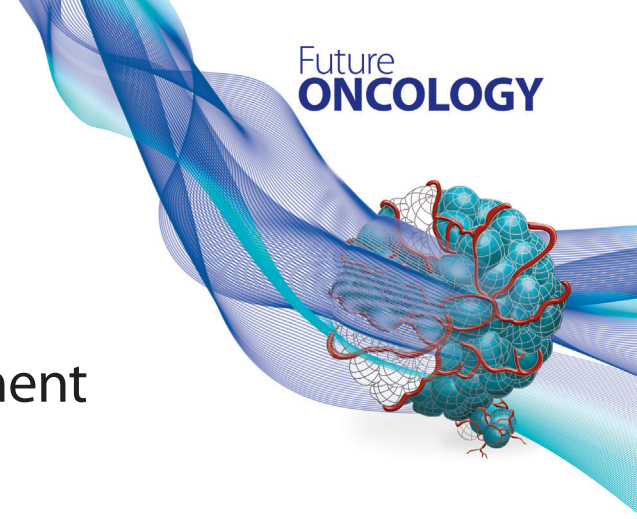

Exploiting molecular biology for diagnosis and targeted management of pediatric low-grade gliomas

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The majority of brain tumors arising in children are low-grade gliomas. Although historically categorized together as pediatric low-grade gliomas (PLGGs), there is significant histologic and genetic diversity within this group. In general, prognosis for PLGGs is excellent, and limitation of sequelae from tumor and treatment is paramount. Advances in high-throughput genetic sequencing and gene expression profiling are fundamentally changing the way PLGGs are classified and managed. Here, we review the histologic subtypes and highlight how recent advances in elucidating the molecular pathogenesis of these tumors have refined diagnosis and prognostication. Additionally, we discuss how characterizing specific genetic alterations has paved the way for the rational use of targeted therapies that are currently in various phase clinical trials.

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KEYWORDS
• pediatric low-grade glioma • pediatrics • targeted therapy

Pediatric low-grade glioma (PLGG) represents the most common brain tumor of childhood [1]. For the majority of children with PLGG these tumors are primarily managed with surgery, with gross total resection often leading to excellent durable disease control [2]. However, tumors residing in critical locations where complete resection is not safe may pose a threat to neurologic function and survival. For instance, about three-quarters of patients with cerebral and cerebellar hemisphere tumors are able to undergo gross total resection, while less than a quarter of children with chiasmic-hypothalamic and midline tumors are able to have a complete resection. For subtotal resections, the volume of residual tumor is predictive of disease progression [2]. Furthermore, some histologic and molecular subtypes of PLGG may have a propensity to recur even after gross total resection. Radiation and cytotoxic or cytostatic chemotherapies have classically been used to improve progression-free survival rates among children with incompletely resected or recurrent tumors. With these treatments, event-free survival has been promising, up to 74% at 10 years [3]. However, concerns over long-term toxicities have led to efforts to reduce the use or dose of radiation and chemotherapy among children at lower risk for progression or at higher risk for toxicity. Conformal radiation is able to better spare normal tissue to limit toxicity [4], though children of younger age still experience significant IQ decline at 5 years after intracranial irradiation [5]. Single or polychemotherapy regimens have been used to delay or obviate the need for radiation therapy. However, chemotherapeutic agents themselves carry risks of significant long term toxicities such as peripheral neuropathies and

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secondary leukemia [6]. Fortunately, there has been substantial progress in understanding the molecular pathogenesis underlying low-grade glioma subtypes, and targeted agents are being developed and tested in clinical trials with the hope of improving progression-free survival while limiting long-term toxicity.

Histologic classifications & molecular refinements

Histologically, PLGGs are defined as WHO grade I or II, with the major histologic subtypes including low-grade astrocytomas, oligodendrogliomas and glioneuronal tumors. We will discuss these histologic subtypes here, highlighting the detection of genetic alterations that provides greater clinicopathologic specificity for children diagnosed with PLGG.

• Low-grade astrocytomas

Low-grade astrocytomas are the most common PLGG, and within this group are pilocytic astrocytoma, pilomyxoid astrocytoma, pleomorphic xanthroastrocytoma, diffuse astrocytoma and subependymal giant cell astrocytoma (SEGA). Low-grade astrocytomas can occur anywhere within the central nervous system but are most commonly found in the posterior fossa, followed by the cerebral hemispheres, midline structures, brainstem and least commonly the spinal cord [7].

Pilocytic astrocytoma, WHO grade I, is the most common of the low-grade astrocytomas. Pilocytic astrocytomas are classically well-circumscribed cystic lesions that contain an enhancing mural nodule on T1-weighted MRI. These tumors generally have an excellent prognosis and are usually not associated with recurrence after complete surgical resection. Very rarely, however, pilocytic astrocytomas may recur after complete resection, disseminate throughout the CSF or undergo malignant transformation [8], highlighting the need to better understand the genetic and molecular underpinnings of typically benign gliomas.

Histologic sections usually demonstrate a neoplastic proliferation of piloid astrocytes in an alternating loose and compact stroma, often containing Rosenthal fibers and/or eosinophilic granular bodies **(Figure 1A)**.

Overall, pilocytic astrocytomas account for approximately 35% of posterior fossa and optic pathway tumors in children [7]. A subset of pilocytic astrocytomas arise in patients with neurofibromatosis type 1 (NF-1) due to germline *NF1* mutation that is accompanied by loss of heterozygosity of the remaining wild-type *NF1* allele in the tumor. Optic pathway pilocytic astrocytomas occur more commonly among children with NF-1, with a third to a half of patients with optic pathway gliomas being present in NF-1 patients [7]. On the other hand, the vast majority of sporadic pilocytic astrocytomas arising in the posterior fossa/cerebellum of non-NF-1 patients harbor a duplication of the 3′ portion of the *BRAF* gene encoding the C-terminal kinase domain. This kinase domain is often fused in-frame with the downstream *KIAA1549* gene to produce a *KIAA1549–BRAF* fusion transcript lacking the N-terminal regulatory domain of the BRAF protein. Other less common fusion partners for the duplicated BRAF kinase domain have been described including *FAM131B*, *RNF130*, *CLCN6* and *GNAI1* [9]. Outside of the posterior fossa *BRAF* duplication and gene fusion is less common, being found in approximately half of cases centered in the diencephalon and cerebral hemispheres. Pilocytic astrocytomas lacking *BRAF* duplication and gene fusion occasionally harbor the $BRAF^{V600E}$ activating missense mutation (6%), somatic mutations in *NF1* (3%) or *PTPN11* (2%), activating mutations within the kinase domain of *FGFR1* (6%) and gene fusions involving *NTRK2* (3%). Indeed, it appears that virtually all pilocytic astrocytomas harbor genetic alterations that activate the Ras–Raf–MEK–ERK signaling pathway [9]. *BRAF* mutations and the resultant signaling aberrations will be discussed in greater detail below.

