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Review

# Children are not just little adults: recent advances in understanding of diffuse intrinsic pontine glioma biology

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Diffuse intrinsic pontine glioma (DIPG) is a high-grade glioma that originates in the pons and is seen exclusively in children. Despite numerous efforts to improve treatment, DIPG remains incurable with 90% of children dying within 2 y of diagnosis, making it one of the leading causes of death in children with brain tumors. With the advent of new genomic tools, the genetic landscape of DIPG is slowly being unraveled. The most common genetic alterations include a K27M mutation in H3.3 or H3.1, which are found in up to 78% of DIPGs, whereas p53 mutations are found in up to 77%. Other recently discovered alterations include amplification of components of the receptor tyrosine kinase/Ras/phosphatidylinositol 3-kinase signaling pathway, particularly platelet-derived growth factor receptor A. Recapitulating such alterations, genetically engineered DIPG preclinical models have been developed, and DIPG xenograft models have also been established. Both models have strengths and weaknesses but can help with the prioritization of novel agents for clinical trials for children with DIPG. As we move forward, it is important that we continue to study the complex and unique biology of DIPG and develop improved preclinical models to increase our understanding of DIPG pathogenesis, allowing translation into successful therapies in the not too distant future.

An estimated 4,000 new malignant and nonmalignant brain tumors are diagnosed annually in children in the United States (1,2). Fifteen percent of malignant central nervous system tumors in children younger than 20 y of age arise in the brainstem, with the majority being diffuse intrinsic pontine glioma (DIPG) subtype (1,3). DIPG is a high-grade glioma (HGG) that originates in the pons and is seen almost exclusively in children with a median age of diagnosis of 6–7 y (4–6). Despite numerous efforts to improve treatment, prognosis remains poor with more than 90% of children dying within 2 y of diagnosis, making it one of the major causes of brain-related death in children (4,7).

A key to improving these outcomes is to gain a better understanding of DIPG tumor biology. Historically, pediatric HGGs were thought to resemble adult HGGs, and children were treated with the same drugs that were being evaluated in adults. However, new biologic, molecular, and genetic data have clearly demonstrated that pediatric DIPG have distinct genetic alterations as compared with adult HGGs and even with pediatric supratentorial HGGs. This strongly suggests that effective therapies for DIPG may be distinct from effective therapies for HGGs. This review will present an overview of DIPG presentation and current therapy and discuss how new unique genetic findings are shaping future research.

## PRESENTATION AND DIAGNOSIS

Patients with DIPG typically present with less than 3 mo of preceding neurological symptoms that can vary based on lesion location. Over 50% present with the "classic" triad of cranial nerve deficits (diplopia and facial asymmetry), long tract signs (hyperrefflexia, upward Babinski, and decreased strength), and cerebellar signs (ataxia, dysmetria, and dysarthria) (8,9). Abducens palsy (cranial nerve VI) is almost always the first clinical sign, and its presence is a highly sensitive predictor of DIPG (9). Signs of increased intracranial pressure are not typically seen, but in <10% of patients the tumor may extend posteriorly and cause obstructive hydrocephalus. DIPG tumors rarely metastasize to distant sites but can expand along known fiber tracts into the cerebellum/ thalamus (10).

DIPG diagnosis is based on clinical history—including physical symptoms and time to presentation—combined with radiologic magnetic resonance imaging findings. Typical DIPG tumors are hypointense with indistinct margins on magnetic resonance imaging T1-weighted images, whereas they are hyperintense on T2-weighted/fluid-attenuated inversion recovery images (**Figure 1**) (4,9). Gadolinium enhancement is often absent or minimal, which distinguishes DIPG from pilocytic astrocytomas or other central nervous system tumors (11). Historically, classic findings on physical exam and imaging were sufficient for DIPG diagnosis, and biopsy confirmation was thought to be an unnecessary risk because it did not change disease management (12). The lack of clear biopsy benefit combined with improved diagnostic imaging capabilities led to magnetic resonance imaging scans becoming the diagnostic standard of care for DIPGs in the United States.

