Management of diffuse intrinsic pontine glioma in children: current and future strategies for improving prognosis

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Practice points

- Diffuse intrinsic pontine glioma (DIPG) is one of the deadliest childhood CNS tumors.
- Radiation therapy is the only standard treatment for affected children.
- Multiple recent genome-wide studies unveiled the molecular characteristics of DIPG, which are distinct from other pediatric and adult high-grade gliomas.
- Although mutations in genes that affect epigenetic mechanisms, particularly *H3F3A* K27M, are common in DIPG, their functional effects are poorly understood. Potential therapeutic interventions based on such abnormalities are currently under investigation.
- Data from genome-wide molecular analyses and preclinical studies support clinical testing of multiple classes of agents in children with DIPG, including receptor kinase, cell-cycle, PI3K and DNA-repair inhibitors.
- Other promising treatment approaches (e.g., immunotherapy) and new ways of delivering anticancer agents (e.g., convection-enhanced delivery) are currently undergoing testing in children with DIPG.
- Although requirement of tumor biopsy in patients with DIPG remains controversial, the molecular analyses of tissue obtained during this procedure may be potentially important in future clinical trials, particularly once a broader range of promising anticancer agents becomes available.
- Multiple research efforts are underway to better understand the biology of DIPG and to test novel therapeutic agents, including high-throughput screening of drugs in DIPG preclinical models.

SUMMARY Diffuse intrinsic pontine glioma (DIPG) is one of the deadliest pediatric central nervous system cancers in spite of treatment with radiation therapy, the current standard of care. The outcome of affected children remains dismal despite multiple clinical trials that investigated radiation therapy combined with chemotherapy. Recently, multiple genome-wide studies unveiled the distinct molecular characteristics of DIPGs and preclinical models of DIPG were developed to mimic the human disease. Both of these accomplishments have generated tremendous progress in the research of new therapies for children with DIPG. Here we review some of these promising new strategies.

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Diffuse intrinsic pontine glioma (DIPG) is one of the deadliest pediatric CNS cancers, accounting for the majority of deaths secondary to brain tumors in this age group [1–3].

Given this dismal prognosis, novel treatment approaches are needed to improve the prognosis and quality of life of these patients.

High-grade gliomas (HGGs), particularly glioblastoma, comprise the majority of histologically confirmed DIPGs in children [4,5]. The diagnosis of DIPG is commonly based on clinical findings consistent with brainstem involvement in the presence of typical MRI characteristics (Box 1). Although the classic MRI features of DIPG were described years ago [6,7], the definition of atypical radiological features, particularly when subtle, remains controversial and subjective. Typical and atypical MRI features of DIPG are shown in Figure 1.

Except in patients with radiographically atypical tumors, biopsies have not been routinely obtained since the early 1990s, when MRI was proven to be a reliable diagnostic tool [8]. However, the role of tumor biopsy in children with DIPG recently regained relevance due to low morbidity and mortality associated with this procedure [9]. For instance, compulsory biopsy of DIPG without therapeutic goals was performed in one recent clinical trial [10], and at least one clinical trial for children with DIPG is stratifying treatment based on the analysis of molecular markers obtained at biopsy. Although molecular analyses of tissue obtained at biopsy has contributed to our understanding of the biology of DIPG [11,12], requirement of tumor biopsy remains controversial.

One concern regarding biopsies is the potential for delaying initiation of radiation therapy (RT) in very symptomatic patients. The current lack of clinically relevant molecular markers to stratify therapies for children with DIPG and the unavailability of promising agents to be used based on this stratification also suggests

that mandatory biopsies may confer risk without personal benefit to patients. For example, testing for the presence of the *H3F3A* K27M mutation, a marker of poor prognosis [13], is easily performed; however, it is difficult to justify a biopsy for the purpose of confirming a known mutation in the absence of available therapeutic options. In future clinical trials, the stratification of children based on molecular data obtained at diagnosis, particularly once more promising targeted therapies become available, may allow for better selection of therapies for affected children [14,15].