Pilomyxoid astrocytoma, WHO grade II, is recognized as a distinct variant of pilocytic astrocytoma with unique histological features and a more aggressive clinical course [10,11]. Similar to pilocytic astrocytoma, pilomyxoid astrocytomas are often solid and cystic in appearance radiographically, but often demonstrate a more prominent solid component [12]. Histologically, pilomyxoid astrocytomas are composed of a neoplastic proliferation of piloid astrocytes in a myxoid stroma and show prominent perivascular arrangement of the tumor cells, while typically lacking Rosenthal fibers and eosinophilic granular bodies [10]. The same *KIAA1549–BRAF* fusion found in pilocytic astrocytomas has also been identified in a subset of pilomyxoid astrocytomas, albeit less commonly, suggesting that this might be a histologic variant of pilocytic astrocytoma rather than a distinct entity [13].

Pleomorphic xanthoastrocytoma, WHO grade II, is a rare but distinct glial neoplasm with a predilection for arising in the superficial areas of the cerebral cortex, particularly in the temporal lobe. Histologic sections typically show a solid, noninfiltrative glial neoplasm composed of markedly pleomorphic astrocytes with occasional cells containing lipidized cytoplasm along with scattered eosinophilic granular bodies. Genetic analysis has shown that *BRAF*^{V600E} mutations are very common in these tumors, as are *CDKN2A* homozygous deletions [13–15]. Radiographically, pleomorphic xanthoastrocytomas are classically peripherally located, well-circumscribed and partially cystic neoplasms, often with enhancing mural nodules [16].

Diffuse astrocytomas, WHO grade II, are infiltrative glial neoplasms in contradistinction to the solid/circumscribed glial tumors described above. Histologically, these tumors are characterized by infiltrating neoplastic astrocytes with elongated and hyperchromatic nuclei with fibrillary glial processes **(Figure 1B)**. Mitotic figures are rare to absent, otherwise a diagnosis of anaplastic astrocytoma (WHO grade III) may be warranted. Diffuse astrocytomas in children appear similar in radiographic appearance to their counterpart diffuse astrocytomas in adult patients, being infiltrative hyperintense lesions on T2/FLAIR MRI. Pediatric-type diffuse astrocytomas are genetically distinct from diffuse astrocytomas arising in adult patients [17], though older teenagers with diffuse astrocytomas arising within the cerebral hemispheres may sometimes have genetic alterations similar to those found in adults [18]. The vast majority of grade II and III infiltrative gliomas arising in adult patients harbor an *IDH1* or *IDH2* mutation (most commonly the R132H substitution in *IDH1*), thought to be an early transforming event during gliomagenesis. However, *IDH* mutations are rare in pediatric low-grade astrocytomas, highlighting one of the distinct differences in pathobiology between these adult and pediatric gliomas [13,19].

Recent genetic analyses of diffuse astrocytomas arising in the cerebral hemispheres of pediatric patients have found rearrangement of *MYB* or *MYBL1* genes, *BRAF*^{V600E} mutation, and *FGFR1* alterations including missense mutations, duplications of the kinase domain, and gene fusions. *MYB* and *MYBL1* encode transcriptional activator proteins, and the rearrangements of these genes in pediatric gliomas typically lead to truncation of their C-terminal negative regulatory domains causing constitutive activation and altered gene transcription [13,20]. The rearrangements of *MYB* and *MYBL1* genes have only been found in PLGGs within the cerebral hemispheres and have not been found in pediatric high-grade gliomas [13].

As compared with infiltrative gliomas arising in the cerebral hemispheres, those arising within midline structures (e.g., thalamus, pons and spinal cord) frequently harbor missense mutations at codon 27 in either of the *H3F3A* or *HIST1H3B* genes, encoding the histone H3 variants, H3.3 and H3.1, respectively [21–26]. These missense mutations cause a lysine to methionine substitution, altering a critical site of post-translational modification within these histone H3 variants that leads to altered gene-expression profiles thought to contribute to tumorigenesis [27,28]. A mutant-specific antibody for histone H3-K27M mutant protein has been developed for immunohistochemical use and is now routinely used in surgical neuropathology for the identification of the diffuse midline gliomas with this important molecular alteration [29–31].

Though only 2% of PLGG as a whole harbor the histone H3-K27M mutation [13], this alteration occurs in a significant subset of low-grade and high-grade diffuse gliomas arising in midline structures where it has significant prognostic implications. In a recent study of diffuse midline gliomas, seven of the nine (78%) pediatric cases that displayed low-grade histologic features at time of initial biopsy were found to have the histone H3-K27M mutation [31]. These seven cases included five diffuse intrinsic pontine gliomas, one thalamic glioma and one third ventricular glioma. The patient with thalamic glioma had a subsequent biopsy six months later that demonstrated high-grade histologic features and was classified as WHO grade III. The patient with third ventricular glioma had a subsequent resection 1 month later that demonstrated high-grade histologic features of glioblastoma and was classified as WHO grade IV. Among the five patients with K27M+ pontine gliomas that displayed only low-grade histologic features, all experienced disease course typical of diffuse intrinsic pontine glioma (i.e., progression and death) despite aggressive therapeutic regimens including radiation, chemotherapy and various targeted small molecule therapies. In at least some of these cases, it is

