

Treatment of high-grade glioma in children and adolescents

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Pediatric high-grade gliomas (HGGs)—including glioblastoma multiforme, anaplastic astrocytoma, and diffuse intrinsic pontine glioma—are difficult to treat and are associated with an extremely poor prognosis. There are no effective chemotherapeutic regimens for the treatment of pediatric HGG, but many new treatment options are in active investigation. There are crucial molecular differences between adult and pediatric HGG such that results from adult clinical trials cannot simply be extrapolated to children. Molecular markers overexpressed in pediatric HGG include *PDGFR α* and *P53*. Amplification of *EGFR* is observed, but to a lesser degree than in adult HGG. Potential molecular targets and new therapies in development for pediatric HGG are described in this review. Research into bevacizumab in pediatric HGG indicates that its activity is less than that observed in adult HGG. Similarly, tipifarnib was found to have minimal activity in pediatric HGG, whereas gefitinib has shown greater effects. After promising phase I findings in children with primary CNS tumors, the integrin inhibitor cilengitide is being investigated in a phase II trial in pediatric HGG. Studies are also ongoing in pediatric HGG with 2 *EGFR* inhibitors: cetuximab and nimotuzumab. Other novel treatment modalities under investigation include dendritic cell-based vaccinations, boron neutron capture therapy, and telomerase inhibition. While the results of these trials are keenly awaited, the current belief is that multimodal therapy holds the greatest promise. Research efforts should be directed toward building multitherapeutic regimens that are well tolerated and that offer the greatest antitumor activity in the setting of pediatric HGG.

Keywords: glioblastoma, integrins, molecular targeted therapy, pediatrics, pontine glioma.

Pediatric high-grade gliomas (HGGs) are usually defined as tumors of glial origin with a grade III or IV histology, according to the World Health Organization (WHO) histological grading system.¹ Besides the most common pediatric HGGs, anaplastic astrocytoma (AA; WHO grade III) and glioblastoma multiforme (GBM; WHO grade IV), there are also less frequently occurring pediatric HGGs of grade III (anaplastic oligodendroglioma, anaplastic oligoastrocytoma, anaplastic ganglioglioma, and anaplastic pilocytic astrocytoma) and grade IV (giant cell glioblastoma and gliosarcoma).¹ Other glial tumors that have histological WHO grades from II to IV but a poor clinical course (e.g., diffuse intrinsic pontine gliomas [DIPG], gliomatosis cerebri) may also be described as pediatric HGG, but this classification is debated. Data on childhood malignancies from the EU show that HGGs are rare, with glioblastomas and anaplastic astrocytomas accounting for 2.8% and 1.9%, respectively, of CNS tumors and 6.8% of CNS tumors located in the pons (likely including DIPG).² The common feature of all these tumors is that they are particularly difficult to treat and usually do not respond to even the most aggressive therapy.³ Surgery and radiation are the usual modes of therapy, especially for AAs and GBMs,¹ and prognosis is better for children than for adults.⁴

In a large analysis of over 6000 pediatric glioma patients <20 years old from the Surveillance, Epidemiology, and End Results database, tumor grade was the most significant independent prognostic factor across age groups, except in children <1 year, where the extent of resection was the most significant indicator of survival.⁵ In 85 children and adolescents with malignant nonpontine gliomas, gross total tumor resection was the strongest predictor of survival.⁶ In order to avoid severe neurological sequelae, infants under the age of 3 years with pediatric HGG do not usually receive primary radiotherapy. Instead, they often

Received April 6, 2011; accepted May 20, 2011.

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receive chemotherapy alone as a first-line approach after resection. Nevertheless, long-term overall survival is usually significantly prolonged for infants compared with older children, which suggests a different tumor biology among the different age groups.⁴

Despite improvements in neurosurgery, radiotherapy, and chemotherapy, the outcome for children with pediatric HGG remains poor. No effective chemotherapy regimens have been identified for any pediatric HGG; 2-year survival rates range from 10% to 30% for supratentorial pediatric HGG and are less than 10% for DIPG,⁷ highlighting the need for new treatment approaches. Improved survival for these patients is likely to require combined therapy, including novel treatments. Current and proposed studies are combining radiation therapy and/or chemotherapy with agents that have shown promise in preclinical studies, such as radiosensitizers, anti-angiogenics, growth factor receptor inhibitors, immunotherapy, and free radical inducers. Other biological therapies, including gene therapy, have been investigated but have not yet demonstrated efficacy.³

In this review, we explore current research efforts to develop new treatment options and identify relevant prognostic and predictive markers for pediatric HGG.

