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# A new hope: Clinical advances in targeted therapies for pediatric diffuse midline glioma

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**Abstract**

Diffuse midline glioma (DMG) is an aggressive pediatric brain tumor with a 1%–2% overall survival, largely due to the ineffectiveness of conventional treatments such as chemotherapy and radiotherapy, as well as the inoperability of the tumors because of their critical location and infiltrative diffuse growth. Recent advances in targeted therapies offer new hope, particularly those addressing key molecular characteristics and newly identified cancer dependencies. Among these are histone deacetylase inhibitors (HDACis), receptor tyrosine kinase inhibitors, and novel agents such as ONC201 and unesbulin that target metabolic and epigenetic pathways respectively. In addition, emerging therapies like FACT inhibitors and polyamine pathway inhibitors are showing promise by disrupting critical cancer cell processes. Immunotherapies, including CAR-T cells targeting surface antigens such as GD2 and B7-H3, cancer vaccines, monoclonal antibodies, and oncolytic viruses, are also gaining traction, offering a new approach by harnessing the immune system to attack tumor cells. Despite these advances, challenges such as drug delivery across the blood–brain barrier and therapeutic resistance persist, necessitating the development of combination therapies and innovative delivery methods. Ongoing research is focused on refining these strategies and exploring additional molecular and immunological targets to improve outcomes for children with DMG.

## Key Points

- Promising DMG-targeted therapies are often limited by poor BBB-penetrability and single-agent efficacy, requiring novel delivery systems and combination therapies.
- Innovative approaches, including immunotherapies and targeting polyamine metabolism and epigenetic drivers, bring new hope.

Diffuse midline glioma, H3K27 altered (DMG), is a highly aggressive, rare brain cancer in children and adolescents, with approximately 300 new cases diagnosed annually in the United States.[1,2] This cancer is usually unable to be resected due to its infiltrative nature and location in critical brain regions such as the brainstem. Current pharmacological therapies, including chemotherapies, are ineffective despite extensive trials,[3,4] and radiotherapy only marginally extends survival.[5] Evidence from microdialysis studies in the brains of rhesus macaques suggested that the blood–brain barrier (BBB) is notably intact in the pons which could notably impede drug penetration.[6] This is further reflected in DMG MRIs, which do not reliably show enhancement, indicating a highly intact BBB.[7] While tumors may enhance after radiotherapy, indicating BBB deterioration, this change is insufficient to significantly improve drug distribution.[8] Consequently, DMG has the poorest prognosis of all pediatric cancers, with less than 10% of patients surviving beyond 2 years.[9] Previously referred to as diffuse intrinsic pontine gliomas (DIPGs) due to their prevalence in the brainstem and more specifically, the pons, DMGs now also include tumors in other midline regions, such as the thalamus, cerebellum, and spine.[10] The