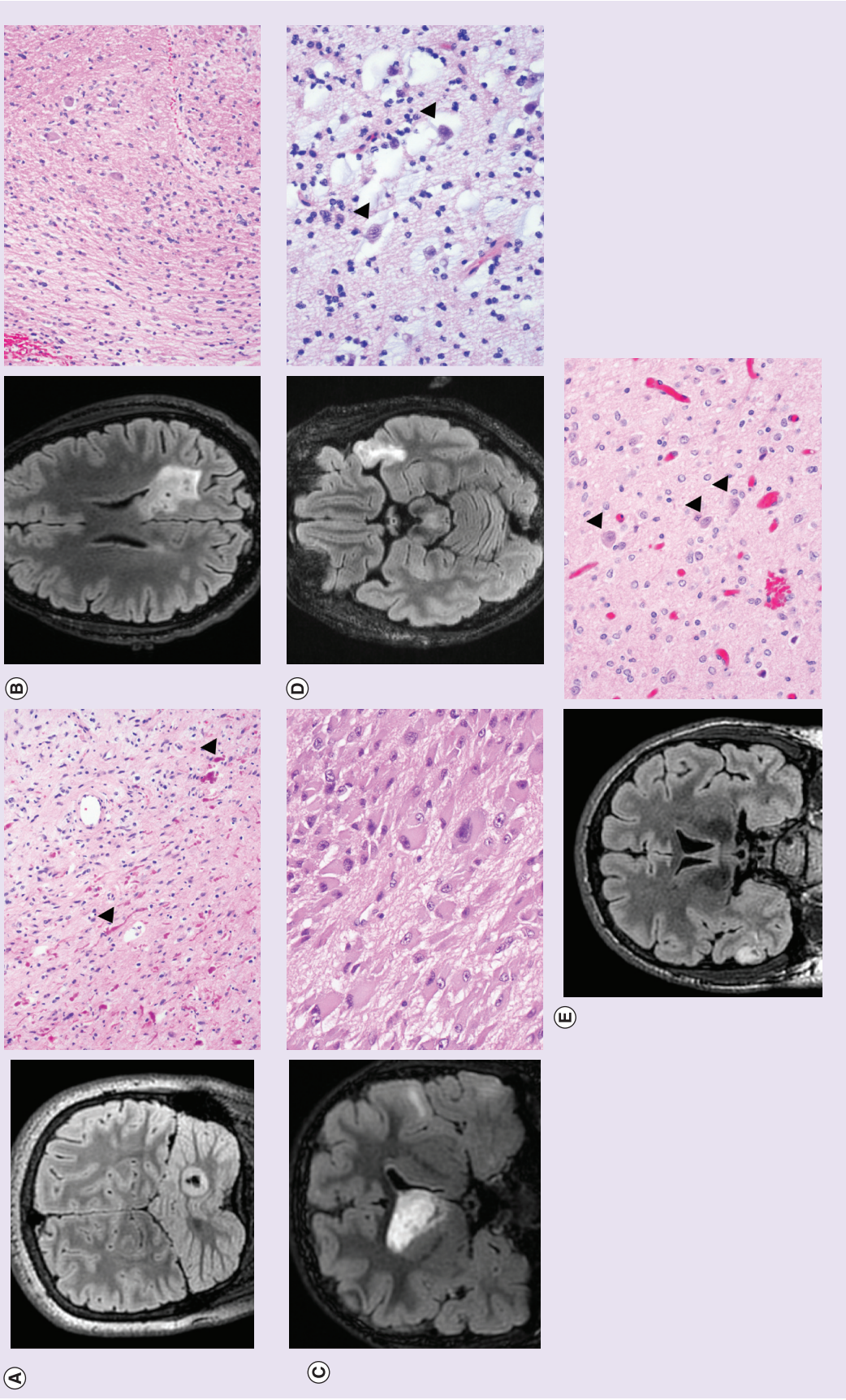

Figure 1. Representative imaging and histology from pediatric low-grade gliomas. (A) Pilocytic astrocytoma, WHO grade I, with *KIAA1549–BRAF* gene fusion. Coronal T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI (left) demonstrating a solid and cystic lesion centered in the cerebellar hemisphere. Hematoxylin and eosin (H&E) stained section (right) demonstrating a compact area of the neoplasm composed of piloid astrocytes containing numerous Rosenthal fibers (examples of which are highlighted by black arrowheads). Molecular analysis (not shown) demonstrated duplication of the 3′ portion of the *BRAF* gene along with fusion to the *KIAA1549* locus. **(B)** Diffuse astrocytoma, WHO grade II, with *MYB* gene rearrangement. Axial T2-weighted FLAIR MRI (left) demonstrating an infiltrative hyperintense lesion within the deep white matter of the parietal lobe and infiltrating into the splenium of the corpus callosum. H&E stained section (right) demonstrating infiltrating neoplastic astrocytes with elongate nuclei and coarse chromatin in a background of densely fibrillar glial processes. FISH (not shown) demonstrated rearrangement of the *MYB* gene in this tumor.

Figure 1. Representative imaging and histology from pediatric low-grade gliomas (cont.). **(C)** Subependymal giant cell astrocytoma, WHO grade I, in a tuberous sclerosis patient. Coronal T2-weighted FLAIR MRI (left) demonstrating an intraventricular mass lesion arising from the caudate nucleus. Also seen are multiple regions of FLAIR-hyperintensity within cerebral cortex and subcortical white matter consistent with cortical tubers. H&E stained section (right) demonstrating a solid neoplasm composed of large epithelioid astrocytes with abundant eosinophilic cytoplasm within a densely fibrillar background. **(D)** Dysembryoplastic neuroepithelial tumor, WHO grade I. Axial T2-weighted FLAIR MRI (left) demonstrating a well circumscribed, cortically based mass lesion in the temporal lobe with internal nodularity. H&E stained section (right) demonstrating a neoplasm of round oligodendrocyte-like cells arranged in linear columns along capillaries and neuronal processes within a mucin-rich stroma containing 'floating' neurons (examples of which are highlighted by black arrowheads). **(E)** Ganglioglioma, WHO grade I, with *BRAF*^{V600E} mutation. Coronal T2-weighted FLAIR MRI (left) demonstrating a well circumscribed, cortically based solid and cystic mass lesion in the temporal lobe. H&E stained section (right) demonstrating a biphasic tumor composed of neoplastic glial cells admixed with large dysmorphic ganglion cells (highlighted by black arrowheads). Molecular testing (not shown) demonstrated the presence of *BRAF*^{V600E} mutation in this tumor.

