Review

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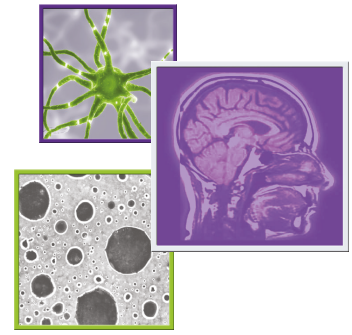

Adult pilocytic astrocytoma in the molecular era: a comprehensive review

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Adult pilocytic astrocytoma (PA) is less prevalent than pediatric PA and is associated with a worse prognosis. In a literature review, we found that 88.3% of the molecular alterations in adult PA are associated with MAPK pathway dysregulation. The most common alterations are fusions of *BRAF*. Understanding of the mechanisms underlying this pathway has evolved substantially, heralding advancements in specific targeted therapy. Here, we review clinical and molecular features of adult PA, characteristics predicting aggressive behavior and approaches to standard and investigational therapies. We highlight epigenetic profiling and integrated diagnosis as an essential component of classifying PA.

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Pilocytic astrocytoma (PA) is a WHO (Geneva, Switzerland) grade I neoplasm with a benign course following surgical resection and a 10-year survival of approximately 95% [1,2]. PA represents the most common primary CNS neoplasm in the pediatric age group (5–14 years) and 75% of the 4969 PAs diagnosed in the US from 2012 to 2016 were diagnosed before age 20. The average annual incidence rate during this timeframe was 994 patients per year. In patients aged 0–14, PA represents 17.8% of total primary brain tumors, compared with adults in whom it represents around 1.5% [3].

Molecular characterization of PA and targeted therapeutic approaches are rapidly evolving. This review details clinical, molecular and treatment paradigms in adult PA.

Clinical features

Adult PAs have a median age at diagnosis of 32.2 years with a slight female predominance [4]. PA incidence decreases with age with only 58 (1.9%) of the 3066 adult PAs in the SEER study [5] and only 3 (2.4%) of the 127 cases in an adult patient series presenting after age 60 [6]. Headache (47.5%) or seizure (13.9%) are the most common presentations, though a substantial portion are found incidentally (24.2%) [4]. In a series of 747 adults, the breakdown of neuroanatomic locations of adult PA were as follows: cerebrum/lobar 29.7%; cerebellum 26.6%; brainstem 10.2%; ventricle 9.5%; spinal cord 7.9%; optic nerve 2.5%. This is distinct from pediatric PA where cerebellar and cerebral/lobar localization occurs in 37.4 and 18.6% of cases, respectively [5].

A population study observed a cancer-specific, 60-month survival of 94–96% in pediatric patients, 92.3% in ages 20–39, 78.6% in ages 40–59 and 63.7% in ages >60 [5]. The declining survival trend with increasing age may be accounted for by comparatively higher *KIAA1549-BRAF* fusion (BK fusion) prevalence in pediatric PA, which is associated with a better prognosis. The largest single series to date of 127 adult patients demonstrated 13% mortality during a median follow-up of 31 months [6], exceeding that expected compared with pediatric PA. In a smaller, single institution cohort of 20 adults, the six that experienced symptomatic progression did so at a median of 16.5 months [7]. Resectability is highly correlated with tumor location and recurrence rate; significantly

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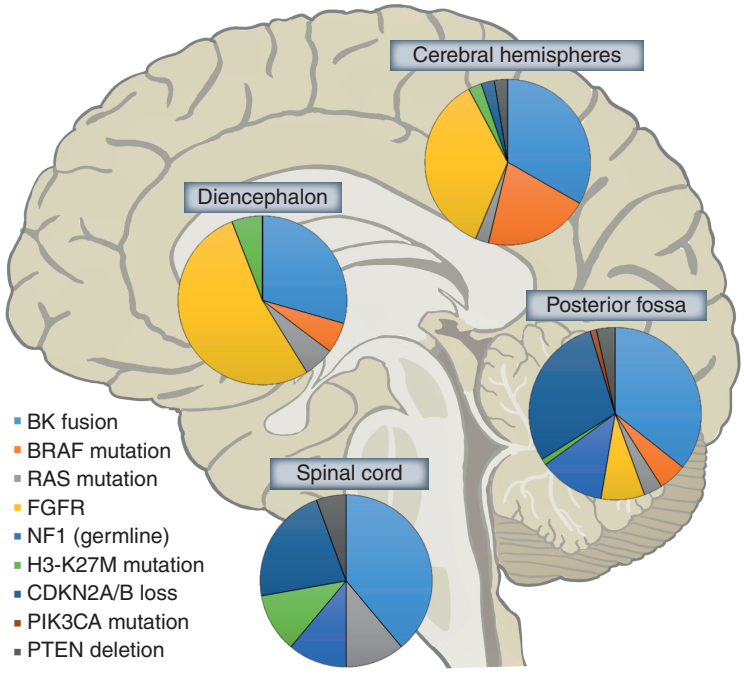

Figure 1. Summary of the relative frequency of alterations by location reported among the cases in Table 1, calculated from a total of 243 mutations in 209 adult pilocytic astrocytoma patients whose tumor location was defined.

higher recurrence rate was noted in incompletely resected (38.9%) versus totally resected (4%) tumors [8]. This contributes to worse prognosis in unresectable locations, such as the brainstem and diencephalon.