Children with DIPG historically were treated in clinical trials that replicated those in adults with glioblastoma [16]. Unfortunately, no combination of chemotherapeutic agents has proven to be efficacious in the treatment of DIPG. A comprehensive review of 29 studies, including 973 patients treated between the years 1984 and 2005, demonstrated no survival benefit with the addition of chemotherapy [16]. To date, RT remains the backbone of treatment for children with DIPG. Dose-escalation and hyperfractionated RT resulted in increased toxicities without improvement in survival [17–22]. Likewise, hypofractionation failed to improve survival [16], although it offered the potential for improved quality of life by reducing duration of treatment [23–25].

Our knowledge of the molecular characteristics of DIPG has increased significantly as a result of genome-wide studies of copy number abnormalities, mutations, gene expression and methylation patterns (Figure 2). It has become clear that DIPG harbors clinical and molecular characteristics that are distinct from other pediatric and adult HGGs [4,5]. This revolution in the knowledge of molecular characteristics, particularly those considered to be actionable therapeutic targets, has led to a paradigm shift in the design of new clinical trials for children with DIPG, which now aim to explore the unique biologic characteristics of these cancers. Here, the authors provide an overview of the most promising current or future therapeutic strategies for children with DIPG.

New agents based on molecular data

In this section, the authors will review promising therapies based on the relevance of their respective targets in the biology of DIPG, as well as discuss the potential of these agents to enhance the activity of RT and/or chemotherapy.

Box 1. Typical radiographic features of diffuse intrinsic pontine glioma on MRI.

- Intrinsic pontine mass with a minor exophytic component
- Lesion involves the majority of the pons (e.g., greater than two-thirds of the pons)
- Hypo- or iso-intense on T1-weighted imaging
- Hyperintense on T2-weighted imaging
- Frequently with ventral involvement of the pons and encasement of the basilar artery

Figure 1. Typical and atypical MRI features of diffuse intrinsic pontine glioma. (A) Provides an example of typical MRI findings seen in DIPG. Patient A was diagnosed at the age of 13.3 years with a 2-week history, was treated with radiation therapy and investigational targeted agents, and survived 7 months. Glioblastoma (WHO grade 4) was confirmed at autopsy. (B & C) demonstrate atypical MRI features characterized by tumors with better delineated margins. Patient B was diagnosed at the age of 3.3 years after a 1-week history. Tumor biopsy showed a primitive neuroectodermal tumor. This patient remains alive more than 3 years after intensive therapy. Patient C was diagnosed at the age of 8.8 years with a 2-week history. Tumor biopsy confirmed a fibrillary astrocytoma (WHO grade 2). This patient was treated with radiation therapy and investigational targeted agents and remains alive and well 31 months after diagnosis.

• Receptor kinase inhibitors

PDGF receptor inhibitors

Compelling preclinical and molecular data support the importance of the PDGF pathway in the pathogenesis of DIPG [26,27]. *PDGFRA* is the most commonly amplified and/or mutated receptor tyrosine kinase in DIPG, with *PDGFRA* amplification and mutations occurring in approximately 30 and 5% of DIPGs, respectively [3,28–30]. Therefore, inhibition of the PDGF pathway is considered a promising therapeutic strategy. Several modest (e.g., dasatinib) and potent (e.g., crenolanib) oral inhibitors of PDGFRA have been tested alone or in combination with RT, although none have shown significant activity or efficacy to date [31,32]. The main limitations of currently available PDGFRA inhibitors are their poor CNS penetration and predominantly cytostatic effect [27]. Limited CNS penetration may be overcome by utilizing PDGFRA inhibitors in combination with other agents designed to improve drug delivery. For example, dasatinib penetration into the CNS is limited primarily by drug efflux transporters (e.g., P-glycoprotein) [33]. The combination of dasatinib with efflux transporter inhibitors may accomplish higher drug concentrations in the CNS [34].