Pathophysiology and Biology of Pediatric HGGs

Efforts to develop effective therapies for HGGs in children may not be able to rely on progress made with adult HGGs. While the histology of HGGs between adults and children appears identical, the biology of the tumors may vary significantly.

EGFR

Whereas deletions of the gene encoding phosphatase and tensin homolog (*PTEN*) and amplifications of the epidermal growth factor receptor gene (*EGFR*) are commonly detected in adult malignant gliomas, analysis of 62 pediatric glioma samples revealed that both features are infrequent in pediatric glioma.⁸ The lower expression of *EGFR* in pediatric GBM is confirmed in other studies.⁹ However, relative to pediatric low-grade gliomas, significant overexpression of *EGFR* has been observed in pediatric HGGs.¹⁰ In a study of 42 children (<20 years old) with HGG, distinct differences were observed in protein expression of EGFR between pediatric and adult HGGs.¹¹ Tumor samples from these children showed a high degree of immunopositivity for EGFR wild type (58%) but low expression of EGFRvIII (2%).¹¹ Another study examined 90 pediatric HGG samples and reported *EGFR* gene amplification in 8 of 74 (11%) evaluable cases (6 GBM and 2 AA).¹² No *EGFRvIII* activating mutations were detected, but *EGFRvIII* deletions occurred in 17% of cases.¹² There are clearly differences between children and adults in the expression of EGFR, but the precise role of EGFR in pediatric HGG requires clarification in further studies.

A recent study provided the first wide-ranging high-resolution genomic analysis of pediatric DIPG.¹³ The group used high-resolution DNA microarray analysis based on single nucleotide polymorphism in the study of 11 biopsies (9 postmortem and 2 pretreatment surgical) from children with DIPG (mean age, 7.6 years).¹³ *EGFR* was not amplified in any sample but EGFR immunopositivity was observed in 7 of 11 cases.¹³ These results are supportive of earlier findings that showed an increase of EGFR expression in high-grade pediatric DIPG.¹⁴ These data collectively suggest that there may be a role for EGFR as a therapeutic target in pediatric HGG.

Akt and PTEN

Elevated levels of Akt are generally observed in adult GBM, and activation of the Akt pathway occurs as a result of *PTEN* mutations.¹⁵ Despite that *PTEN* mutations are infrequent in pediatric HGG, overexpression of activated Akt was observed in 42 of 53 childhood malignant tumors.¹⁶ Akt activation was seen at relatively comparable frequencies in lesions of grade III and grade IV in this group. There was a trend toward an association between activated Akt and poorer prognosis¹⁶: 1-year event-free survival was 59% in samples with Akt overexpression and 91% for those with no overexpression ($P = .16$, log-rank test) and 1-year overall survival was 78% and 100% ($P = .06$, log-rank test), respectively. Examination of a further series of 32 pediatric GBM samples also showed an association between Ras/Akt activation and poor survival.¹⁷

PDGFR α

Alpha-type platelet-derived growth factor receptor (PDGFR α) is overexpressed in pediatric HGG (including DIPG) compared with adult HGG, highlighting the potential of PDGFR α as a therapeutic target for pediatric HGG.⁹ In a study of 63 pediatric HGG samples, *PDGFR α* amplification and *CDKN2A/B* deletion were the most frequent focal events.¹⁸

While AA and GBM are largely indistinguishable from one another on a molecular level, DIPG has molecular characteristics that set it apart from other pediatric HGGs. A study of 11 DIPG samples found evidence of amplification of *PDGFR α* in 4 (36%) tumor samples.¹³ Immunohistochemistry revealed strong expression of PDGFR α in 7 of the 11 samples and weak expression in the remainder. Low-level gains in poly(ADP-ribose) polymerase-1 were also identified in 3 cases.¹³

IDH1

Mutations in *IDH1* are common in adult secondary GBM (85%) but are rare in primary GBM (5%). An analysis of 78 pediatric HGG samples, including 7 DIPG, from patients <23 years old found that *IDH1*

mutations were rare.⁹ A study of a series of 41 childhood astrocytomas revealed a zero incidence of *IDH1* mutations.¹⁹ A further study of pediatric primary malignant gliomas (AA or GBM) from the Children's Oncology Group observed *IDH1* mutations in 7 of 43 tumors.²⁰ Strikingly, all of these *IDH1* mutations occurred in children ≥ 14 years old, with none occurring in younger children. No *IDH2* mutations were observed. It was not reported whether the 7 *IDH1*-positive cases were in AA or GBM tumor samples. Recently, pyrosequencing was used to detect *IDH1* mutations in HGG samples from 47 young patients (6 weeks–23 years old), and a similar mutation rate (11 of 47 tumors) was reported.²¹ Mutations in *IDH1* were more common in AA (5 mutations in 14 tumors) than in GBM samples (6 of 33).²¹