Molecular era

Activation of the canonical, mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway is a well-characterized driver of oncogenesis in multiple cancer types including melanoma and glioblastoma. Under nonpathologic conditions, MAPK/ERK signaling components are ubiquitous in the brain and are involved in various processes, such as memory formation, pain perception and neurogenesis. The influence of the MAPK/ERK pathway on neurogenesis is central in the differentiation of mesencephalon and metencephalon, which may be related to the frequent posterior fossa presentations in the young [9,10]. Across all cancer types, the most common oncogene mutations in MAPK pathway regulation affect *KRAS* followed by *BRAF* [11], and the most common oncogenic *BRAF* mutation is the valine to glutamate, or *BRAFV600E*, mutation resulting in a constitutively activated kinase [12]. PA is regarded as a single-hit disease resulting from MAPK dysfunction via a variety of mechanisms, including sporadic *BRAF* fusion in 60–70%, various single amino acid alterations in the *BRAF* gene in up to 10% [13] and inherited germline mutations of the *NF1* gene in approximately 15%, which all lead to constitutive MAPK activation [14].

We identified 273 adult PAs in the literature with a reported genetic alteration (N = 336 alterations, Table 1). 88.3% of these alterations drive the MAPK pathway. The frequency of the mutations by location is shown in Figure 1.

BRAF fusions

Focal duplication of chromosome 7q34 was reported as a frequent chromosomal abnormality in PA. The 7q34 duplication is associated with gene fusion between *KIAA1549* and the *BRAF* oncogenes (BK fusion) [43,44]. The product for all *BRAF* fusions is absence of an inhibitory N-domain leading to constitutively active BRAF kinase. The BK fusion is the most common molecular event in PA across all ages, occurring in up to 68% of cases of sporadic PA [45,46]. It is more common in pediatric PA than adults and is more frequent in infratentorial PA (54 vs 32% of supratentorial). In a retrospective series of 105 PA, the fusion was observed in 79% of ages 0–10, 51% of ages 11–20, 42% of ages 21–30, 30% of ages 31–40 and 7% of those older than 40 [17]. In a series of 45 adult PA, 20% had the BK fusion [6]. The BK fusion has been associated with an improved progression-free survival (PFS) and overall better prognosis in pediatric low grade gliomas [22,47], but the impact on adult survival could not be established in two large series [6,17]. Figure 2 shows a thoracic intramedullary, nonenhancing astrocytic neoplasm with BK fusion that

Table 1. Review of reported adult pilocytic astrocytoma genetic aberrations (n = 336).

Study/publication	Year	Genetic events	n	Comments	Ref.
Yu *et al. Neurology*	2009	BK fusion	1	Seven adults among 70 total PAs studied	[14]
Rodriguez *et al. Acta Neuropathol.*	2011	BK fusion	13	51 adults among 92 total PAs studied two patients had coexisting BK fusion and *PTEN* deletion	[15]
		PTEN deletion	5		
Cin *et al. Acta Neuropathol.*	2011	BK fusion	14	27 adults among 125 total PAs studied, noting a few novel fusions in peds cases	[16]
		BRAF mutation	2	One V600E and one ins598T	
Hasselblatt *et al. Neuropathol. Appl. Neurobiol.*	2011	BK fusion	37	37 adults among 105 total PAs studied	[17]
		NF1 mutation	1		
Schindler *et al. Acta Neuropathol.*	2011	BRAF V600E mutation	2	22 adult PAs among 1320 total nervous system tumors studied	[18]
Jones *et al. Nat. Genet.*	2013	BK fusion	5	14 adults among 104 total PAs studied	[19]
		KRAS mutation	2		
		BRAF V600E mutation	1		
		FGFR1 mutation	5		
Cykowski *et al. J. Neurooncol.*	2013	BK fusion	1	One adult among 10 total PAs studied	[20]
Yeo *et al. Clin. Neuropathol.*	2013	BRAF V600E mutation	1	Case report	[21]
Theeler *et al. Neuro Oncol.*	2014	BK fusion	9	127 total adult PAs studied Note: One KRAS mutation coexisted with BK fusion	[6]
		NF1 mutation	5		
		RAS mutation	4		
		PIK3CA mutation	1		
Becker *et al. J. Neuropathol. Exp. Neurol.*	2015	BK fusion	4	11 adults among 69 total PAs studied	[22]
Fontebasso *et al. Oncotarget*	2015	BK fusion	20	57 adults among 118 total PAs studied†	[23]
		BRAF mutation	4	Two V600E, one ins598T and one K656M/T658P	
		FGFR1 mutation	3		
Trabelsi *et al. Neurochirugie*	2015	BK fusion	1	Case report	[24]
Orillac *et al. Acta Neuropathol. Commun.*	2016	H3-K27M mutation	1	Case report	[25]
Strowd *et al. Am. J. Med. Genet.*	2016	NF1 mutation	3	Three adults among seven total PAs studied	[26]
Pathak *et al. Brain Pathol.*	2017	BK fusion	12	59 total adult PAs studied. Note: One patient had both BK fusion and FGFR-TKD, one patient had both BRAF V600E and FGFR1 mutations	[27]
		BRAF V600E mutation	1		
		FGFR1 mutation	7		
		FGFR-TKD duplication	3		
Lehtinen *et al. BMC Cancer*	2017	BK fusion	1	Two adults among four total PAs studied	[28]
Reers *et al. Clin. Neuropathol.*	2017	H3-K27M mutation	1	Case report	[29]
Ballester *et al. Mol. Case Stud.*	2018	FGFR1 mutation	1	Case report	[30]
Ishida *et al. Pathol. Int.*	2018	BK fusion	1	Case report	[31]
Kaley *et al. J. Clin. Oncol.*	2018	BRAF V600E mutation	2	Basket trial that included two PA	[32]
Liao *et al. Medicine*	2018	BK fusion	1	Case report	[33]
Morita *et al. J. Neurosurg.*	2018	H3-K27M mutation	1	Case report	[34]
Reinhart *et al. Acta Neuropathol.*	2018	BK fusion	11	102 anaplastic PA analyzed, 83 stratified to be MC AAP‡, of which 70 were adult Note: 36 cases had >1 alteration	[35]
		BRAF V600E mutation	1		
		FGFR1 mut/fus	11		
		NF1 mutation	19		
		CDKN2A/B loss	60		
		KRAS mutation	2		
Ahn *et al. J. Neuropathol. Exp. Neurol.*	2019	BK fusion	1	Case report	[36]
Olar *et al. Clin. Pathol.*	2019	BK fusion	1	Three adults among five total PAs studied	[37]
		PIK3CA mutation	1		
		PTEN deletion	3	(One patient had all three listed mutations)	