ALK2 inhibitors

Recurrent activating mutations in *ACVR1*, which encodes the activin A type receptor (ALK2), have been identified in 20–30% of DIPGs [5,12,35]. ALK2 is a bone morphogenetic protein (BMP) receptor kinase known to activate pathways integral to normal development and tissue repair [36]. Activating *ACVR1* mutations trigger the BMP signaling pathway, leading to phosphorylation of downstream proteins (e.g., SMAD), followed by upregulation of the signaling targets ID1/2 [35]. The ID proteins subsequently promote cell cycle progression via interactions with the Rb1 and p21 pathways [37]. Interestingly, germline gain-of-function mutations in *ACVR1*, some of them identical to those found in DIPG, have been described in fibrodysplasia ossificans progressiva [36,38–40], a rare autosomal dominant disorder. It remains unclear how activation of the BMP signaling pathway influences the tumorigenesis of DIPG. Small-molecule inhibitors of the BMP signaling pathway have been investigated in the management of several disease models, including cancer [41]. Recently, the highly selective ALK2 inhibitor K02288 was shown to inhibit the BMP-induced SMAD pathway [42]. However, further preclinical models are needed to investigate its potential therapeutic role in DIPG.

Mutations	<i>H3F3A</i> ^{K27M} (56–80%)
	<i>HIST1H3B/C</i> ^{K27M} (16–31%)
	<i>TP53</i> (50–72%)
	<i>ACVR1</i> (<i>ALK2</i> ; 17–33%)
	<i>PIK3CA</i> (0–20%)
	<i>PIK3R1</i> (7–8%)
	<i>PDGFRA</i> (5–12%)
	<i>BCOR/BCORL1</i> , <i>NF1</i> , <i>PPM1D</i> , <i>PTEN</i>
CNAs	<i>PDGFRA</i> ampl (15–30%)
	<i>CDK4/6</i> ampl (7–18%)
	<i>CCND1/2/3</i> ampl (~15%)
	<i>MYCN/MYC</i> (3–6%)
	1q gain, <i>MET</i> , <i>IGF1R</i>

Figure 2. Prevalence of specific mutations and copy number abnormalities seen in diffuse intrinsic pontine glioma.

CNA: Copy number abnormality.

EGF receptor & other receptor tyrosine kinase inhibitors

Although *EGFR* amplification and/or mutations (e.g., *EGFRvIII*) are the most common genetic aberrations found in glioblastoma in adults [43,44], genome-wide studies have demonstrated these genetic abnormalities in only a minority of DIPGs [28,29,45,46]. *EGFRvIII* expression by immunohistochemistry was observed in approximately half of a small cohort of DIPGs [47], but it remains unclear whether its expression alone harbors any meaningful clinical significance. Several clinical trials continue to investigate the role of EGF receptor (EGFR)-targeting agents in children with DIPG, including monoclonal antibodies (e.g., nimotuzumab [48,49]), small-molecule inhibitors (e.g., erlotinib [10,50]), and *EGFRvIII*-targeting peptide vaccines [51]. However, the rationale for this approach remains weak given the uncommon findings of EGFR pathway abnormalities in DIPG [5].

Although one study demonstrated *MET* and *IGF1R* amplification in 26 and 19% of DIPGs, respectively [29], subsequent studies found these abnormalities to be less common. Therefore, c-Met inhibitors may only be useful in a minority of children with DIPG. To our knowledge, small-molecule inhibitors of IGF1R have not yet been tested in children with cancer.