In France, biopsies have been routinely performed since 2003 for both atypical and typical DIPG evaluation and have been associated with minimal morbidity and high diagnostic

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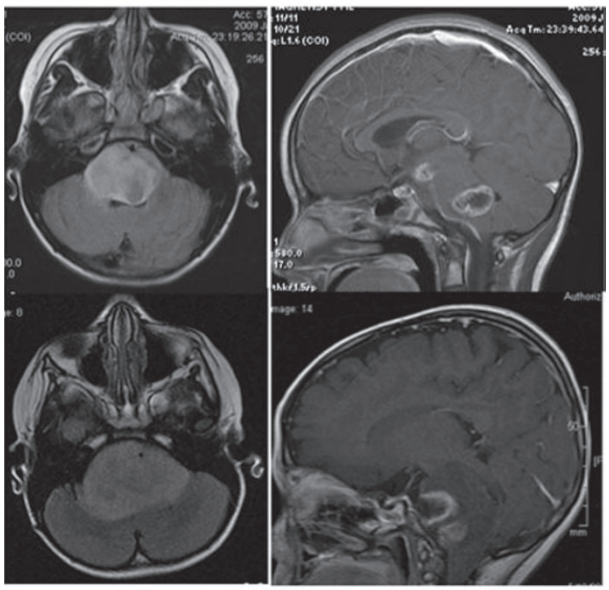


**Figure 1.** Typical magnetic resonance imaging images of diffuse intrinsic pontine glioma (DIPG). Axial fluid-attenuated inversion recovery (left) and sagittal T1 with contrast (right) of a (**a**) more dorsally located DIPG and (**b**) more ventrally located DIPG. Figure reproduced with permission from Oxford University Press.

yield (13). This surgical success has also led to an increase in available DIPG tumor samples, providing much of the new biologic, molecular, and genetic data that are currently advancing our understanding of DIPG biology. Some of the identified biologic markers have been shown to correlate with progression-free survival and may be useful to stratify patients in future clinical trials (14). With the potential for new therapeutic and diagnostic options combined with the relative low morbidity, the benefit of biopsies is being reconsidered and may eventually be included as part of the routine diagnostic evaluation for DIPG in the United States (15).

## TREATMENT

### Radiotherapy

The standard treatment for patients with DIPG remains conventional radiotherapy with a treatment dose of 54–60 Gy, fractionated over a 6-wk period (7). This treatment has been shown to transiently improve neurologic function or temporarily stabilize disease in 70% of patients, but the effect on overall survival is minimal, with mean progression-free survival of 5.8 mo compared with 5 mo without radiotherapy (4). In an effort to improve quality of life for children with DIPG, hypofractionated radiotherapy was evaluated, shortening the time children spent in the hospital. Limiting radiation to 3 Gy daily for 3 wk reduced toxicity without negatively impacting overall survival (16).

### Chemotherapy/Targeted Agents

With the known benefit of radiation, numerous studies have looked at combining traditional cytotoxic chemotherapeutic agents or targeted therapeutics before, during, and after radiotherapy. In 2006, Hargrave *et al.* (7) reviewed 29 clinical studies completed in 1984–2005, and in 2012, Jansen *et al.* (17) reviewed an additional 26 studies completed in 2005–2011, and none of the studies demonstrated improvement in the response rate, event-free survival, or overall survival in children with DIPG. It is worth noting that numerous combinations of cytotoxic agents have been evaluated in this disease, but very few combinations of targeted agents have been tested.

### Drug Delivery Strategies

The lack of efficacy of systemic chemotherapy or targeted agents may be due to the inherent resistance of DIPG cells to such treatments, or alternatively due to inadequate delivery of these agents into the tumor. In support of the latter explanation, it is well-known that DIPGs have a relatively intact blood–brain barrier as evident by the minimal contrast enhancement with magnetic resonance imaging. This impermeability is due to tight junctions between the endothelial cells combined with limited transcellular transport and expression of adenosine triphosphate–binding cassette transporters such as multidrug resistance (MDR) and adenosine triphosphate–binding cassette subfamily G member 2 on the endothelial cells. To bypass the blood–brain barrier in DIPG, several approaches have been evaluated thus far, with limited success, including hyperosmotic solutions (18), bradykinin analogs (19), and an MDR inhibitor (20). No survival benefit was seen, and additionally, the MDR inhibitor increased chemotherapy toxicity.