Note: Only adults identified to have genetic aberrations in each series are included here.
† Cases previously reported by Jones *et al.* 2013 [19] are excluded.
‡ Methylation class anaplastic astrocytoma with piloid features.
PA: Pilocytic astrocytoma.

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Table 1. Review of reported adult pilocytic astrocytoma genetic aberrations (n = 336) (cont.).

Study/publication	Year	Genetic events	n	Comments	Ref.
Rodriguez *et al. Brain Pathol.*	2019	BK fusion	7	25 adults among 36 total PAs studied Five adults had mutation by sequencing, an additional five adults had NF1 clinical features	[38]
		BRAF activating mutation (non V600E)	1		
		NF1 mutation	5		
		H3-K27M mutation	3		
		CDKN2A homozygous deletion	3		
Sievers *et al. Acta Neuropathol.*	2019	FGFR1 mutation	1	One adult among nine total PAs studied	[39]
Trisolini *et al. J. Neurooncol.*	2019	BRAF mutations	10	108 total adult PAs studied Three BRAF V600E, six p.Thr599_Val600insThr, one p.Val600_Lys601>Glu	[40]
		FGFR1 mutation	15		
Chiang *et al. Acta Neuropathol.*	2020	KRAS G12R	2	Two adults among 23 total PAs studied	[41]
Lucas *et al. Acta Neuropathol.*	2020	FGFR1	3	Three adults among eight total PAs studied	[42]

Note: Only adults identified to have genetic aberrations in each series are included here.
†Cases previously reported by Jones *et al.* 2013 [19] are excluded.
‡Methylation class anaplastic astrocytoma with piloid features.
PA: Pilocytic astrocytoma.

Figure 2. Spinal cord pilocytic astrocytoma with BK fusion. A 21-year old man with T2-hyperintense **(A)**, nonenhancing **(B)** intramedullary thoracic lesion determined to be a pilocytic astrocytoma based on presence of *KIAA1549*(exon12)-*BRAF*(exon9) fusion.

highlights the diagnostic utility of the fusion in cases that are difficult to classify histologically. Pediatric PA with BK fusions and other Class I and II *BRAF* mutations have both a constitutively activated MAPK pathway driving tumor growth and express markers of oncogene-induced senescence [48]. Oncogene-induced senescence may underly the unpredictable pattern of PA growth with periods of tumor growth and growth arrest. Recent characterization of mediators in the senescence-associated secretory phenotype may lead to development of predictive biomarkers and potential therapeutic targets [49]. Notably, markers of oncogene-induced senescence and the senescence-associated secretory phenotype have not been characterized in adults nor associated with the clinical outcomes in adult PA.

The list of novel *BRAF* and *RAF* gene fusions is continually expanding among PA and now includes *FAM131-BRAF*, *SRGAP3-RAF1*, *RNF130-BRAF*, *CLCN6-BRAF*, *MKRN1-BRAF* and *GNAI1-BRAF* [16,50,51], highlighting an important area for continued investigation to characterize diagnostic, prognostic and therapeutic significance. *BRAF* fusions are reported in multiple other glial neoplasms, including 50–77% of pilomyxoid astrocytoma, 18–21% of ganglioglioma [52,53], and in isolated cases of pleomorphic xanthoastrocytoma and ependymoma [54,55].

BRAF fusions are class 2 mutations and are not responsive to first-generation BRAF inhibitors (e.g. vemurafenib); however, next generation BRAF inhibitors do appear to display preclinical activity against PA with *BRAF* fusions [56], which will be discussed below. The downstream effect of losing the wild-type BRAF inhibitory domain is deregulated

MEK/ERK signaling and subsequent cell proliferation. As such, there is growing interest in utilizing MEK-inhibitors in the treatment of PA. Two cases of pediatric PA have been reported with durable symptomatic and radiographic response to trametinib, a MEK1 and MEK2 kinase inhibitor [57]. A Phase I trial of the MEK inhibitor selumetinib demonstrated a 20% sustained partial response among 38 pediatric patients with low grade glioma [58]. In association with the slow-growing nature of PAs and corresponding low Ki67 indices (generally <5%), the response to MEK inhibition has been noted to occur after months of treatment, suggesting activity confined to a small subset of proliferating cells [59].