• Inhibitors of essential cellular pathways

Cell cycle inhibitors

Specific abnormalities in the Rb1 pathway consisting of focal amplification of genes encoding some of its components (e.g., *CCND1*, *CCND2*, *CCND3* [cyclins D1, D2 and D3, respectively], *CDK4* and *CDK6*), have been identified in approximately a third of DIPGs [29]. Other genetic abnormalities affecting this pathway, including *RB1* mutations/loss and *CDKN2A* loss, were uncommon in DIPG. D-type cyclins and cyclin-dependent kinases (CDK), particularly *CDK4/6*, are known to form complexes that phosphorylate and inactivate Rb1, thereby releasing transcription factors required for cell cycle progression. Since *RB1* remains intact in the majority of DIPGs [12], *CDK4/6* inhibitors keep Rb1 in its activated form, leading to inhibition of abnormal proliferation. The *CDK4/6* inhibitor PD-0332991 (palbociclib) was tested in a preclinical DIPG mouse model, and the combination of PD-0332991 with RT produced only modest improvement in survival [52]. Potential limitations of most *CDK4/6* inhibitors include their cytostatic mechanism of action and the uncommon loss of *CDKN2A* in DIPG, which is thought to confer sensitivity to these agents. Since concomitant amplification of genes from both Rb1 and receptor tyrosine kinase pathways occur in approximately 20% of DIPGs [29], the combination of such inhibitors should be tested in the future.

PI3K pathway inhibitors

There is a strong rationale for the use of PI3K pathway inhibitors in children with DIPG based on the prevalence of intrinsic molecular abnormalities in key components of this pathway, particularly activating mutations (e.g., *PIK3R1* and *PIK3CA*) and focal amplifications [12,29,30]. PI3K pathway activation also results from upstream activating changes in receptor kinases. Although several clinical trials already used rapamycin analogs and AKT inhibitors [53,54], specific PI3K inhibitors have not yet been tested in children. In a nonbrainstem glioblastoma xenograft murine model, the PI3K/mTOR inhibitor NVP-BEZ235 was shown to significantly impair DNA repair, thereby leading to tumor sensitization to RT and temozolomide and extending survival [55].

Hedgehog pathway inhibitors

Upregulation in the Hedgehog (Hh) signaling pathway has been demonstrated in DIPG via activation of patched 1 [56]. However, intrinsic

molecular abnormalities within this pathway were uncommon in DIPG [4]. In early preclinical work, activation of the Hh pathway enabled self-renewal of DIPG cells in a culture model; however, Hh pathway dysregulation alone was not found to be sufficient for DIPG formation in a murine model [57]. Although the Hh pathway may play a role in early DIPG formation, the potential usefulness of Hh pathway inhibitors in the treatment of children with DIPG remains unclear. Studies using Hh pathway inhibitors (e.g., smoothened inhibitor) are currently underway in children with DIPG.

• DNA repair mechanisms

PARP inhibitors

There is a strong rationale for combining DNA repair or cell cycle checkpoint inhibitors with RT and/or chemotherapy in the treatment of children with DIPG. PARP1/2 are nuclear proteins involved in sensing DNA damage and consequently activating DNA-repair proteins to escape apoptosis [58–60]. PARP-1 overexpression has been observed in DIPG, as well as low gains in chromosome 1q corresponding to the location of *PARP1* [28]. PARP1 overexpression may facilitate repair of damage secondary to RT or alkylating agents like temozolomide, which historically have been used in the treatment of DIPG [61–63]. Veliparib (ABT-888) is a PARP1/2 inhibitor shown to impair tumor growth in synergy with RT and alkylating agents [64]. A Phase I/II trial of ABT-888 with concurrent RT and temozolomide in children with newly diagnosed DIPG is currently underway. In addition, defects in DNA-repair mechanisms including deletions or loss of heterozygosity in the breast cancer 2, early-onset (*BRCA2*) pathway have been identified in approximately half of DIPGs [28], suggesting that select patients may be particularly susceptible to PARP inhibition as a therapeutic strategy.