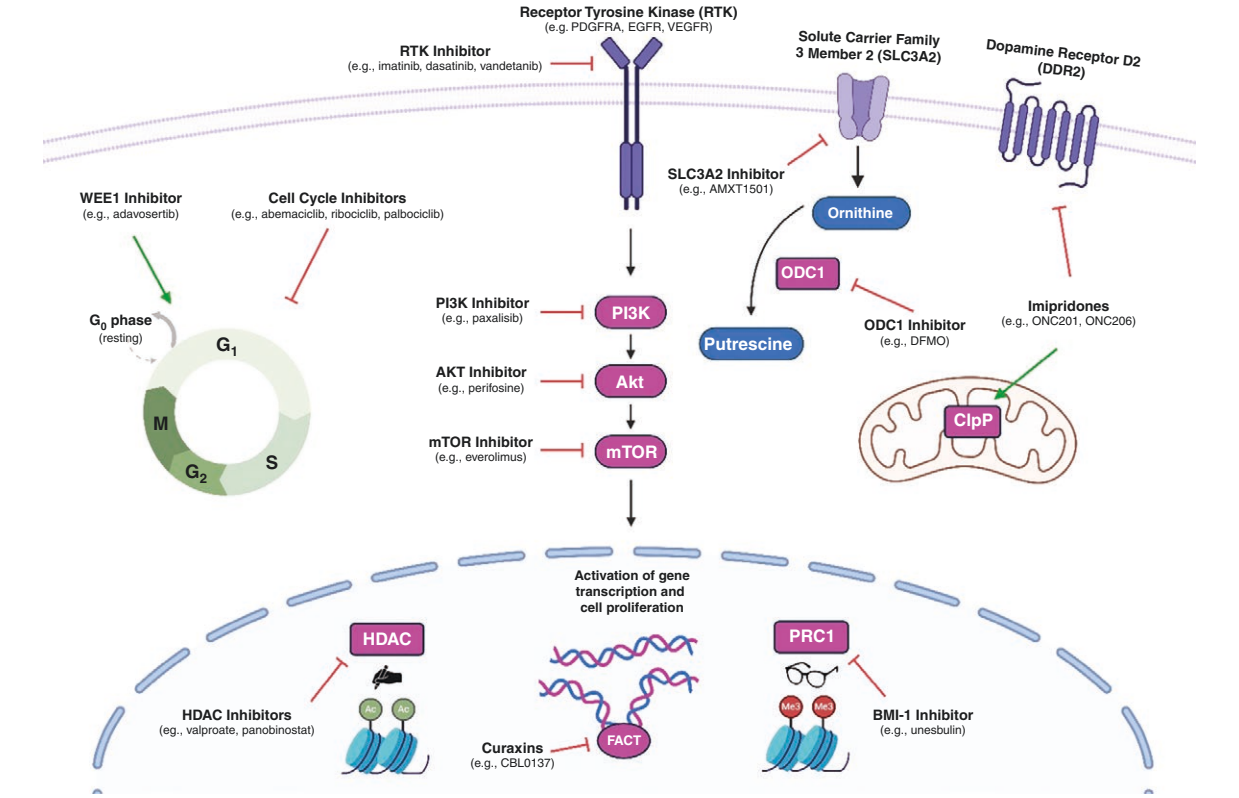


**Figure 1.** Therapeutic strategies targeting key pathways in pediatric diffuse midline glioma (created with BioRender). Pediatric diffuse midline gliomas (DMGs) harbor alterations to cell cycle signaling, which can be targeted with inhibitors to wee1-like protein kinase (WEE1) to promote premature G2-M progression or cell cycle inhibitors to prevent mitosis. Several receptor tyrosine kinases (RTKs), including platelet-derived growth factor receptor A (PDGFRA), are dysregulated in DMGs. This activates the phosphoinositide 3-kinase/Ak strain transforming/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway, leading to gene transcription, cell survival, and proliferation. Several trials have explored single and combination therapies targeting different points in this pathway. Histone deacetylases (HDACs) are histone "writers" that deposit acetylation marks on histones, a marker of active transcription. DMGs overexpress H3K27 acetylation (H3K27Ac) and paradoxically, increasing H3K27Ac with HDAC inhibitors is a potential therapeutic strategy by detoxifying oncohistones. Trapping the facilitating chromatin transcription complex (FACT) to DNA using the novel agent CBL0137 can disrupt cancer cell stemness and proliferation. BMI-1 is a subunit of the polycomb repressive complex 1 (PRC), a reader complex that interprets the H3K27 trimethylation (H3K27Me3) mark, driving cancer initiation and maintaining stem-like properties in cells. It is overexpressed in DMGs and the inhibition of BMI-1 has promoted cell senescence in preclinical study. DMGs rely on polyamines for proliferation, and the emerging strategy of targeting polyamine uptake and synthesis through solute carrier family 3 member 2 (SLC3A2) and ornithine decarboxylase (ODC1) inhibitors is under clinical investigation. Novel imipridones have multiple mechanisms of action, including inhibiting dopamine receptor D2 as well as activating ATP-dependent Clp protease proteolytic subunit (ClpP). Blunt arrows from compounds indicate negative regulation; pointed arrows from compounds indicate positive regulation. Akt, Ak strain transforming; BMI-1, B lymphoma Mo-MLV insertion region 1 homolog; ClpP, ATP-dependent Clp protease proteolytic subunit; HDAC, histone deacetylase; mTOR, mammalian target of rapamycin; ODC1, ornithine decarboxylase; PI3K, phosphoinositide 3-kinase; PRC1, polycomb repressive complex 1; RTK, receptor tyrosine kinase; SLC3A2, solute carrier family 3 member 2; WEE1, wee1-like protein kinase

lack of pediatric tumor models initially hampered DMG therapy development, as biopsies were traditionally avoided due to perceived risks. With technological advancements in stereotactic surgery, biopsies have an improved safety profile and are more commonly performed at diagnosis and/or progression.[11–13] The availability of tumor tissue has rapidly accelerated research efforts, enabling the identification of novel molecular targets, investigation of targeted therapies, and advancement of translational research (Figure 1). This review provides an update on targeted therapies for DMG that have undergone clinical trials or are currently in development.