BRAF mutations

The second most common mechanism of MAPK pathway dysregulation in PA is hotspot activation due to substitutions at position 600 in the *BRAF* gene. *BRAFV600E* mutations are found in approximately 50% of cutaneous melanomas [12,60] and is an identified driver in a subset of colorectal cancer, non-small-cell lung cancer, papillary thyroid cancer, cholangiocarcinoma, hairy cell leukemia, multiple myeloma and Langerhans cell histiocytosis [61,62]. It is found in approximately 20% of extra-cerebellar PA and is variably associated with other CNS tumors, including 60% of (pleomorphic xanthoastrocytoma) and 20% of gangliogliomas [18]. Of the 273 adult PAs with genetic aberrations reviewed here, 9.2% had *BRAF* mutations.

As a class 1 mutation, the *BRAFV600*-mutated kinase is a constitutively active monomer that activates downstream MEK independent of dimerization [63]. As such, *BRAFV600E* is a prototypical target in metastatic melanoma therapy with the widespread use of the BRAF inhibitors vemurafenib, dabrafenib and encorafenib. These are US FDA approved and improve PFS and overall survival in melanoma, though resistance to these therapies often occurs within a year [64]. Basket trials that have included high and low grade glial neoplasms have suggested a 25% response rate to vemurafenib [32]. Case reports have also noted response in *BRAFV600E* mutated anaplastic pleomorphic xanthoastrocytomas and gangliogliomas [65–67].

Resistance and toxicities, such as cutaneous skin lesions appear to be the result of paradoxical activation of the MAPK pathway via inhibitor bound BRAF recruitment to the plasma membrane, facilitating upregulated heterodimerization with CRAF and transactivation of MAPK activity [68]. Use in combination with a MEK inhibitor, such as trametinib, cobimetinib or binimetinib can improve clinical responses and prevent development of premalignant skin lesions; however, resistance still develops in most melanoma cases [69,70]. An anaplastic ganglioglioma that developed resistance to vemurafenib after 14 months of response was rescued by the addition of cobimetinib, producing a durable complete response 16 months after beginning the combination [71].

Several class 2, non-*BRAFV600* mutations have been characterized in gliomas at undefined frequencies resulting in RAS independent dimers, unresponsive to first-generation BRAF inhibitors which only target monomers [63]. Rational means of targeting such mutations is with paradox-breaking BRAF inhibitors, such as PLX8394, which provides BRAF inhibition without promotion of dimer formation [72].

NF1 & *RAS* mutations

NF1 is among the most common tumor predisposition syndromes in which neurofibromin, a negative regulator of Ras, is inactivated [73]. CNS tumors occur in up to 20% of NF1 patients, 70% of which arise along the optic pathway. Nearly all optic pathway gliomas in NF1 are PAs although clinical behavior does not significantly differ by histology. Up to two-thirds of optic pathway gliomas in children do not require therapy and some regress [26]. Higher mortality has been observed in NF1-associated gliomas when diagnosed in adulthood, occurring outside of the optic pathway or when symptomatic at discovery [26]. Up to 33% of PA with anaplasia develop in patients with a clinical diagnosis of NF1 [74], which have a more aggressive clinical course and have recurring mutations in *ATRX* leading to alternative lengthening of telomeres (ALT) [38].

MEK inhibition appears to be a potential target for tumor control in NF1 tumors, based on responses in neurofibromas to selumetinib. In 24 children with NF1 and treated with selumetinib, 71% had at least a 20% volume reduction, and none of the cases had tumor progression [75]. Selumetinib has been shown to have a down-regulating effect on VEGF and angiogenic factors [58], corresponding to anecdotal evidence that bevacizumab can be effective against NF1-related PA. The MEK inhibitor mirdametinib has also recently demonstrated plexiform neurofibroma volume reduction in 42% of adolescent and adult Phase II subjects [76].

A low frequency of somatic *KRAS* mutations (<5%) have been identified in PA [9]. In contrast with the predominant anatomic predilection of *BRAF* fusions (cerebellar) and *BRAF V600* mutations (supratentorial) [18], somatic *RAS* mutations seem to have a predilection for the midbrain/tectal region. A series of 23 tectal gliomas

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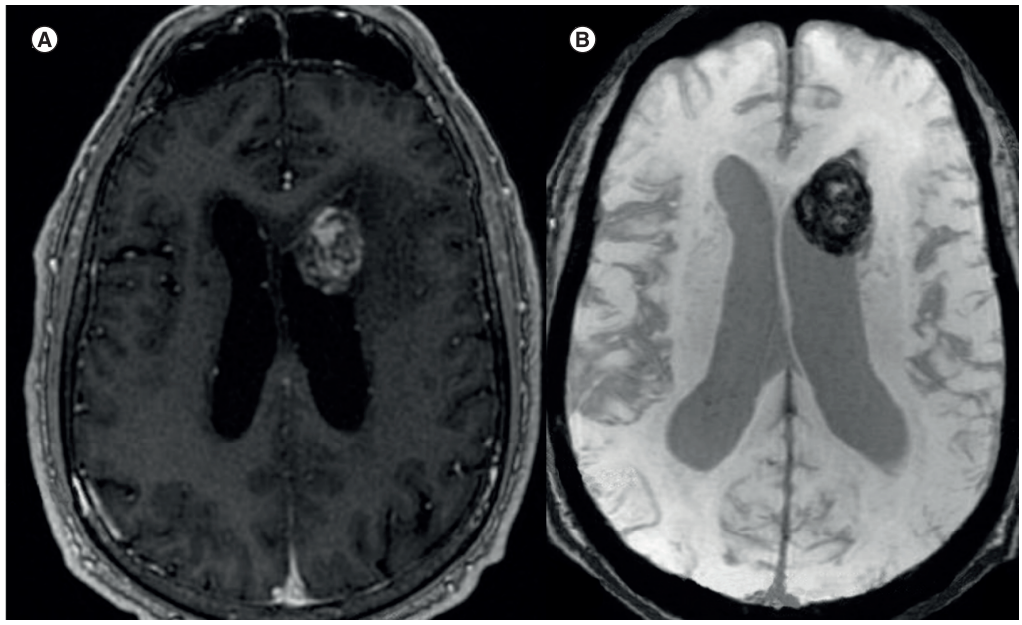