WEE1 inhibitors

WEE1, a major regulatory kinase at the G2 checkpoint, is highly overexpressed in DIPGs [65,66]. Inhibition of WEE1 in cells exposed to DNA-damaging agents leads to abrogation of G2 arrest, with subsequent premature termination of DNA repair and cell death [67]. In preclinical studies, small-molecule inhibitors of WEE1 showed a synergistic interaction with RT against both DIPG and glioblastoma models *in vivo* [65–67]. A Phase I trial is currently investigating the use of MK-1775, an oral WEE1 inhibitor, concurrently

with RT. Another clinical trial will combine this agent with chemotherapy in children with recurrent/progressive cancer.

PPM1D inhibitors

PPM1D is a p53-inducible phosphatase whose overexpression leads to oncogenesis via suppression of multiple targets including p53. [68] *PPM1D* amplification and mRNA overexpression have been identified in various tumor models [68]. Recently, *PPM1D* mutations were identified in more than a third of DIPGs harboring the *H3F3A* K27M mutation [69]. Interestingly, mutations in *PPM1D* and *TP53* were mutually exclusive. Furthermore, gain-of-function *PPM1D* mutations may abrogate the cell cycle checkpoint by suppressing activation of DNA repair proteins [69]. Small-molecule inhibitors of PPM1D are currently under investigation in preclinical studies [70,71], with potential for utility in a select subgroup of DIPG patients.

• Epigenetic regulation

Molecular abnormalities affecting epigenetic mechanisms are among the most prevalent and potential promising targets in DIPG. *H3F3A* K27M, which is found in approximately two-thirds of DIPGs [13,56,72], leads to a decrease in K27 di- and tri-methylation by potently inhibiting the histone methyltransferase EZH2 [73,74]. This mutation also leads to decreases or increases in the transcription of specific subsets of genes [74]. In an *in vivo* DIPG model, *H3F3A* K27M in association with p53 loss caused proliferation of ectopic cell clusters but did not lead to tumor formation [73]. Despite a recent surge in research focused on understanding the potential therapeutic opportunities associated with histone mutations in DIPG, their full functional consequences remain unclear. There is some evidence that EZH2 inhibitors, which are promising agents for different types of cancer, may be useful in *H3F3A*-mutated DIPGs, as EZH2 was shown to co-immunoprecipitate with transcriptionally activated genes in the presence of H3.3K27M-mutant protein [74]. Since H3.3 mutations have been identified concomitantly with abnormalities in *PDGFRA* and *ACVR1* [4,13,75,76], further preclinical studies that investigate combinatorial therapies are warranted.

Immunotherapy

Immunotherapy has emerged as one of the most promising treatment strategies for several types

of cancers in adults [77]. Multiple preclinical studies have tested different strategies in brain cancer models, including peptide-based vaccines [78], dendritic-pulsed vaccines [79,80], chimeric antigen receptors [81], natural killer cells [82] and immune checkpoint (CTLA-4 and PD-1/PDL-1) inhibitors [83]. None of these studies focused exclusively on DIPG, and only a few clinical trials are underway or under planning to use these strategies in children with DIPG. One such recent pilot study demonstrated the feasibility of subcutaneous vaccinations with glioma-associated antigen epitope peptides (EphA2, IL-13R α 2 and survivin) in HLA-A2-positive children with newly diagnosed DIPG. These vaccinations were well-tolerated, with preliminary evidence to suggest potential immunologic and clinical responses [78]. However, this study raised several important concerns: some of these strategies may be applicable only to a select group of patients (e.g., patients not requiring corticosteroids); a

high prevalence of pseudoprogression (approximately 20% of cases) led to serious neurologic toxicities in a few patients. Nonetheless, immunotherapy should be further investigated in children with DIPG, and future studies are needed to evaluate how best to integrate this approach with other treatment modalities.