TP53

TP53, which encodes the p53 protein, is often mutated in adult malignant glioma. In 77 cases of nonbrainstem malignant gliomas (AA or GBM) from 17 children aged < 3 years and 60 children aged 3–17 years, the overall incidence of *TP53* mutation was 34%.²² When stratified by age, the incidence of mutations was significantly greater in older children (24/60; 40%) than in those < 3 years (2/17; 12%). There were also numerically more mutations in the GBM tumor group compared with the AA tumor group (40% vs 26%; NS). In a series of 41 pediatric astrocytomas, alterations affecting p53 function were reported in 6 of 31 cases analyzed, with homozygous deletion in 1 case and *TP53* sequence alterations in 2 grade III and 3 grade IV tumors.¹⁹

Expression of p53 and mutations in *TP53* were analyzed in nonbrainstem malignant glioma samples from 231 children with HGG.²³ Overexpression of p53 was significantly associated with dramatically reduced progression-free survival (PFS) at 5 years, while mutations in *TP53* were nonsignificantly associated with adverse prognosis. These data are important in initiatives to identify robust prognostic markers to guide treatment decisions in pediatric HGG.

BRAF

In 41 pediatric astrocytoma samples, DNA analysis revealed a missense activating *BRAF* mutation (*BRAF*^{V600E}) that occurred in grades II–IV samples (including AA and GBM) but not in grade I pilocytic astrocytomas.¹⁹ This grade-linked oncogenic *BRAF*^{V600E} mutation appears to define a subset of malignant pediatric astrocytomas in which there is also deletion of the gene for cyclin-dependent kinase inhibitor 2A.¹⁹ The development of targeted *BRAF* signaling pathway inhibitors would seem to be a rational therapeutic approach for certain subsets of pediatric gliomas. Indeed, several such therapeutics are currently being investigated in clinical trials for various types of cancer.²⁴

MGMT

The *MGMT* gene encodes a DNA-repair enzyme. High levels of O⁶-methylguanine-DNA methyltransferase (*MGMT*) activity in tumor tissue can reduce the efficacy of alkylating chemotherapies.²⁵ From the Children's Cancer Group (CCG) 945 study, 109 samples of pediatric HGG from children > 36 months old who had received alkylator-based chemotherapy were examined.²⁶ The majority of samples (97/109) showed little or no *MGMT* immunoreactivity, but 12 samples showed *MGMT* overexpression. A low 5-year PFS (8.3%) was observed in the 12 patients with *MGMT* overexpression, compared with a 42.1% 5-year PFS in the remaining 97 patients. There was a significant association between *MGMT* expression and poor overall survival ($P = .02$).²⁶

Promoter methylation of the *MGMT* gene compromises DNA repair and has been associated with prolonged survival in adult GBM patients receiving alkylating agents.²⁵ Several studies indicate that methylation of the *MGMT* gene promoter may be prognostic for survival in pediatric HGG.^{26–28} These findings are discussed in more detail in the next section.

Another mechanism that has been implicated in reducing the efficacy of alkylating chemotherapies, independent of tumor *MGMT* status, is deficiency in mismatch repair (MMR).²⁹ Until recently, the role of this mechanism in pediatric HGG had not been well characterized. The frequency of microsatellite instability, a marker of defective MMR, was examined in 68 pediatric HGG samples from newly diagnosed patients. Only 3 tumors had microsatellite instability involving 3 or more markers, and outcome was not unusual in these 3 patients. In contrast, immunohistochemistry showed that 25 tumors (37%) exhibited *MGMT* overexpression.²⁹ The contribution of microsatellite instability to tumorigenesis in pediatric malignant astrocytomas appears to be minimal.

Molecular Differences Among Subtypes of Pediatric HGG

In DIPG, there may be a somewhat better prognosis for younger children than in the older population,³⁰ and this may be driven in part by distinct molecular differences between the 2 diseases. More research is required to understand these differences; however, this is burdened to some extent by the difficulties in obtaining biopsy samples from diffuse tumors and the complicated issues of postmortem consent for autopsy.³¹

The biology of pediatric GBM has been characterized in more detail than that of DIPG. Current understanding of the disease, its subtypes, and its molecular differentiation from adult GBM is hindered by a lack of data directly addressing these questions, but related studies provide some information. For example, the clustering of copy number aberrations in the DNA of pediatric GBM cells is different from that of adult GBM.^{13,18} Detailed mapping studies have revealed differences in mutations at locations 1q, 7q, and 10q. Thus, while

there are similarities in the genomic events driving gliomagenesis in patients of all ages, the pediatric disease harbors a spectrum of copy number aberrations distinct from adult tumors.¹⁸ Adult and pediatric GBM also differ in their expression of EGFR and the EGFRvIII mutant.¹² These differences underscore the need to develop therapeutic modalities that are specific to pediatric HGGs, given the observed differences in the molecular characteristics of these diseases.