Direct infusion of the therapeutic agent into the tumor bed via convection-enhanced delivery (CED) is another approach that is now gaining momentum. CED uses a stereotactically placed catheter with attached pump to provide positive pressure and maintain convective flow, bypassing the blood–brain barrier and providing direct localized drug delivery into the tumor (21). Based on moderate success in adult trials, a recent pediatric DIPG phase I trial using CED with topotecan demonstrated technical feasibility, but with increased chemotherapy volume and flow, there was significant morbidity (22). The optimal infusion rate, infusion time, and agent continue to be debated, but CED remains a promising approach for the treatment of DIPG.

## GENETIC ALTERATIONS

With the advent of genomic tools, the genetic landscape of DIPG is slowly being unraveled. Several molecular studies have shown that DIPG have distinct alterations when compared with adult HGG and non-DIPG pediatric HGG (**Table 1**). For example, Paugh *et al.* (23) completed the largest DIPG autopsy cohort study with $n = 43$ and found that gain of 1q is more common in DIPG compared with that in adult HGG, and alternately, epidermal growth factor receptor amplification, which is one of the most frequently amplified genes in adult HGG, was rarely seen in DIPG autopsy samples. Compared with nonbrainstem HGG, DIPG have an increased frequency of 17p loss and an absence of *CDKN2A* or *CDKN2B* deletion (23,24). In the first comprehensive genomic analysis of diagnostic biopsy DIPG samples, Puget *et al.* (25) demonstrated that DIPG have distinct gene expression profiles compared with pediatric supratentorial HGG, with increased expression of homeobox and HLH

**Table 1.** Distinct molecular genetics of DIPG as compared with pediatric high-grade glioma (non-DIPG) and adult high-grade gliomas

| | DIPG (%) | Pediatric high-grade glioma (non-DIPG) (%) | Adult high-grade glioma (%) | References |
|---|---|---|---|---|
| ATRX mutation | 9 | 31 | 14 | (28,36) |
| CDKN2A/B deletion | 0–9 | 8–26 | 55 | (23,25,37–39) |
| EGFR amplification | 0–18 | 0–19 | 40–55 | (37–44) |
| H3F3A mutation-K27M | 60–71 | 19 | <3 | (28,30,36) |
| H3F3A mutation-G34R/V | 0 | 13–14 | <3 | (28,30,36) |
| Hist1H3B (K27M) | 18 | 3 | NA | (30) |
| IDH1/2 mutations | 0 | 10–16.3 | 42 | (28,37,45,46) |
| TP53 mutation | 40–77 | 21–54 | 33–43 | (27,28,38,47,48) |
| SETD2 mutation | NA | 15 | 8 | (49) |
| PDGFR-A amplification | 13–36 | 4–10 | 11 | (23–25,29,37,39) |
| Loss 10q/PTEN | 3–64 | 20–38 | 42–80 | (23,25,27,29,37,39,42) |
| Gain 1q | 23–64 | 13–43 | 8–9 | (37,39,50) |
| Gain 2q | 26 | 8 | 3 | (23) |
| Gain 8q | 28 | 5 | 5 | (23) |
| Gain 9q | 28 | 10 | 8 | (23) |
| Gain 7p/7q | 14/9 | 13/15 | 70/74 | (23,37) |
| Loss 16q | 49 | 18–26 | 7 | (23,37,50) |
| Loss 17p | 31–64 | 4–25 | 9 | (23,24,39,50) |
| Loss 20p | 26 | 3 | 1 | (23) |
| Loss 21q | 2 | 21 | 3 | (23) |
| Loss 3q | 0 | 21 | 4 | (23) |
| Loss 4q | 7 | 21–54 | 2 | (23,24,37,43) |