## Epigenetic Therapies

Approximately 80% of DMGs are driven by the lysine-to-methionine substitution at position 27 in histone H3 (H3K27M),[14] occurring in one of two genes encoding for the histone H3 isoforms, either in the *H3F3A* (~60% of DMGs) or *HIST1H3B* (~20% of DMGs) genes. This mutation has been shown to sequester the catalytic unit of the polycomb repressive complex 2 (PRC2), specifically the enhancer of zeste homolog 2 (EZH2). EZH2 is responsible for depositing the repressive methylation

**Table 1.** Epigenetic Therapies in Clinical Studies or Clinical Development

| Target | Compound | Clinical trial title | Clinical trial identifier | Start date | Status |
|---|---|---|---|---|---|
| HDAC | Valproate | Valproic Acid in Treating Young Patients With Recurrent or Refractory Solid Tumors or CNS Tumors | NCT00107458 | 2005-05 | Completed |
| HDAC | Valproate + bevacizumab | Valproic Acid, Radiation, and Bevacizumab in Children With High-Grade Gliomas or Diffuse Intrinsic Pontine Glioma | NCT00879437 | 2009-09-01 | Completed |
| HDAC | Valproate + temozolomide | International Cooperative Phase III Trial of the HIT-HGG Study Group (HIT-HGG-2013) (HIT-HGG-2013) | NCT03243461 | 2018-07-17 | Completed |
| HDAC | Vorinostat | Vorinostat and Radiation Therapy Followed by Maintenance Therapy With Vorinostat in Treating Younger Patients With Newly Diagnosed Diffuse Intrinsic Pontine Glioma | NCT01189266 | 2010-08-09 | Completed |
| HDAC | Vorinostat + temsirolimus | Vorinostat and Temsirolimus With or Without Radiation Therapy in Treating Younger Patients With Newly Diagnosed or Progressive Diffuse Intrinsic Pontine Glioma | NCT02420613 | 2015-10-05 | Active, not recruiting |
| HDAC | Panobinostat | Trial of Panobinostat in Children With Diffuse Intrinsic Pontine Glioma (PBTC-047) | NCT02717455 | 2016-06-28 | Completed |
| HDAC | Panobinostat + everolimus | A Study of Panobinostat in Combination With Everolimus for Children and Young Adults With Gliomas | NCT03632317 | 2019-10 | Withdrawn |
| HDAC | MTX110 (Panobinostat nanoparticle formulation) | MTX110 by Convection-Enhanced Delivery in Treating Participants With Newly-Diagnosed Diffuse Intrinsic Pontine Glioma (PNOC015) | NCT03566199 | 2018-05-22 | Completed |
| HDAC | MTX110 | CED of MTX110 Newly Diagnosed Diffuse Midline Gliomas | NCT04264143 | 2020-03-10 | Completed |
| HDAC | Panobinostat | Non-Invasive Focused Ultrasound (FUS) With Oral Panobinostat in Children With Progressive Diffuse Midline Glioma (DMG) | NCT04804709 | 2023-12 | Active, not recruiting |
| HDAC + Proteosome inhibitor | Panobinostat + marizomib | Phase I Study of Marizomib + Panobinostat for Children With DIPG | NCT04341311 | 2020-08-10 | Terminated |
| HDAC/PI3K | Fimepinostat | Fimepinostat in Treating Brain Tumors in Children and Young Adults | NCT03893487 | 2019-08-07 | Active, not recruiting |
| FACT | CBL | CBL0137 for the Treatment of Relapsed or Refractory Solid Tumors, Including CNS Tumors and Lymphoma | NCT04870944 | 2022-01-28 | Recruiting |
| BMI-1 | Unesbulin (PTC596) | A Phase 1b Study of PTC596 in Children With Newly Diagnosed Diffuse Intrinsic Pontine Glioma and High-Grade Glioma | NCT03605550 | 2018-08-01 | Active, not recruiting |