hypothesized that the small nature of the biopsy from these tumors, that are located in deep or critical structures within the midline, failed to capture tissue within the tumor containing high-grade histologic features. Alternatively, these biopsies may have occurred early within the course of tumor progression before development of any high-grade histologic features. With the exception of a couple rare case reports of children with K27M+ gliomas with indolent behavior [32,33], the vast majority of K27M+ gliomas in children have aggressive clinical course regardless of the grade of histologic features observed in biopsy specimens. Multiple large studies have corroborated this finding [34,35]. This is in contrast to K27M+ gliomas located in the thalamus of adult patients, where histone H3 status does not uniformly appear to portend worse prognosis [26,32].

Finally, SEGA, WHO grade I, is seen as a solidly enhancing mass within the lateral ventricles without invasion of adjacent brain parenchyma on T1-weighted MRI sequence. Hematoxylin and eosin stain shows these solid neoplasms to be composed of large epithelioid astrocytes containing abundant eosinophilic cytoplasm accompanied by a densely fibrillar background **(Figure 1C)**. SEGA is virtually always associated with the genetic syndrome tuberous sclerosis, resulting from germline mutations in *TSC1* or *TSC2*, and up to 20% of children with tuberous sclerosis develop SEGAs [36].

• Low-grade oligodendrogliomas

Pediatric oligodendrogliomas are infiltrative tumors that may be low-grade (WHO grade II) or anaplastic (WHO grade III). Histologically and radiographically, pediatric oligodendrogliomas are quite similar to the adult type. A recent case series of 50 pediatric oligodendrogliomas reported histologic features of uniform round cells with perinuclear halos and secondary structures such as perineuronal satellitosis in all cases, with a subset of tumors containing calcifications and/or microcysts. However, the genetic profile of pediatric oligodendroglioma appears to be distinct from those oligodendrogliomas arising in adult patients. Whereas virtually all oligodendrogliomas in adult patients have mutation of the *IDH1* or *IDH2* genes, codeletion of chromosomes 1p and 19q, TERT promoter mutation and mutations in *CIC* or *FUBP1*, these alterations are uncommon in their pediatric counterpart, being only present in tumors arising in

older teenagers [37]. Whole-genome sequencing has found a duplication of the 3′ portion of the *FGFR1* gene encoding the intracellular kinase domain portion of the protein in three of five pediatric oligodendrogliomas [13]. Other smaller series corroborate the lack of 1p19q codeletion in pediatric-type oligodendroglioma, with the presence of 1p19q codeletion occurring only in the 'adult-type' usually in older teenagers and young adults [13,38–40]. In contrast to the adult-type, the prognostication by molecular subtype of pediatric oligodendroglioma has been quite difficult given the rarity of the tumor and small patient series present in the literature [37].

• Glioneuronal tumors

Glioneuronal tumors, WHO grade I, are mixed tumors composed of both neoplastic glial and neuronal components. They most commonly arise within the cerebral hemispheres, usually within the temporal lobes, and also have predilection for the cervicomedullary junction. Subtypes of glioneuronal tumors include dysembryoplastic neuroepithelial tumor (DNT), ganglioglioma and desmoplastic infantile ganglioglioma (DIG).

Histologically, DNT is composed of round oligodendrocyte-like cells arranged in linear columns along neuronal processes and capillaries, surrounded by mucin-rich stroma with 'floating' neurons. **(Figure 1D)**. The *BRAF*^{V600E} mutation is present in 15–51% of DNT [41], and *FGFR1* alterations are present in 58–82% [14,42]. Radiographically these tumors usually are not associated with mass effect or peritumoral edema. DNT tumors have low or no gadolinium enhancement, and they appear bright on T2-weighted imaging [43].

Ganglioglioma is a biphasic tumor made up of large dysmorphic ganglion cells admixed with neoplastic astrocytes **(Figure 1E)**. The frequency of the *BRAF*^{V600E} mutation ranges between 18 and 58% among low-grade glioneuronal tumor series [15,44–45]. On MRI, ganglioglioma tumors may be of low intensity on T1-weighted images and hyperintense on T2-weighed images. However, imaging characteristics are largely nonspecific to gangliogliomas, and diagnosis is usually established by histology.

Other neuroepithelial tumors

Other very rare glial neoplasms that may occasionally arise in pediatric patients include astroblastoma and angiocentric glioma. Astroblastomas typically occur in children and adolescents and are usually located within the cerebral hemispheres. They are solid, noninfiltrative neoplasms characterized by glial cells radially arranged vessels with extensive vascular sclerosis and lacking the perivascular fibrillarity seen in ependymomas. The genetic alterations that drive astroblastomas are unknown. Given the rarity of this entity, the biologic behaviors of these tumors are not well understood, and astroblastomas were thus not assigned a grade in the 2007 WHO Classification.

Angiocentric glioma, a WHO grade I tumor, mainly occurs in children and young adults (mean age at diagnosis is 17 years) [46]. Angiocentric glioma was first reported in 2005 [47] and recognized as a distinct clinicopathologic entity in the 2007 WHO classification [46]. Angiocentric glioma is a stable or slow growing cerebral pediatric tumor for which surgical resection is generally curative. Angiocentric gliomas are epileptogenic lesions; most patients have a several year history of presurgical epilepsy. Histologically, this tumor is characterized by an angiocentric pattern of growth, monomorphous bipolar cells, and features of ependymal differentiation. Superficial cerebrocortical location is typical, and on MR imaging angiocentric gliomas are well-delineated solid, T2-hyperintense, nonenhancing cortical lesions that usually extend into the subcortical white matter. There is usually focal enlargement of the affected cortical gyrus, and calcifications are rare. Recently, the *MYB–QKI* gene fusion was found to be a specific genetic alteration in angiocentric gliomas and was demonstrated to be the single genetic driver of these rare glial tumors [48].