A summary of the molecular markers investigated to date and found to have relevance to pediatric HGG is shown in Table 1.

Indicators of Prognosis in Pediatric HGGs

Indicators of prognosis in children with HGGs are still in development. A range of markers have been studied, but none is currently used for stratification or as a guide for therapeutic decision making. At present, the strongest indicator of prognosis in pediatric HGGs is extent of resection. The European protocol HIT-GBM-C reported that in patients with tumors that are completely resected prior to combination radiotherapy and chemotherapy, 5-year overall survival was $63 \pm 12\%$. In contrast, the 5-year overall survival rate for all patients ($n = 97$, including complete resection, subtotal resection, partial resection, biopsy only, and no surgery) was only $19 \pm 4\%$.³⁴ Tumor location was another prognostic factor identified in the HIT-GBM database, with the poorest outcomes associated with pontine locations and the best outcomes occurring in tumors of the cerebral hemispheres.³⁵

In addition to successful surgery, a number of molecular markers have been examined, many on the basis of their putative involvement in the biology, oncogenesis, and pathogenesis of pediatric HGG. Despite the lack of association between microsatellite instability

and outcome, MGMT expression has been shown to correlate with outcome in childhood malignant gliomas.^{26–28} A retrospective study of 10 pediatric patients with GBM showed MGMT promoter methylation in 4 of 10 patients and revealed a significant association between MGMT promoter methylation and prolonged survival ($P = .0005$).²⁷ Average survival in the 4 patients with a methylated MGMT promoter was 13.7 months, compared with 2.5 months for the remaining 6 patients with an unmethylated promoter. Furthermore, MGMT promoter methylation was associated with better response to temozolomide ($P = .007$).²⁷ A later study of 25 relapsed pediatric HGGs also demonstrated an association between methylation of the MGMT gene and increased median event-free survival (5.5 months vs 0.9 months, $P = .015$).²⁸

Poor outcomes in childhood malignant gliomas have been associated with p53 overexpression^{22,23}; however, TP53 mutations correlated with tumor aggression, whereas p53 overexpression did not.³⁶

Loss of expression of PTEN has been associated with poorer outcomes in childhood gliomas.³⁷ Interestingly, mutations and deletions in PTEN are associated with elevations in Akt activity; however, genomic alterations to PTEN were found to occur less frequently in pediatric HGGs than in adult HGGs.¹⁶ Other progression-associated genes include RPS2, RPS8, RPS18, and RPL37A (all upregulated), and APOD, SORL1, SPOCK2, PRSS11, and ID3 (all downregulated).³⁸

Reduced expression of the tissue inhibitor of metalloproteinases-1 gene (TIMP-1) predicts longer patient survival in glioblastoma patients, but this has not been studied specifically in children.³⁹ Coexpression of the extracellular matrix metalloproteinase inducer and matrix metalloproteinase-2 is associated with higher-grade pediatric gliomas and worse outcome.⁴⁰ Bcl-2 overexpression has also been suggested as a negative prognostic marker in pediatric glioblastomas.⁴¹

Table 1. Molecular markers in pediatric HGGs

Marker	Relevance to Pediatric HGGs
ADAM3A	DNA analysis of 38 predominantly pretreatment pediatric HGG samples (including 13 DIPGs) revealed homozygous loss at 8p12 in 16% of cases. This novel deletion includes the ADAM3A gene. ³²
Akt	Ras/Akt activation is observed in pediatric HGG and may be associated with poor prognosis. ^{16,17}
BRAF	The BRAF ^{V600E} mutation has been found in pediatric high-grade astrocytomas. ¹⁹
CDKN2A/B	Approximately 50% of supratentorial tumors expressed CDKN2B and ~75% of infratentorial tumors were positive for CDKN2B expression. Pediatric DIPG samples showed high-frequency loss of 17p and 14q and lack of CDKN2A/CDKN2B deletion. ³²
EGFR	A lesser degree of EGFR amplification is observed in children compared with adult HGG. ^{8,11} Marked overexpression of EGFR is observed in pediatric HGG relative to pediatric low-grade glioma. ¹⁰
EGFRvIII	EGFRvIII deletion mutations have been observed in pediatric HGG, but no EGFRvIII activating mutations. ¹²
IDH1	Until recently, most data had shown that IDH1 mutations were rare or absent in pediatric HGG. ^{9,34} However, a recent study found IDH1 mutations in 7 AA/GBM tumors from children ≥ 14 years old. ²⁰
MGMT	MGMT overexpression is rare in pediatric HGG but is associated with poor prognosis. ²⁶ Methylation of the MGMT promoter may be prognostic for survival in pediatric HGG. ^{26–28}
PDGFRA	PDGFRA is overexpressed in the majority of pediatric HGG cases and amplified in ~1/3 of pediatric HGG, including DIPG. ^{9,13,18}
P53	p53 overexpression and mutation correlate independently with adverse outcome and appears to vary with age. ^{22,23}
VEGF 121 isoform	The VEGF 121 isoform, which promotes mitogenesis and vascular permeability, has been linked with AA and GBM. ³³