Figure 3. *FGFR1*-mutated pilocytic astrocytoma. Left lateral ventricular heterogeneously enhancing mass **(A)** with intralesional hemorrhage **(B)** in a 71-year old man. The lesion had pilocytic histology, *FGFR1* mutations c.1059A>G and c.2032G>T with methylation class consistent with pilocytic astrocytoma.

harbored the hotspot *KRAS G12R* mutation in 19 patients (including 2 adults) (82.6%) [41]. KRAS inhibitors are in development [77] and their role in targeted glioma therapy is unclear.

FGFR & other rare events

Mutations, fusions or duplications of the upstream regulators of the MAPK pathway, including the tyrosine kinase *FGFR1*, can drive pathologic MAPK activation in adult PA. Duplication events and various mutations of *FGFR1* have been described as a frequent occurrence in up to 60% of dysembryoplastic neuroepithelial tumors [78]. In PAs, hotspot point mutations in *FGFR1* have been implicated in altered autophosphorylation and increased kinase activity [19]. *FGFR1* mutations were found in 15 of a series of 108 adult PA (patients age >15) and were associated with a significantly higher prevalence among sporadic optic pathway PA (6/9) [40]. *FGFR1* mutations increase in frequency in older PA patients (Figure 3) [79]. Compared with wild-type in a series of 69 PAs, the 7% of cases with *FGFR1* mutation had decreased overall and event-free survival [22]. FGFR1 inhibitors, such as ponatinib and lucitanib have been utilized in other solid malignancies but their role in CNS tumors has yet to be defined. Erdafitinib is now US FDA approved for urothelial carcinoma but has unknown CNS penetrance [80], though two advanced glioblastoma patients with *FGFR3-TACC3* rearrangements experienced clinical improvement [81]. A basket trial with the FGFR inhibitor, TAS-120, also includes glial neoplasms (NCT02052778).

NTRK fusions have additionally been identified as a rare event involved in multiple solid malignancies, including colorectal cancer and papillary thyroid carcinoma. In a whole genome assessment of 96 PAs, three pediatric patients were found to have oncogenic *NTRK* fusions implicated in tumorigenesis [19]. The prevalence of this mutation in adults is unknown. *NTRK* fusions have demonstrated responsiveness to TRK inhibition [82] resulting in US FDA approval of larotrectinib and entrectinib for solid tumors harboring the fusion, the latter with comparatively better CNS penetration [80].

High risk features of PA

PA with anaplastic histology has been shown to behave more aggressively and portend a worse outcome. Histologic features designating an anaplastic PA include brisk mitotic activity (at least 5 mitoses per 10 high power fields), hypercellularity, cytologic atypia with or without necrosis, in an otherwise well-circumscribed, noninfiltrative lesion [38]. Rodriguez *et al.* have recently demonstrated that the majority of PA with anaplasia harbor ALT (69%) and ATRX loss (57%); this included a significant proportion of NF1 patients. In addition to MAPK activation,

dysregulation of the PI3K/Akt pathway has been reported in clinically aggressive and anaplastic PA, with the majority of PA with PI3K/Akt activation being of adult age [37,74] Anaplasia was found to herald a worse prognosis with median overall survival of 13 months [38]. In a recent retrospective series of 102 anaplastic PA, 83 cases (89% adults) clustered into a novel methylation class designated anaplastic astrocytoma with piloid features [35], leading to cIMPACT's recent recognition of high-grade astrocytoma with piloid features as a distinct entity [83]. High-grade astrocytoma with piloid features has been associated with MAPK pathway gene alterations in 75% and *CDKN2A/B* deletion in 80% [35]. *CDKN2A/B* deletion is similarly strongly associated with poor survival outcomes in IDH-mutated glioma [84]. *H3-K27M* has been implicated in development of anaplastic histology and aggressive behavior in circumscribed, nondiffuse glioma [85]. Though generally regarded as a defining mutation in grade IV diffuse midline gliomas, three case reports of less aggressive adult PA harboring *H3-K27M* mutations imply that this mutation may not have the same prognostic significance in PA [25,34,86]. Aneuploidy, most frequently involving chromosomes 5, 7, 6 and 11, preferentially affects adult compared with pediatric PA (45 vs 17%), and is typically found in noncerebellar and non-BK fusion cases, suggesting another possible driver for worsened outcome in adults [23].