Genetic mutations & cellular signaling aberrations

Though histomorphology has historically guided the diagnosis of PLGG subtypes, integration of histopathology with emerging genomic data is helping to refine PLGG subtypes to provide meaningful prognostic information. Some of the first insights into the molecular pathobiology came from the genetic syndromes NF-1 and tuberous sclerosis. We have come to understand that PLGGs are genetically distinct from low-grade gliomas in adult patients, particularly the infiltrative gliomas. Thus we will focus our discussion here on some of the most important genetic alterations and signaling pathway alterations in the pediatric-type low-grade gliomas. The

strong association between the genetic syndromes NF-1 and tuberous sclerosis with PLGG, as well as the fact that early insights into the molecular pathogenesis of PLGG came from understanding these syndromes, merit a discussion of neurofibromatosis and tuberous sclerosis.

• NF-1 & Ras–Raf–MAPK pathway

NF-1 is inherited as an autosomal dominant syndrome, characterized by the development of neurofibromas and astrocytomas. The association between low-grade gliomas and NF-1 is strong, with up to 15% of children with NF-1 developing a PLGG before adulthood; the most common being pilocytic astrocytomas and diffuse astrocytomas [49]. The NF-1 syndrome results from mutation of *NF1*, a tumor suppressor gene residing on chromosome 17q. The majority of *NF1* mutations result in protein truncation, causing disruption of its functional domain, Ras–GAP related-domain (Ras–GRD). Ras–GRD accelerates the conversion of the active GTP-bound Ras into its inactive GDP-bound form, downregulating the Raf and PI3K transduction pathways **(Figure 2)**. Truncation of NF-1 and disruption of Ras–GRD results in dysregulation of the Raf and PI3K pathways and promotion of cellular proliferation [50,51].

Indeed, dysregulation of the Ras–Raf–MAP kinase pathway plays an important role in the molecular pathogenesis of PLGG. Within the Ras-Raf-MAP kinase pathway, Raf regulates the MEK/MAP kinase cascade, itself a regulator of cellular differentiation and proliferation **(Figure 2)** [1,17]. Notably, there has been much attention over the last decade on a specific member of the Raf family, *BRAF*, that is now recognized as one of the most commonly mutated genes in both pediatric and adult cancers [1,43,52]. There have been two major *BRAF* genomic alterations characterized in PLGG, the $BRAF^{V600E}$ missense mutation and *BRAF* gene duplication/fusions.

The $BRAF^{V600E}$ mutation results from replacement of valine by glutamic acid within the activation loop of the enzyme. This substitution mimics phosphorylation of the active cite causing constitutive activation of BRAF kinase domain [43], thus leading to dis-inhibition of the MEK/MAP kinase cascade **(Figure 2)**. The $BRAF^{V600E}$ mutation is sufficient for NIH3T3 fibroblast transformation *in vitro* [53]. Interestingly, $BRAF^{V600E}$ also promotes proliferative transformation of human neural stem cells followed by senescence, and it has been hypothesized that this 'oncogene-induced senescence' may be one underlying mechanism for the low-grade pathogenesis of pilocytic astrocytomas [53,54]. In the whole-genome sequencing study by Zhang and colleagues, $BRAF^{V600E}$ mutations were detected in 70% of pleomorphic xanthoastrocytomas, 23% of diffuse astrocytomas, 33% of gangliogliomas and 6% of pilocytic astrocytomas [13].

In addition to the $BRAF^{V600E}$ missense mutation, genetic duplication/fusion mutations are common in PLGG. The gain of chromosomal region 7q34, that contains the *BRAF* locus, is the most common copy number alteration in sporadic (non-NF-1 related) PLGG. Tandem insertion of this locus is frequently at the *KIAA1549* gene [43,55–56], and the *KIAA1549–BRAF* fusion gene codes for a BRAF protein that lacks its auto-inhibitory domain and is thus constitutively active [57]. Over 90% of pilocytic astrocytomas arising in the cerebellum in children without NF-1 have *KIAA1549–BRAF* gene fusions, whereas approximately half of pilocytic astrocytomas arising outside the cerebellum have the *KIAA1549–BRAF* fusion [9,13]. The other *BRAF* fusion transcripts that have been characterized, including *GNA11*, *MACF1*, *MKRN1*, *CLCN6*, *SRGAP3*, *FAM131B* and *RNF130*, all result in loss of the N-terminal inhibitory domain of BRAF [9,13,58–61], resulting in constitutive activation of the BRAF kinase domain and dysregulation of the downstream MAPK signaling pathway [43].

• Tuberous sclerosis & the mTOR pathway

Tuberous sclerosis results from germline mutations in either of the genes hamartin (*TSC1*) or tuberin (*TSC2*) [62–64], and SEGA is strongly associated with tuberous sclerosis. Both TSC1 and TSC2 function together as a tumor suppressor protein complex within the mTOR signaling pathway. The TSC1–TSC2 complex converts active GTP-bound Rheb into its inactive GDP-bound form **(Figure 2)** [62,65]. Mutations in *TSC1* or *TSC2* can result in loss of function of the protein complex, resulting in unopposed activation of Rheb-GTP. This disinhibited activation of the mTOR signaling cascade promotes the development of hamartomatous lesions and helps drive the tumorigenesis of SEGAs [62,64].