ATRX, α-thalassemia/mental retardation syndrome X-linked; CDKN2A/B, cyclin dependent kinase inhibitor 2A/2B; DIPG, diffuse intrinsic pontine glioma; EGFR, epidermal growth factor receptor; NA, not available; PDGFR-A, platelet-derived growth factor receptor A; PTEN, phosphatase and tensin homolog.

genes in the DIPG samples. In contrast to adult HGGs, isocitrate dehydrogenase 1/2 mutations were not observed in this cohort (26). In addition, two distinct DIPG subgroups were identified: oligodendroglial, associated with platelet-derived growth factor receptor A amplification, and a second mesenchymal and proangiogenic phenotype (25).

Other known mutations in DIPG include p53 mutations in 75% of DIPG samples, PIK3CA mutations in 15% of DIPG samples, and platelet-derived growth factor receptor A mutations in 10% of DIPG samples (25,27,28). In addition to mutations, up to 50% of DIPGs harbor gains in components of the receptor tyrosine kinase/Ras/phosphatidylinositol 3-kinase/v-akt murine thymoma viral oncogene homolog 1 (AKT) signaling network with gains in platelet-derived growth factor receptor A as the most commonly amplified component. Other components include *c-MET* (also known as hepatocyte growth factor receptor), insulin-like growth factor receptor, insulin-like growth factor 2, hepatocyte growth factor, *AKT1*, Kirsten rat sarcoma viral oncogene homolog (*KRAS*), *PIK3CA*, *PIKC2G*, epidermal growth factor receptor, erythroblastic leukemia viral oncogene homolog-4 (*ERBB4*), and *AKT3*. Cell-cycle control genes are also gained in up to 30% of DIPGs such as *CDK4*, *CDK6*, and D-type cyclins (23,24,29). Finally, in addition to the above-mentioned loss of 17p, there are other broad chromosomal gains and losses that are particularly common in DIPGs compared with nonbrainstem HGG, including gains of chromosomes 2q, 8q, and 9q and losses of 16q and 20p (23,29).

Using whole-genome sequencing, Wu *et al.* (30) examined 7 DIPGs combined with targeted sequencing from 43 additional DIPG and nonbrainstem HGG samples. Over 75% of DIPG samples were found to have mutations in either *H3F3A* or *HIST1H3B*, which encode histone variant H3.3 or H3.1, respectively, resulting in a Lys27Met amino acid substitution. This residue is in the highly conserved N-terminal of the histone H3 protein, and mutations can effect the epigenetic regulation of gene expression, including nucleosome structure and interactions between the histone complex and transcription modifiers. Previous studies have shown either methylation or acetylation of histone H3 Lys27 can control gene activation (31,32). Most recently, Lewis *et al.* (33) demonstrated that the K27M histone mutation is a gain of function mutation that inhibits the activity of the polycomb repressive complex 2. The exact mechanism by which inhibition of polycomb repressive complex 2 contributes to DIPG pathogenesis is currently the subject of intense investigation.

## DIPG MOUSE MODELS

Prior to 2010, researchers used adult glioma cell lines implanted into the brainstem of immunodeficient mice as DIPG

preclinical tools, with the assumption that the genetic alterations of adult gliomas are similar to the genetic alterations of DIPG. Since 2010, two complementary approaches have been used to develop improved DIPG mouse models: DIPG xenograft models and genetically engineered DIPG mouse models. Monje *et al.* (34) have successfully cultured DIPG tumor cells from postmortem tissue and implanted them into immunodeficient mice to create the first-published DIPG xenograft model. The advantages of this approach are that this model arose from an actual DIPG human tumor with all of its complex genomic heterogeneity. A disadvantage of this model is that using immunodeficient mice removes the immune system as a component of DIPG biology. A second disadvantage is that this modeling approach assumes that the complex stroma–tumor interactions in DIPG are conserved when one uses human tumor cells with a mouse stroma.