Histology consistent with pilomyxoid astrocytoma, which predominantly arises in younger patients and in the hypothalamic/chiasmatic region, is noted to be independently associated with a more aggressive course and decreased overall survival, in part due to a propensity for leptomeningeal seeding [87]. At least half of these tumors are associated with BK fusion [53] and the known tendency for pilomyxoid astrocytoma to mature into PA suggests these are the same entity existing together on a spectrum [88]. Similarly, diffuse leptomeningeal glioneuronal tumor demonstrates a high rate of BK fusion in up to 75% [89], and a subset of these (up to 22%) can demonstrate anaplastic progression with worsened outcome [15]. Despite its nomenclature, multiple cases of focal, circumscribed parenchymal tumor have resolved to diffuse leptomeningeal glioneuronal tumor on methylome analysis [90], making the classification of this entity unclear. Epigenetic analysis is further aiding the delineation of rosette-forming glioneuronal tumor from PA and other low grade neuroepithelial tumors with overlapping genomic signatures, with recent description of *FGFR1* p.N546 or p.K656 mutation with *PIK3CA* or *PIK3R1* appearing to represent a molecular signature specific to rosette-forming glioneuronal tumor [42].

Histologically benign PAs and glioneuronal tumors sometimes reclassify to other tumor types when DNA methylation profiling is employed, including to more aggressive tumors such as high-grade astrocytoma with piloid features [91,92]. Conversely, 5% of 160 glioblastomas were reclassified as a lower grade entity, including multiple gangliogliomas and at least one PA [93], highlighting that challenging histologic cases may require further differentiation with DNA methylation profiling. In summary, aggressive behavior is at least in part mediated by molecular factors that need to be defined in the context of clinical, histomorphological, genetic and epigenetic factors. An integrated diagnosis including histology, mutational profile and methylome profile is emerging as the most accurate way to identify and differentiate PAs with anaplastic features from other high-grade CNS neoplasms.

Treatment

Standard therapies

Gross total resection, the mainstay of therapy, is associated with greater than 95% 10-year survival in pediatric PA [2]. A majority of adults have favorable outcomes after gross total resection, with evidence of both improved PFS and overall survival [8], though long-term outcome is generally not as favorable as in pediatric PA. A retrospective aggregation of 254 adult patients with an average follow-up of 77 months showed a 28% rate of subsequent recurrence after gross total resection [4] compared with a recurrence rate of 10% reported in pediatric patients [94]. A watch-and-wait approach after maximal resection remains the standard of care for most adult PA patients.

Adjuvant radiation therapy (RT) was associated with significantly decreased PFS in the largest, single-center retrospective study [6] and was one of three factors associated with decreased survival in a population study of adult PA [5]. Conversely, a retrospective series of 30 adult PA showed 5- and 10-year PFS rates of 91 and 60%, respectively, compared with 42 and 17% without adjuvant RT [95]. A prospective series of adults that included three biopsy-only patients who underwent adjuvant RT showed no evidence of disease progression at 18.8, 16.1 and 2.1 years [96]. A 2013 review of the literature suggested that malignant transformation of PA occurs mainly in tumors treated with prior irradiation [97]. In a study of anaplastic PA with ALT and ATRX loss, nine of 15 patients in whom anaplasia arose from a precursor PA had received prior irradiation, and in a separate series of three adult anaplastic PA patients with ATRX loss, all had received prior RT, one tumor had a *PIK3CA* mutation, and another