Sporadic mutations within the mTOR signaling pathway in children without tuberous sclerosis have also been shown to be important in the pathogenesis in PLGG. The PI3K–Akt–mTOR

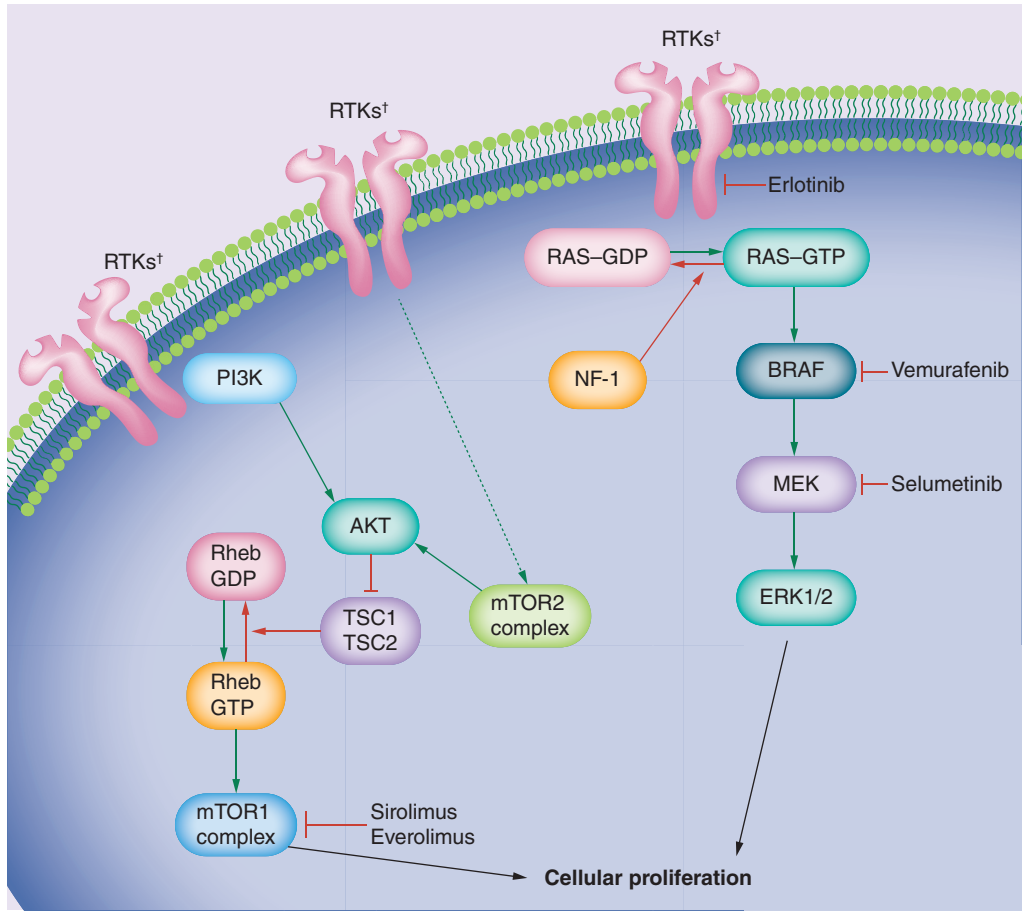

Figure 2. BRAF and mTOR signaling pathways shown with targeted therapies. Green arrows represent activating steps that ultimately lead to cellular proliferation. The dashed green arrow represents indirect activation. Red arrows represent de-activating steps that ultimately inhibit cellular proliferation. Blind-ended arrows represent inhibitory interactions.
†RTKs include EGFR, PDGFRA, NTRK2 and FGFR1.
RTK: Receptor tyrosine kinase.

signaling pathway normally integrates both extracellular and intracellular signals to integrate cellular metabolism, proliferation and survival [66]. mTOR is a multiprotein serine–threonine kinase, that itself is composed of two protein complexes, mTORC1 and mTORC2.

In high nutritional states, mTOR undergoes a conformational change that facilitates mTORC1 activation by Rheb. Activated mTORC1 then activates p70S6 kinase that results in formation of phospho-S6 and phospho-4EBP1, driving translation and cellular proliferation **(Figure 2)**. Approximately half of PLGG show enhanced expression of phospho-S6 and phospho-EBP1 [1], and expression of these two proteins is associated with worse progression-free survival [67].

Like mTORC1, the mTORC2 component also responds to cellular nutritional status as well as redox states. As a regulator of cellular proliferation, mTORC2 activates Akt. Akt is a serine–threonine kinase that plays a critical role in cellular metabolism and proliferation, and Akt has been implicated in numerous human cancers. Akt phosphorylation is associated with a more clinically aggressive pilocytic astrocytoma [68].

Both the Ras–Raf–MAPK and mTOR pathways are affected by alterations of the *FGFR1* gene that encodes the transmembrane receptor tyrosine kinase FGFR1. A variety of *FGFR1* alterations have been found in PLGG including somatic missense mutations, duplication of the 3′ portion of the gene encoding the kinase domain and rearrangement usually involving fusion with *TACC* genes. These alterations in *FGFR1* lead to its constitutive activation of downstream signaling

pathways including both Ras–Raf–MEK–ERK and PI3K–Akt–mTOR [13].

• Targeted systemic agents

Elucidation of oncogenic mutations within the Ras–Raf–MAPK and PI3K–AKT–mTOR pathways has led to the development of agents that specifically target oncogenic proteins within these pathways for the treatment of pediatric gliomas. As described above, the *BRAF*^{V600E} mutation is prevalent among pleomorphic xanthoastrocytomas, diffuse astrocytomas, gangliogliomas and pilocytic astrocytomas. The enzyme inhibitor vemurafenib specifically inhibits *BRAF*^{V600E} from activating MEK, and has been shown to have strong clinical activity in *BRAF*^{V600E}-positive melanoma. The clinical success in melanoma has led to great interest in using vemurafenib in other *BRAF*^{V600E}-positive cancers. A multicenter trial under the auspices of the Pacific Pediatric Neuro-Oncology Consortium (PNOC002) is enrolling children with recurrent or refractory *BRAF*^{V600E} gliomas to evaluate the safety and pharmacokinetic characteristics of vemurafenib. Dabrafenib is a selective ATP-competitive inhibitor of the *BRAF*^{V600E} kinase, approved in unresectable or metastatic melanoma with the *BRAF*^{V600E} mutation. NCT01677741 is currently enrolling children with *BRAF*^{V600E}-positive relapsed or refractory solid tumors, including high-grade and low-grade gliomas **(Table 1)**. Preliminary results of NCT01677741 demonstrating good tolerability and manageable toxicity were recently presented [69].