New drug delivery modalities

Convection-enhanced delivery (CED) is an experimental modality in the treatment of malignant gliomas, in which continuous infusion of a therapeutic agent through a site-directed catheter is utilized to facilitate a pressure gradient in an effort to bypass the blood–brain barrier. Allhenn *et al.* and Bidros *et al.* comprehensively reviewed the literature on the delivery of diverse agents via CED in adult malignant gliomas, including chemotherapy, immunotoxins and targeted therapies [84,85]. Several clinical trials have used conventional chemotherapeutic drugs (e.g.,

Figure 3. In an optimal model, children with diffuse intrinsic pontine glioma and their families receive care from a primary neuro-oncology team, with early integration of pediatric palliative care that begins at the time of diagnosis and continues throughout the illness trajectory in cooperation with the primary medical team.

topotecan and carboplatin) or investigational agents (e.g., IL-13-pseudomonas exotoxin [IL-PE38QQR] and ^{124}I -8H9) in the treatment of children with DIPG. Despite the appeal of delivering high concentrations of the desired agents to specific targets, this technique continues to have several shortcomings, including reliance on skilled well-trained neurosurgeons and variable drug distribution due to nonuniform tumor shape, presence of cystic structures or fiber tracks, and proximity to pial or ependymal surfaces. Based on these limitations, CED will require further testing and refinement of technical issues before it can be incorporated into larger clinical trials for children with DIPG.

Quality of life

Despite the significant advances described above, the majority of children with DIPG unfortunately succumb to their disease within a short time. During their illness trajectory, these children suffer from multiple symptoms, including pain, fatigue, depression, nausea and vomiting, seizures and other neurologic deficits [86]. Experience shows that children with cancer who receive optimal palliative care at the end of life have improved symptom control and less suffering [87,88]. Leaders in the field of pediatric palliative care have advocated that an ideal model for compassionate care involves early integration of palliative care principles by the primary neuro-oncology team in conjunction with parallel involvement from a subspecialty palliative care team, with both parties journeying cooperatively with the patient and family from the time of diagnosis to the end-of-life (Figure 3) [89,90].

Conclusion & future perspective

Similar to other nonbrainstem pediatric and adult glioblastoma [91], molecular tumor heterogeneity in DIPG is believed to partially account for its resistance to current targeted therapies. Although this profound diversity has already been shown by FISH analysis [29], more detailed and sophisticated research is underway to further refine our knowledge about the molecular heterogeneity of DIPG [3–5,29,30]. Following a similar paradigm of personalized therapies adopted recently in several adult cancers [92], our hope is that a better understanding of the molecular landscape of each patient's tumor will lead to the use of optimal personalized combinations of agents for children with DIPG.

Analogous to other glioblastomas, the molecular characteristics of DIPGs likely vary over time, particularly in the context of therapy. Since single or multiple biopsies of the tumor to assess molecular characteristics may not be feasible or reliable, the assessment of such characteristics in other body fluids (e.g., blood and cerebrospinal fluid) is particularly appealing in patients with DIPG. For example, assessment of specific tumor characteristics in tumor-derived microvesicles in the plasma and cerebrospinal fluid has been conducted in adults with glioblastoma [93]. Future studies should investigate the feasibility of indirectly assessing the molecular characteristics of DIPG by analyzing tumor DNA and RNA shed into more easily accessible body fluids.

Finally, multiple reliable preclinical models of DIPG that mimic the human disease have been established [26,57]. Similar to what has already been done for other pediatric brain cancers [94,95], multiple groups are performing high-throughput screening of thousands of agents in these DIPG models. It remains uncertain whether testing *in vitro* and *in vivo* will reliably predict effective agents in human disease. However, we hope that such testing may at least lead to more efficient design of clinical trials. For example, drugs that fail in the laboratory will likely no longer undergo testing in children with DIPG.

Despite the fact that DIPG remains a lethal cancer, for the first time, scientists and clinicians currently possess the appropriate tools and knowledge to significantly impact the lives of children affected by this fatal disease. We hope that future trials for children with DIPG will continue to explore the unique molecular characteristics of these tumors with concomitant application of relevant therapeutic strategies built on our rapidly increasing body of knowledge.

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