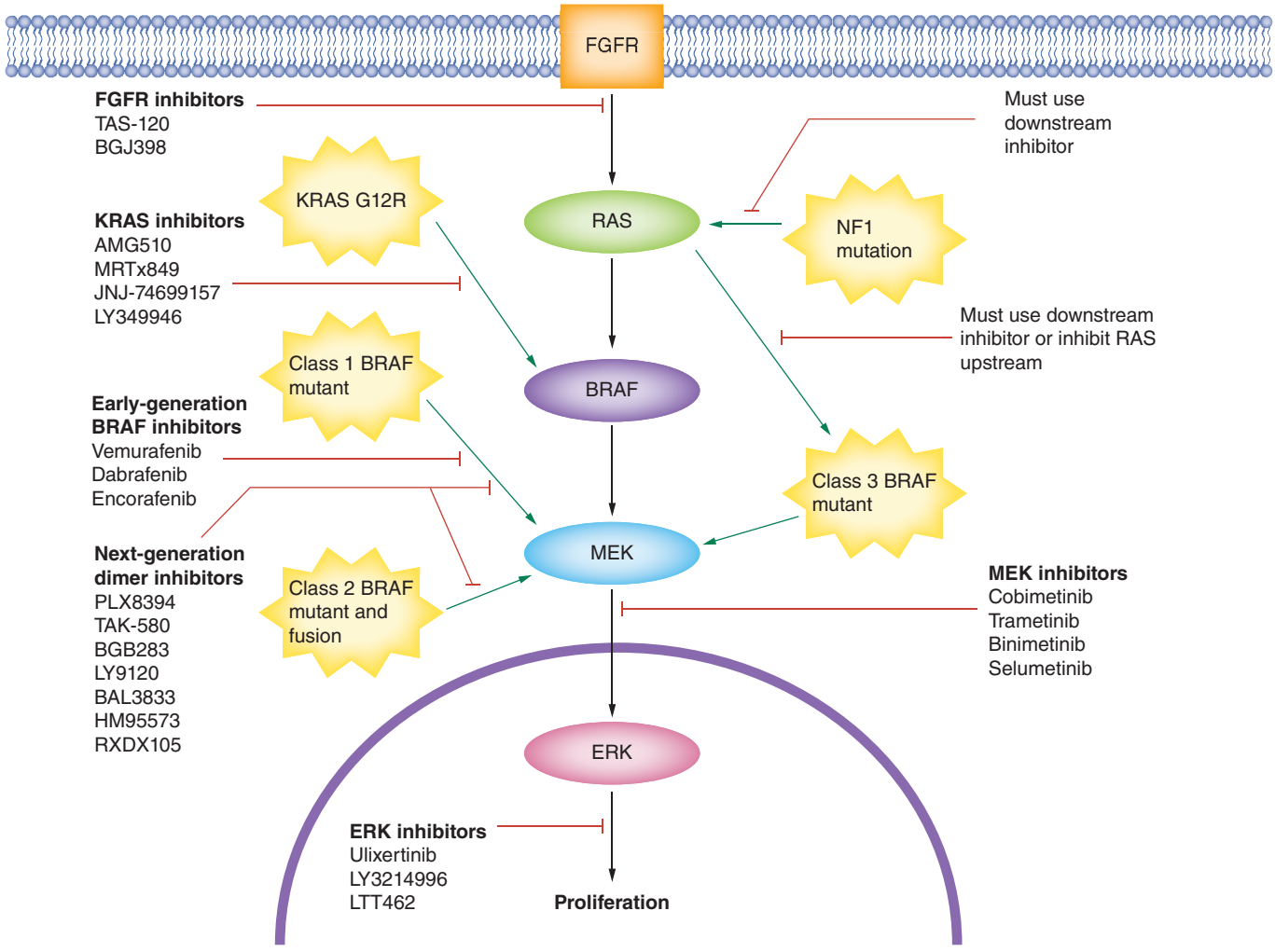

Figure 4. Overview of targetable regions of the MAPK pathway and therapies currently available or undergoing trial.

a *PDGFR* mutation [37,74]. Whether RT should be avoided due to deleterious impact on tumor biology in some cases, or whether RT should be employed in an adjuvant setting in cases with STR, requires prospective study.

PA does not have MGMT promotor methylation and temozolomide does not appear to be effective based on case reports and anecdote [98,99]. Platinum-based chemotherapy regimens used in pediatric low-grade gliomas, such as carboplatin and vincristine [100], may be an option in adult patients with progressive tumors but the effectiveness is unknown. Further addition of etoposide did not show benefit in a Phase III study in children, and whether adults would benefit from chemotherapy intensification is unknown [101]. Single-agent vinblastine has shown benefit in pediatric PA [102] and while not studied in adults, this may be another cytotoxic chemotherapy option for select patients. Specific examples of when pediatric chemotherapy regimens may be considered would include sporadic optic pathway PAs in young adults as a strategy to delay the need for radiotherapy. Multi-disciplinary treatment planning of adult PA patients is of paramount importance given the lack of prospective evidence to guide treatment decisions with regards to timing of radiotherapy and use of chemotherapy in the adjuvant and recurrent settings.

Targeted therapies

MAPK pathway dysregulation is central to PA oncogenesis and rational pursuit of molecular targets has become an essential quest over the past decade [43,103]. Figure 4 illustrates current and experimental therapies targeting the various components of the pathway. In short, identification of a class II or III *BRAF* mutation precludes the use of an early generation BRAF inhibitor, which can only target class I mutations. Class I *V600E*-mutant low-

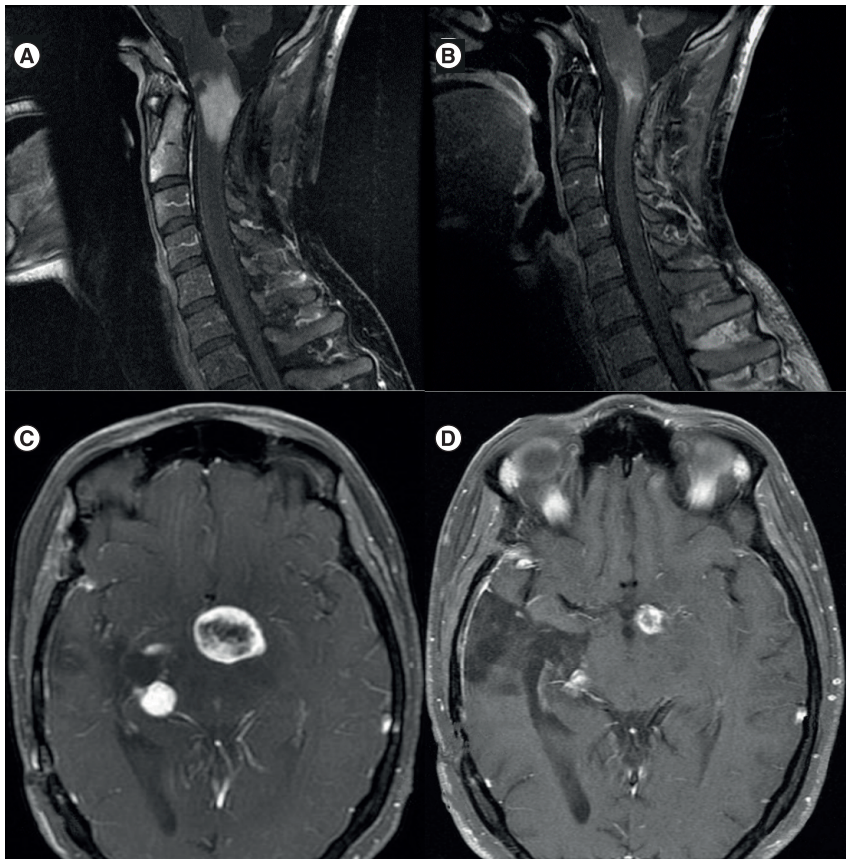

Figure 5. Responses to therapy. (A) 28-year old man with *BRAFV600E*-mutated pilocytic astrocytoma at the cervicomedullary junction and marked response to dual BRAF pathway inhibition with dabrafenib and trametinib at 3-month interval **(B)**. **(C)** A 21-year old woman with sporadic optic pathway glioma with significant response to bevacizumab shown at 2-month interval **(D)**.