As is the case in other cancers, molecular analysis of pediatric HGGs is likely to be useful in determining therapeutic targets for novel therapies in children. The impetus behind research into molecular targeted therapies for HGG carries the promise of major improvements in the therapeutic management of children with HGGs and may also assist investigators in developing completely new therapeutic modalities such as dendritic cell vaccines or T-cell-based immunotherapeutics.⁴²

Treatment Options for Pediatric HGG

Multiagent Chemotherapy

Temozolomide is the standard treatment for adult patients with HGG, but there is no standard chemotherapy backbone that is universally acknowledged in the setting of pediatric HGG. For DIPG in particular, there is no established role for chemotherapy, and radiation is the standard treatment. However, it is mostly palliative in nature, with <10% of children surviving beyond 2 years, despite the majority showing transient improvement in neurologic symptoms following radiotherapy.⁴³

Very few randomized trials of adjuvant chemotherapies have shown substantial benefits in children with pediatric HGG. Postsurgical use of chloroethyl-cyclohexyl nitrosourea (CCNU or lomustine), vincristine, and prednisone have long been studied as an adjuvant to standard surgical treatment and radiotherapy. The 5-year event-free survival initially reported with this regimen was 46% for pediatric patients in the radiotherapy and chemotherapy group, and 18% for patients in the radiotherapy alone group.⁴⁴ An 8-drug chemotherapeutic regimen consisting of vincristine, carmustine, procarbazine, hydroxyurea, cisplatin, cytosine arabinoside, prednisone, and dimethyl-triazenoimidazole-carboxamide was tested in children younger than 2 years old with mixed results.⁴⁵ In the first instance, 39 patients were administered the regimen, and the PFS rate at 3 years was 36%. However, PFS was greater in patients with AA (44%) than in patients with GBM (0%).⁴⁵ A subsequent study in older children with astrocytomas comparing the 8-drug regimen to vincristine, lomustine, and prednisone found no statistical superiority of either regimen and recorded a greater incidence of myelosuppression and hearing loss for the 8-drug combination.⁴⁶ Furthermore, subsequent review of tumor histopathology in patients enrolled in this study revealed that nearly one-third actually had low-grade gliomas rather than high-grade tumors and that the PFS rate for those with true HGGs (19% ± 3%) was no better than the historical rates reported with surgery and radiation alone.⁴⁷ These findings drew attention to the need for central pathology review in pediatric HGG clinical trials investigating the efficacy of chemotherapy.⁴⁷

However, the authors noted that the acute toxicity of high-dose chemotherapy in patients with neurological impairment was an important concern. The concept of intensive chemotherapy following surgery has been

explored further in a study of 97 pediatric patients with HGGs treated under the HIT-GBM-C protocol.³⁴ Patients were administered standard fractionated radiochemotherapy in the form of radiation plus simultaneous cisplatin 20 mg/m² for 5 days, plus additional etoposide and vincristine. This was followed by a single cycle of cisplatin plus etoposide and ifosfamide (PEI) during the last week of radiotherapy. Subsequent maintenance therapy consisted of 6 cycles of PEI followed by oral valproic acid. Complete resection was achieved in 21 patients, and the 5-year overall survival rate for these patients with total resections was 63% vs. 17% for historical controls. The overall survival was 19% at 60 months from diagnosis. Notably, there was no benefit from intensive chemotherapy in patients with residual tumor tissue.³⁴

Current evidence is suggestive of a possible role for high-dose chemotherapy for the treatment of HGG. Survival benefits of high-dose myeloablative chemotherapy followed by autologous bone-marrow transplantation were demonstrated in pediatric patients with astrocytomas, including small groups of patients with GBM.⁴⁸ However, high-dose thiotepa and etoposide-based chemotherapy regimens with autologous bone-marrow rescue failed to prolong survival in 16 children with DIPG compared with conventional therapy.⁴⁹ In addition, these regimens were highly toxic, and all patients suffered severe hematologic toxicity, neutropenia, and mucositis.⁴⁹ The Children's Cancer Group trial 9933 compared 3 high-dose chemotherapy regimens administered in 4 cycles prior to radiotherapy and found no difference in outcomes compared with standard therapy.⁵⁰ One can conclude from all of these data that high-dose chemotherapy is unlikely to be a viable therapeutic option for pediatric HGG and that alternative strategies are necessary.