Enthusiasm over targeted agents in general should be met with some degree of caution. A Phase II trial of sorafenib, a multikinase inhibitor targeting BRAF, VEGFR, PDGFR and c-kit, was terminated early because of a rapid and unexpectedly high rate of progression in children with PLGGs [70]. *In vitro* studies suggest this finding may be due to paradoxical activation of ERK by sorafenib. A proportion of *BRAF* mutated tumors have *BRAF* mutations other than the V600E missense mutation, including alternative missense mutations, duplications, fusions and deletions that have been shown to decrease the efficacy of *BRAF*^{V600E}-targeted inhibition. For instance, in cells expressing KIAA1549–BRAF, these fusion kinases function as homodimers that are resistant to PLX4720 (a research analog of vemurafenib) and PLX4720 leads to paradoxical activation of MEK and ERK [71].

However, some tumors harboring BRAF alteration do have sensitivity to MEK inhibition [72]. Trametinib is a MEK inhibitor shown to have clinical activity against melanoma, colorectal, hepatocellular and non-small-cell lung cancers. Selumetinib (AZD6244), another MEK inhibitor, has been shown to have activity against a pilocytic astrocytoma xenograft harboring the *BRAF*^{V600E} mutation [73]. A Phase I study of AZD6244 by the Pediatric Brain Tumor Consortium (PBTC-029B) has been completed [74], and a Phase II study is currently underway **(Table 1)**. Furthermore, the maximal tolerated dose of selumetinib has been evaluated in children with histologically confirmed recurrent or refractory PLGG under the auspices of the Pediatric Brain Tumor Consortium. In addition, the National Cancer Institute is sponsoring a Phase II trial of selumetinib for children with recurrent or refractory PLGGs **(Table 1)**.

There has also been progress in targeting the mTOR pathway. Sirolimus is an allosteric inhibitor of mTORC1, and the binding of mTORC1 with sirolimus interferes with mTORC1 activation of S6 kinase, itself a regulator of translation. A clinical response to sirolimus in a tuberous sclerosis child with SEGA harboring a *TSC2* gene mutation was first reported in 2008 [75]. Everolimus is a derivative of sirolimus and has been used for multiple cancer types in adults [76]. The efficacy of sirolimus was first reported in SEGA in 2006 [77]. Among children with tuberous sclerosis and progressive SEGA tumors, 75% of tumors respond to everolimus [78] and everolimus is now US FDA approved for the treatment of SEGA in children with tuberous sclerosis. Kieran and colleagues reported the results of a prospective Phase II study of everolimus for children with recurrent PLGG after initial treatment with carboplatin-containing chemotherapy regimens. Of the 23 children enrolled, four patients had a partial response (greater than 50% decrease on MRI), 13 had stable disease and six children had progressive disease within 1 year. This trial met the goal of greater than 25% response rate defined *a priori* for everolimus to be considered a promising regimen for further study [79].

Sirolimus has also been evaluated in combination with erlotinib, an EGF receptor inhibitor. In this feasibility and efficacy study, 19 children with recurrent PLGG received the two-drug regimen. Of these children there was one partial response, five were stable and ten had progressive

Table 1. Clinical trials for pediatric low-grade gliomas.

Trial Identifier	Title	Sponsor
NCT01748149 (enrolling)	PNOC002: Safety, Phase 0, and Pilot Efficacy Study of Vemurafenib, an Oral Inhibitor of *BRAF*V600E, in Children With Recurrent/Refractory *BRAF*V600E-mutant Gliomas	Pacific Pediatric Neuro-Oncology Consortium
NCT01089101 (enrolling)	A Phase 1 and Phase II and Retreatment Study of AZD6244 (PBTC-029B) for Recurrent or Refractory Pediatric Low Grade Glioma	Pediatric Brain Tumor Consortium/National Cancer Institute
NCT01734512 (enrolling)	PNOC 001: Phase II Study of Everolimus for Recurrent or Progressive Low-grade Gliomas in Children	Pacific Pediatric Neuro-Oncology Consortium
NCT01158651 (ongoing, enrollment complete)	A Phase II Study of RAD001 (Everolimus) for Children With NeurF1 and Chemotherapy-Refractory Radiographic Progressive Low Grade Gliomas	Pediatric Oncology Experimental Therapeutics Investigators' Consortium
NCT01677741 (enrolling)	A Study to Determine Safety, Tolerability and Pharmacokinetics of Oral Dabrafenib In Children and Adolescent Subjects	GlaxoSmithKline
NCT00901849 (completed)	Phase 1 Study of Tarceva and Rapamycin For Recurrent Low-Grade Gliomas in Children With or Without Neurofibromatosis Type 1 (NF1)	Children's Research Institute

disease during the planned 1 year of therapy, and three children discontinued therapy due to toxicity or compliance issues. There was tumor stabilization for at least 12 months in six children, and two children experienced tumor control for over 1 year after therapy completion [80].

Kaul and colleagues recently showed that the KIAA1549–BRAF fusion is sufficient to induce glioma-like lesions *in vivo* in a cell type-specific and mTOR-dependent manner, and mTOR inhibition blocks KIAA1549–BRAF fusion induced S6 activation and proliferation in neural stem cells. These data also provide preclinical evidence for use of mTOR inhibitors for sporadic PLGGs [81]. Overall, there is strong biologic evidence supporting the notion that molecular markers will define PLGG subgroups most likely to respond to mTOR inhibition, and clinical evidence is still being gathered. A PNOC Phase II study of everolimus is enrolling children with recurrent or progressive PLGGs with the aim of seeking a molecular signature that predicts responses to mTOR inhibition **(Table 1)**.