Alternately, a genetically engineered mouse model of DIPG was developed by Becher and colleagues (33,35), using the replication-competent ASLV long terminal repeat with a splice acceptor (RCAS)/tv-a system to overexpress PDGF-B in nestin-expressing cells, in conjunction with ink4a-ARF loss initially and more recently with p53 loss and H3.3K27M (**Figure 2**). Tumors generated using this model have histologic and immunophenotypic similarities to human DIPG samples, and this mouse model has been successfully used in preclinical screening. Advantages of the genetically engineered mouse modeling approach include the ability to generate tumors in the brainstem of immunocompetent mice and the ability to study the contribution of each genetic alteration to DIPG biology. In addition, tumors arising with this approach are infiltrating, invading the surrounding normal brain as seen in human DIPG (**Figure 2d**). However, this genetic mouse modeling approach also has limitations. First, it is likely that DIPGs that form in this model do not have the full genetic complexity of the human disease. Second, most of the tumors arise from nestin-expressing progenitors that reside in the floor of the fourth ventricle or aqueduct of the neonatal dorsal brainstem, whereas the majority of human DIPGs are thought to arise from the ventral brainstem (34,35). Finally, tumors arising with this model are more focal and usually do not occupy the entire pons as the human tumors do. In summary, both models can help to prioritize the translation of novel agents into clinical trials for children with DIPG, and preclinical models of adult gliomas should no longer be used to prioritize the translation of novel agents to treat children with DIPG.

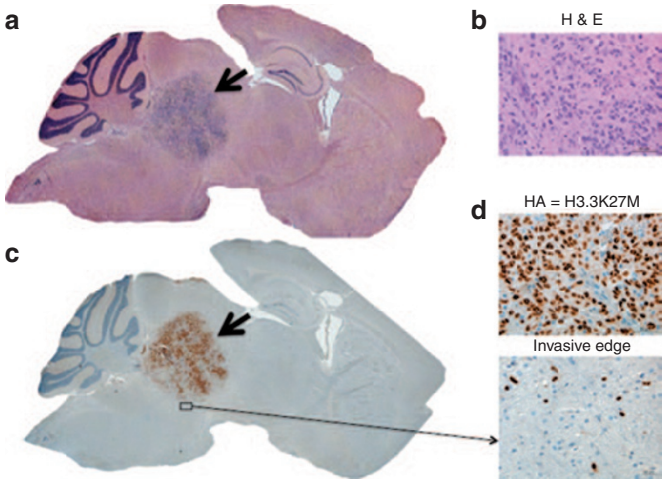


**Figure 2.** A genetically engineered mouse model of H3.3K27M mutant diffuse intrinsic pontine glioma (DIPG). (**a**) Sagittal hematoxylin and eosin (H&E) image of DIPG-bearing mouse (PDGF-B; H3.3K27M; p53 loss). The arrow points to tumor. (**b**) 40× Magnification H&E of DIPG-bearing mouse from **a**. Scale bar = 50 µm. (**c**) Sagittal immunohistochemistry image for hemagglutinin (HA) (H3.3K27M is (HA) tagged) of DIPG-bearing mouse from **a**. The arrow points to tumor. (**d**) 40× Magnification immunohistochemistry images for HA from **c**. Top image is of the tumor bulk and the bottom image is of the invasive edge. Scale bar = 50 µm.

## FUTURE DIRECTIONS

Previously, novel agents that were being evaluated in clinical trials for DIPG were primarily chosen based on antitumor activity in adult glioma preclinical models; this is no longer the case. Currently, genetically engineered DIPG preclinical models and DIPG xenograft models guide future clinical trials for DIPG, and ideally, agents that demonstrate efficacy in both genetic and xenograft models should be prioritized for translation into a clinical trial (34,35).

With the recent explosion in knowledge regarding the genetic alterations in DIPG, it will be important to determine the prognostic implication of each. In addition, prognostic factors that have been identified retrospectively should be evaluated prospectively in future clinical trials for children with DIPG. As we move forward, it is important that we continue to study the complex and unique biology of DIPG and develop improved preclinical models to increase our understanding of DIPG pathogenesis, allowing translation into successful therapies in the not too distant future.

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