A small number of other studies of chemotherapies have also been negative. In a phase II window study of procarbazine and topotecan, neither drug demonstrated efficacy; however, this study showed that alkylator resistance appeared to be based on mismatch repair deficiency and high levels of alkylguanine transferase (AGT).⁵¹ Pegylated liposomal doxorubicin administered with oral topotecan has provided evidence of tumor responses, but toxicities are high and probably need to be managed by individualizing the dose of doxorubicin.⁵²

Temozolomide

A dose-finding study of temozolomide in recurrent pediatric brain tumors identified 85 mg/m² for 42 days as the maximum tolerated dose and recorded responses in 4 of the 28 patients treated.⁵³ However, further studies of temozolomide in children found no benefit over conventional treatment.^{54,55}

Although temozolomide has generally been found to be nonsuperior to other chemotherapeutic agents in pediatric HGG, promoter methylation of *MGMT* is predictive of greater sensitivity to alkylating chemotherapies, including temozolomide. It is therefore possible that greater activity could be observed for temozolomide

in patient populations enriched for tumors harboring methylated *MGMT*.²⁷ Within the context of a phase I study, the combination of temozolomide with O⁶-benzylguanine, which acts to deplete AGT levels, was found to have modest activity in recurrent pediatric malignant gliomas.⁵⁶

The ACNS0126 study examined the effect of temozolomide with radiotherapy followed by temozolomide maintenance therapy in patients aged 3–21 years with AA, GBM, DIPG, or gliosarcoma. The results show that temozolomide failed to improve outcome when compared with historical data.^{57,58} There remains the possibility that temozolomide may have a role in multi-drug regimens in children, and this premise is worthy of further investigation. Lomustine and temozolomide have been studied in phase I to determine the maximum tolerated dose of temozolomide in this combination. Results of this trial in terms of antitumor activity are difficult to determine, owing to the radiographic changes induced by concomitant radiotherapy.⁵⁹

Angiogenesis Inhibitors

The vascular endothelial growth factor (VEGF) inhibitor bevacizumab has also been studied in pediatric HGGs, and despite data that support the involvement of VEGF in the pathogenesis of pediatric GBM,³³ clinical data from small studies have not met the expectations of researchers. In a study of 10 patients with supratentorial HGGs and 2 with DIPGs, responses to bevacizumab were inferior to those in adult patients; this points to possible further age-related genetic differences in patients with HGGs.⁶⁰ Bevacizumab was also investigated in combination with irinotecan in 31 children with recurrent malignant glioma and intrinsic brainstem glioma, and although the combination was well tolerated, it lacked efficacy, with no sustained responses observed.⁶¹ In a phase II study in 12 pediatric patients with brainstem gliomas and one with GBM, thalidomide was administered with radiotherapy with no effect on the time to disease progression or time to death versus historical controls. Toxicities, however, were increased for the thalidomide regimen versus the same controls.⁶²

Molecular Targeted Therapy and Other Strategies

Imatinib, which targets PDGFR, was one of the first molecular targeted therapies investigated for pediatric malignant gliomas. A phase II dose was determined⁶³; however, to date no efficacy studies have been reported. Other molecular targeted agents that have been studied in pediatric HGGs include erlotinib, gefitinib, and tipifarnib. Tipifarnib is a farnesyl transferase inhibitor under investigation for the treatment of acute myeloid leukemia. It is well tolerated but has minimal activity in pediatric HGGs.⁶⁴ The EGFR inhibitors erlotinib and gefitinib, while acting on the same biological pathway, may have different activity profiles in HGGs. Erlotinib plus radiotherapy was examined in a phase I pharmacokinetic study in children, adolescents, and

young adults (median age at diagnosis, 10.7 years). No relationship was observed between dose and exposure to the drug, which may make further clinical development of erlotinib in this age group problematic.⁶⁵ Gefitinib, on the other hand, was also studied in a radio-chemotherapy regimen, and researchers found activity commensurate with amplification of EGFR expression. Gefitinib is being advanced to phase II trials.⁶⁶ A phase II study examined lobradimil, a bradykinin agonist that increases permeability of the blood-brain barrier, in combination with carboplatin in 41 patients aged 2–19 years with primary brain tumors.⁶⁷ No objective responses were observed in DIPG and pediatric HGG.⁶⁷

Therapies in Development for Pediatric HGGs

The continuing unmet medical need for new therapeutic options against HGGs has stimulated research on a range of new drugs and therapeutic modalities (Table 2).