Though not directly compared in clinical trial, BRAF, MEK and mTOR inhibitors appear to have favorable toxicity profiles compared with the chemotherapies commonly used for progressive or recurrent PLGGs. Some of the major toxicity profiles of chemotherapy, including neurotoxicity (vincristine), hypersensitivity (carboplatin), cytopenia (temozolomide, vinblastine, platinum agents), infertility (alkylating agents) and secondary malignancies [82], appear distinct from the targeted agents. In a Phase II trial of everolimus, two patients had to discontinue treatment due to oral sores [79]. Children who received erlotinib combined with sirolimus on a Phase I–II study most commonly experienced grade 1–2 rash (58%), oral apthous ulcers (47%) and diarrhea (37%). No children required removal from this trial due to toxicity [80]. In a Phase I study of dabrafenib, among 29 patients, one child had a dose limiting grade 3 maculopapular rash. The serious adverse events reported as related to dabrafenib included hypotension (one patient), disseminated intravascular coagulation (one patient), fever (one patient) and arthralgia (one patient) [69]. In a Phase I study of the MEK inhibitor, selumetinib, the most common toxicity was rash. Dose-limiting toxicities were headache, rash, mucositis and elevation of amylase and lipase [74].

Conclusion & future perspective

The histologic subtypes of PLGGs are diverse, and the work in high-throughput genetic sequencing and gene expression profiling is adding both clarity and complexity to the way these childhood tumors are being diagnosed, prognosticated and treated. Fortunately, PLGG have excellent prognoses in general, though tumors progressive or recurrent after surgical resection pose significant challenges in management.

Going forward, it will continue to be a fine balance between the benefits of aggressive treatment with minimization of long-term toxicities. Targeted agents, including those that act within the Ras–Raf–MAPK and PI3K–AKT–mTOR pathways, may provide durable disease control for tumors at risk for progression. By augmenting and perhaps replacing chemotherapies and radiotherapy, which both have considerable toxicities, molecularly targeted agents will hopefully transform progressive and incurable PLGG into a chronic manageable disease. The results of current and future clinical trials will be met with anticipation.

EXECUTIVE SUMMARY

Introduction & histologic classifications & molecular refinements

- Pediatric low-grade gliomas (PLGG) are defined as WHO grade I or II. Major histologic subtypes include low-grade astrocytomas, oligodendrogliomas and glioneuronal tumors.
- Prognosis of PLGG is generally excellent, though long-term sequelae from tumor, surgery or cytotoxic therapies can be significant.

Low-grade astrocytomas

- Pilocytic astrocytomas, well circumscribed cystic tumors that can be cured surgically, are the most common low-grade astrocytoma.
- Sporadic pilocytic astrocytomas arising in the posterior fossa harbor *BRAF* duplication and gene fusion.
- Outside the posterior fossa sporadic pilocytic astrocytomas lacking *BRAF* duplication and gene fusion may harbor the *BRAF*^{V600E} missense mutation, *NF1* or *PTPN11* somatic mutations, activating *FGFR1* mutations and gene fusions involving *NTRK2*.
- Virtually all pilocytic astrocytomas harbor genetic alterations that activate the Ras–Raf–MEK–ERK signaling pathway.
- Diffuse astrocytomas are infiltrative gliomas. Pediatric-type diffuse astrocytomas are genetically distinct from the adult-type, lacking *IDH* mutations.
- Immunohistochemistry for histone H3-K27M mutant protein plays an important role in the diagnosis of midline diffuse gliomas, as this mutation is associated with a poor prognosis.

Low-grade oligodendrogliomas

- Pediatric oligodendrogliomas are infiltrative tumors that are histologically and radiographically similar to the adult type.
- The genetic profile of pediatric oligodendroglioma is distinct from the adult type.
- *IDH1*/*IDH2* mutations, codeletion of chromosomes 1p and 19q, TERT promoter mutation and mutations in *CIC* or *FUBP1* are rare in children.

Glioneuronal tumors

- Up to 60% of gangliogliomas tumors harbor the *BRAF*^{V600E} mutation.

Genetic mutations & cellular signaling aberrations

- Integrating histopathology with genomic data is helping to refine pediatric-type low-grade glioma subtypes.

Neurofibromatosis 1 & Ras–Raf–MAPK pathway

- Neurofibromatosis 1 (NF-1) is an inherited autosomal dominant syndrome. Up to 15% of NF-1 children develop PLGG.
- Majority of *NF1* mutations result in protein truncation, causing disruption in its ability to regulate the Ras–Raf–MEK–ERK signaling pathway.
- A Raf family member, *BRAF*, is one of the most commonly mutated genes in human malignancy.
- The major *BRAF* alterations known are the *BRAF*^{V600E} missense mutation and *BRAF* gene duplication/fusions. Both are implicated in the pathogenesis of PLGG.

Tuberous sclerosis & the mTOR pathway

- Tuberous sclerosis results from germline mutations in *TSC1* or *TSC2*.
- Mutations in *TSC1* or *TSC2* lead to disinhibited activation of the PI3K–Akt–mTOR signaling cascade, promoting SEGA tumorigenesis.
- mTORC2 activates Akt, which plays an important role in cell proliferation.

Targeted systemic agents

- Agents that target the Ras–Raf–MEK–ERK and PI3K–AKT–mTOR pathways are being developed and tested for use in PLGG.

EXECUTIVE SUMMARY (CONT.)

Targeted systemic agents (cont.)

- Vemurafenib specifically inhibits $BRAF^{V600E}$ from activating MEK. Vemurafenib is being evaluated in the multicenter trial PNOC002 for recurrent *$BRAF^{V600E}$*-positive PLGG.
- Everolimus is an inhibitor of mTORC1 and has resulted in clinical responses in SEGA tumors. Phase II studies are evaluating everolimus for refractory PLGG.

Conclusion & future perspective

- Genome sequencing and identification of genetic alterations in PLGG subtypes are changing the way these tumors are diagnosed, prognosticated and managed.
- In general PLGG have excellent prognoses, though progressive or recurrent tumors after resection pose significant challenges.
- Minimizing long-term sequelae of tumor and treatment is paramount.
- Characterizing genetic alterations has led to the rational use of molecular targeted therapies that are in various phase clinical trials.

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