mark on histone H3.[15] This mutation exerts a dominant effect, which prevents the spreading of the methylation mark, causing a global loss of H3K27 trimethylation (H3K27Me3), including on wild-type histones and DNA hypomethylation,[16,17] thus causing an aberrant increase in gene expression and epigenetic reprogramming.[18] However, this model has recently been challenged, with evidence suggesting that regions of H3K27M oncohistones that colocalize with H3K27 acetylated sites exclude PRC2, thereby preventing methylation.[19,20] Regardless of the precise mechanism, it is evident that the *H3K27M* mutation and subsequent histone hypomethylation are essential to DMG tumorigenesis. However, the H3 protein itself remains "undruggable," prompting significant interest in developing therapies aimed at reversing or modulating the aberrant epigenetic changes observed in DMGs (Table 1).

## Histone Deacetylase Inhibition

DMGs exhibit elevated levels of H3K27 acetylation (H3K27ac), a reversible histone modification associated with active transcription.[18,21] This hyperacetylation may contribute to the oncogenic process in DMGs by disrupting PRC2, which is crucial for repressing genes that maintain cellular differentiation. The resulting accumulation of PRC2 at genes normally activated during differentiation could further drive a stem-like phenotype in DMG.[20,22]

Paradoxically, a therapeutic strategy involves enhancing the existing hyperacetylation using histone deacetylase inhibitors (HDACis). HDACis may reverse PRC2 sequestration on H3K27M by inducing polyacetylation of the H3 N-terminus, potentially detoxifying oncohistones.[23–25] In addition, enhanced H3K27 acetylation induced by HDAC

inhibitors can promote abnormal retroviral transcription, leading to viral mimicry, tumor cell death, and immune cell activation.[26] This effect extends beyond DMGs to other cancers with H3K27-alterations.[27]

Several HDACis have undergone clinical trials (Table 1). Early trials investigated *valproate (valproic acid)* and *vorinostat* (NCT00107458, NCT01189266); however, poor BBB-penetrability and limited efficacy as monotherapies have shifted the focus toward developing combination therapies (NCT00879437, NCT03243461, and NCT02420613). No combinations with these HDACis have demonstrated clinical efficacy so far.

*Panobinostat*, a pan-HDAC inhibitor targeting class I, II, and IV HDACs emerged as a promising drug candidate in several drug screens in DMG primary cultures and patient-derived xenograft (PDX) models.[28–32] Panobinostat was shown to restore H3K27Me3 in DMG cells, leading to reduced tumor cell growth and increased cell death.[28] These cell models are crucial for gathering preclinical data to improve clinical translation, considering the unique molecular characteristics of DMGs compared to other gliomas and adult cancers.[33] However, an initial phase I clinical trial involving 53 patients revealed that panobinostat had limited tolerability, with myelosuppression identified as the primary dose-limiting toxicity (NCT02717455).[34] Although the trial did not demonstrate significant clinical efficacy, some patients had periods of stable disease, but disease progression occurred in all cases. Pharmacokinetic analyses revealed that while panobinostat was well absorbed into plasma, it exhibited a shorter half-life and higher clearance compared to previous observations in adults. Furthermore, subsequent studies showed limited panobinostat penetration into the CNS and cerebrospinal fluid (CSF), which likely contributed to its reduced effectiveness in treating DMG.[35–37] Unfortunately, panobinostat was withdrawn from the US market because the manufacturer could not complete the FDA-mandated post-marketing trials, and it is no longer available for DMG clinical trials.

To address BBB penetration, a phase I/II trial assessed *MTX110* (NCT03566199), a nanoparticle formulation of panobinostat delivered via convection-enhanced delivery (CED).[38] CED bypasses the BBB by infusing the drug directly into the tumor site via an implanted catheter. The first reported use of CED in DMG treated a single patient with carboplatin, a chemotherapy with limited BBB permeability.[39] This case report established the safety and feasibility of CED in DMG, with improvement of clinical signs, despite tumor progression outside the zone of drug infusion. CED of MTX110 had limited systemic toxicity in DMG PDX rat models and prolonged survival.[40] Patients received repeated perfusions of MTX110 every 4–8 weeks and gadoteridol dye to track the distribution of the drug in the brain. MTX110 was well tolerated, and patients in this trial experienced a median overall survival of 26 months, surpassing historical controls (9–12 months).[38] The small sample size requires validation in a larger cohort.