Integrin Inhibitors

The integrin inhibitors represent a promising area of research. Integrins are a family of cellular adhesion receptors that are important in the function of cell types associated with tumor progression.⁶⁸ Cilengitide, an inhibitor of $\alpha v \beta 3$ and $\alpha v \beta 5$ integrins, has demonstrated antitumor activity and good tolerability when administered in combination with standard chemotherapy in a phase III trial of adults with newly diagnosed GBM.⁶⁹ The study also showed a survival benefit in those patients whose tumor had a methylated *MGMT* promoter.

Cilengitide was examined in 31 children ≤ 21 years old with primary CNS tumors in a phase I study to

Table 2. Investigational therapies in pediatric HGGs

Therapy	Mode of Action
Cilengitide	$\alpha v \beta 3$ and $\alpha v \beta 5$ integrin inhibitor
Enzastaurin (LY317615)	Anti-angiogenic; protein kinase C inhibitor
MK-2206	Akt inhibitor
IL13-PE38QQR	Cytotoxic to Interleukin-13-expressing cells
Nimotuzumab	EGFR inhibitor
Cetuximab	EGFR inhibitor
MK-0752	Gamma secretase inhibitor
RO4929097	Gamma secretase inhibitor
Valproic acid	Histone deacetylase inhibitor
Vorinostat	Histone deacetylase inhibitor
LDE225	Inhibitor of the Smoothed G-protein-coupled receptor
ABT-888	Poly(ADP-ribose) polymerase inhibitor
Dendritic cells	Vaccine
Bevacizumab	VEGF inhibitor
Cediranib (AZD2171)	VEGF receptor tyrosine kinase inhibitor

establish the maximum tolerated dose and to assess any dose-limiting toxicities.⁷⁰ Cilengitide was infused over 1 hour in doses ranging from 120 to 2400 mg/m². Three patients completed 1 year of therapy, 1 patient with GBM had a complete response, and 2 patients had stable disease. No dose-limiting toxicities to cilengitide were observed, and the 1800 mg/m² dose was selected for further study in ongoing phase II trials with the Children's Oncology Group.

EGFR Inhibitors

The EGFR inhibitor cetuximab is being investigated in children with high-grade pontine gliomas and astrocytomas as part of a sequenced radiochemotherapy regimen. Study NCT01012609, conducted by the Pediatric Oncology Experimental Therapeutics Investigators Consortium, is recruiting patients to undergo external-beam radiotherapy and concurrent cetuximab, followed by cetuximab and irinotecan. The primary endpoint of the study is the proportion of patients achieving 1 year PFS.⁷¹

Nimotuzumab is an h-R3 monoclonal anti-EGFR antibody under investigation for the treatment of pediatric HGG. A phase II trial examined nimotuzumab in 47 patients aged 4–17 years with refractory or relapsed pediatric HGG, including GBM, AA, or DIPG.⁷² Nimotuzumab was infused as an induction dose at 150 mg/m² weekly for 6 weeks followed by a consolidation therapy of 4 infusions over 3 weeks. Objective response was achieved in 14 of 46 patients: partial response in 4 patients and stable disease in 10 patients. Median overall survival was extended for responders (10 months) compared with nonresponders (4 months).⁷² A phase III trial in 42 patients aged 3–16 years with newly diagnosed DIPG and life expectancy <4 weeks were treated with nimotuzumab combined with radiotherapy.⁷³ The results to date indicate a cytotoxic effect and good tolerability; the final results are eagerly awaited.

Anti-angiogenesis

The Pediatric Brain Tumor Consortium (PBTC) is investigating enzastaurin, an inhibitor of protein kinase C, as a potential inhibitor of VEGF-stimulated neo-angiogenesis in a range of tumors, including those of the CNS. Study PBTC-023 is under way and aims to determine the maximum tolerated dose of enzastaurin in patients up to 21 years of age with refractory primary CNS tumors.⁷⁴ The VEGF receptor tyrosine kinase inhibitor cediranib (AZD2171) is also being investigated in the PBTC-020 phase I trial in children under 21 years old with recurrent or progressive CNS tumors.⁷⁵