grade glioma appear to benefit from treatment with dabrafenib in the relapsed or refractory pediatric setting [104]. Randomized trials are currently evaluating targeted therapy versus chemotherapy in pediatric low-grade gliomas, including dabrafenib plus trametinib versus carboplatin plus vincristine (NCT02684058, 18 years or younger included), selumetinib versus carboplatin plus vincristine (NCT03871257, 21 years or younger included) and trametinib versus carboplatin plus vincristine in the Phase III LOGGIC trial (18 years or younger included) [105]. Downstream MEK-inhibition is a reasonable adjunct for *V600E* mutants as shown in Figure 5A and B, as well as for class II mutations and fusions [63]. While these strategies are limited by the lack of prospective data and understanding of resistance mechanisms in adults, MEK inhibition employed in progressive pediatric low-grade glioma has demonstrated benefit, including 100% disease control in 18 children treated with trametinib [106] and up to 40% sustained partial response in recurrent, refractory or progressive cases treated with selumetinib [107].

Currently under study are paradox-breakers, such as PLX8394, and pan-RAF kinase inhibitors, such as TAK580, which can prevent or overcome RAF dimer formation thereby circumventing paradoxical MAPK activation [108]. For *NF1* mutations, downstream MEK inhibition is potentially beneficial as discussed previously. Further study is warranted to further define parallel PI3K-mTOR pathway inhibition as a strategy to overcome resistance to BRAF/MEK inhibition, with some suggested benefit of this approach in a series of five V600-mutated gliomas employing vemurafenib combined with everolimus [109]. Questions that often come up in multi-disciplinary discussion include the optimal timing of off-label, targeted therapy whether in an adjuvant setting, before or after radiation at recurrence, or as monotherapy for recurrent disease.

Bevacizumab also appears to be beneficial based on small studies. In an 11-patient adult series using bevacizumab alone in four patients or with temozolomide in seven patients with unresectable PA, all but one achieved a partial response [110]. A more recent series of four adults demonstrated significant clinical and radiographic response,

including two complete responses, which were durable across follow-up that ranged from 7 to 37 months [111]. These correspond with a retrospective series of high grade gliomas in five adult NF1 patients, at least two of which may have represented transformed PA, which showed postrecurrence response to bevacizumab ranging from 10 to 72 months [112]. Figure 5C and D shows a sporadic optic pathway PA in an adult patient that had a durable response to bevacizumab after failing radiotherapy and cytotoxic chemotherapy at recurrence.

Future perspective

Future directions include synthesis of histologic, molecular and epigenetic data to personalized therapeutic approaches in adult PA. Prospective clinical data will be difficult to obtain due to tumor rarity and multi-institution cooperation, for example with use of registries, will be needed. The variety of targeted therapeutics undergoing investigation foretells an expanding range of therapeutic options, but determining the optimal targets, use of single versus multiple targeted agents and the timing of targeted therapeutics will require innovative clinical research efforts in adult PA.

Executive summary

- Adult pilocytic astrocytoma (PA) represents approximately 25% of all PAs and are associated with inferior survival outcomes compared with pediatric PA.
- In adult PA, MAPK alterations are the most common genomic alterations reported in the literature.
- BK fusions are the most common MAPK alteration, but the prevalence of BK fusion is lower in adult than pediatric cases and the association with survival outcomes in adults is unknown.
- *BRAF, NF1* and *FGFR1* mutations are additional well-characterized drivers of MAPK signaling in adult PA.
- Transformation of PA to an aggressive cancer can occur as recognized by the newly described entity, high-grade astrocytoma with piloid features.
- Markers of aggressive behavior include anaplasia, alternative lengthening of telomeres and *ATRX* loss, *CDKN2A/B* deletion and aneuploidy.
- Integrated diagnosis improves accuracy in distinguishing entities with morphological and molecular features that overlap PA, such as pleomorphic xanthoastrocytoma, pilomyxoid astrocytoma, diffuse leptomeningeal glioneuronal tumor or rosette-forming glioneuronal tumor. Some high grade or difficult to classify glial neoplasms may be re-classified as PA with an integrated diagnostic approach including DNA methylation profiling.
- Resection is the cornerstone of adult PA therapy, with possible individualized roles for chemotherapy, radiation or bevacizumab.
- Targeting the MAPK pathway is evolving as a treatment option for some adult PA patients.
- Off label use of first-generation BRAF and MEK inhibitors may be an option for carefully selected patients.
- Future testing of the next generation of MAPK-targeted therapies will require coordinated clinical research efforts.

Author contributions

TA Gregory is the primary author. LB Chumbley contributed toward editing and figures. JW Henson contributed toward editing and figures. BJ Theeler contributed toward drafting and editing.

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