Dendritic Cell-Based Vaccinations

In the context of novel treatment modalities, dendritic cell-based vaccinations have been a source of optimistic discussion for some time. Autologous dendritic cells pulsed with acid-eluted tumor peptides have been shown to activate T-cell responses in patients with GBM.⁷⁶ In a phase II

trial of therapeutic dendritic cell vaccination in 32 adult GBM patients, time to tumor progression and time to survival were both significantly longer in patients who responded to the vaccine—the first report of an association between T-cell activity and clinical response in humans.⁷⁷ Dendritic cell-based immunotherapy has now been tested in children; 45 children pretreated with surgery and radiochemistry were vaccinated, and this included 33 children with HGG.⁷⁸ The vaccinations were given in an outpatient setting and only mild adverse events were noted. There were 6 long-term survivors (overall survival >24 months) among the relapsed pediatric HGG patients (including 3 GBM) and 4 of these patients were still alive at last follow-up, at between 36 and 86 months. These results support further investigation of this novel therapy in pediatric HGG.⁷⁸

Valproic Acid

The histone deacetylase inhibitor valproic acid, which often forms part of highly intensified chemotherapy regimens, has the potential to sensitize cells to other chemotherapeutic agents. In a study of 66 pediatric patients (age range, 1–19 years) who received valproic acid therapy to treat their glioma-associated seizures, response rates were encouraging: partial responses were observed in 3 patients, stable disease in 4 patients, and progressive disease in only 2 patients.⁷⁹ Other histone deacetylase inhibitors, such as vorinostat (which is in phase I/II trials for the treatment of younger patients with newly diagnosed DIPG⁸⁰), are also now under investigation.

Other Therapies in Development

In addition to therapies that have been studied in completed clinical trials in pediatric patients with GBM, further therapies are under development in clinical trials that are currently in their recruitment phases. These therapies include boron neutron capture therapy⁸¹ (an experimental form of radiotherapy using a neutron beam that interacts with boron injected into a patient), cytomegalovirus-specific cytotoxic T cells,⁸² IL-13-PE38QR⁸³ (an enzymatically active portion of *Pseudomonas* exotoxin A conjugated with human interleukin-13), and the Smoothened inhibitor LDE225.⁸⁴ Telomerase inhibition has also been suggested as a novel therapy for GBM, as human telomerase reverse transcriptase is expressed at high levels in most pediatric astrocytomas.⁸⁵

Gamma secretase inhibitors, such as MK-0752 and RO4929097, are being developed to inhibit Notch signaling, which has been described as critical for GBM cell survival.⁸⁶ Activation of Akt is a common finding in pediatric malignant gliomas,¹⁶ and Akt inhibition is an additional potential therapeutic target. Finally, the poly(ADP-ribose) polymerase inhibitor ABT-888 is being studied in combination with temozolomide in young patients with recurrent or refractory CNS tumors.⁸⁷

Concluding Remarks

The treatment of pediatric HGG suffers from a lack of therapy options, partly because these tumors are invasive and aggressive. However, pediatric HGG cases are relatively rare, making large randomized clinical trials difficult. It is therefore uncertain why many standard adult therapies display little activity in children, although distinct molecular differences between children and adults have been observed. Multimodal therapies have shown the greatest promise in the treatment of pediatric HGG, and it remains unlikely that any one therapy or drug will provide a therapeutic panacea for HGG in children.

Until recently, physicians have generally attempted to treat pediatric HGG patients on the basis of data from trials in adults. A growing body of molecular evidence now demonstrates that this is not the best approach and that the prognosis and treatment of pediatric HGGs require research to develop specific markers and therapies that are effective in younger patients. This research should focus chiefly on immune therapies, anti-angiogenic agents, novel forms of radiotherapy, new cytotoxic chemotherapy regimens, and specific, molecular targeted therapies designed with the pediatric tumor in mind. Multimodal therapies should be built in a

stepwise manner, to ensure patient safety while delivering focused and effective treatment. Organizations such as the Children's Oncology Group and other groups around the world will be important drivers of research in this area.

Acknowledgments

The authors would like to thank Anna Palmer of GHG Publishing for assistance with the preparation of this manuscript, and Merck KGaA, Darmstadt, Germany, who provided funding for this editorial assistance.

Funding

Merck KGaA, Darmstadt, Germany.

Conflict of interest statement. T.McD.—none to declare. D.A.—none to declare. C.K. was financially supported by Merck KGaA regarding the setup and performance of a clinical trial (HIT-HGG-CilMetro) investigating the combination of cilengitide and metronomic temozolomide in pediatric patients with relapsed HGG and was supported by a research grant from Celgene to further develop immunotherapy strategies for HGGs.

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