A similar phase I CED clinical trial involved an implantable subcutaneous pump administering MTX110 (NCT04264143). This trial delivered the drug in two 48-h pulses, spaced 7 days apart. The results were promising, with one patient achieving a survival time of 35 months and a median OS of 16.5 months. Likewise, despite the favorable trend in OS, the small sample size of just 9 patients limits the generalizability of these findings.[41] A larger trial, incorporating more infusion pulses, will be necessary to confirm and potentially enhance these outcomes.

Another emerging technology to enhance drug delivery across the BBB is focused ultrasound (FUS), which uses microbubbles to temporarily open the BBB at the tumor site. In a phase I trial of oral panobinostat enhanced with a Delsona FUS device, two of three patients showed neurological improvements (NCT04804709).[42] However, the trial was suspended with the withdrawal of panobinostat from the market. Considering that FUS was well tolerated, further safety and efficacy studies in DMG are warranted.

Irrespective of the delivery modality, resistance to panobinostat has been observed in both clinical and preclinical studies.[28,43] This highlights the urgent need for novel combinatorial strategies. A high-throughput drug combination screen with panobinostat found a potent synergy with *marizomib*, a BBB-penetrant proteasome inhibitor. While this combination was highly cytotoxic in DMG cells, its antitumor effectiveness in orthotopic animal models was only modest, prolonging survival by ~20%. Despite this limited efficacy, any survival benefit is considered promising in the context of DMG, given the aggressive nature of this cancer. A phase I clinical study involving only 4 patients reported a median survival of 95 days (NCT04341311).[44] Unfortunately, the study was terminated after the sponsor withdrew, following the discontinuation of marizomib's clinical development—likely due to insufficient efficacy in a phase III glioblastoma trial.[45] This underscores the necessity of developing more effective combinatorial strategies with panobinostat potentially leveraging therapies that target multiple DMG vulnerabilities.[29]

*Fimepinostat* (CUDC-907) is a first-in-class dual inhibitor of HDAC and phosphatidylinositol 3-kinase (PI3K). It was found to inhibit cell growth of and radiosensitize pediatric high-grade glioma (pHGG) cell lines, including DMG cells, and also extended the survival of mouse PDX models.[46] This preclinical efficacy suggests that this compound is BBB penetrable and could overcome the limitations of previous HDACis. It entered a phase I clinical trial for pediatric patients with brain tumors, including DMG (NCT03893487). Preliminary data showed that fimepinostat was detected intratumorally at levels similar to its observed $IC_{50}$ in vitro. Some grade 3 haematological toxicities were observed including 9/31 patients who experienced thrombocytopenia, which is similar to other HDACi-treated cohorts. The median OS for DMG patients was 13 months, which is similar to historical controls for radiotherapy alone[47]; however, survival analyses have yet to be posted.[48] Although many HDACis show preclinical efficacy, they do not prolong patient survival. Further research is needed to enhance BBB penetrability and find a synergistic combination therapy.

## Facilitates Chromatin Transcription Complex (FACT) Inhibition

The facilitates chromatin transcription (FACT) complex is a histone chaperone composed of two subunits of the suppressor of Ty 16 (SPT16) and structure-specific recognition

protein 1 (SSRP1). This complex is predominantly enriched at the coding regions of transcribed genes and plays a vital role in nucleosome maintenance during transcription.[49] FACT is highly expressed in stem cells and cancers, especially those with poor prognoses.[50,51] Its increased expression in cancer cells likely responds to elevated chromatin destabilization due to higher replication demand,[49] making FACT an appealing molecular target.

A recent high throughput screen on DMG cells identified that antimalarial drugs including quinacrine, mefloquine, and primaquine were effective at inhibiting cell viability.[52] Quinacrine was used as the basis for the development of a new class of compounds known as curaxins, which function as DNA intercalators, trapping soluble FACT and perturbing chromatin structure.[53] The reduction in soluble FACT impairs NF-κB signaling and activates p53, thereby exerting anticancer effects. *CBL0137*, the lead clinical curaxin, has improved water solubility and reduced off-target effects.[54] In DMG models, CBL0137 demonstrated potent anticancer activity and significantly extended survival by over 30% when combined with panobinostat in orthotopic mouse models.[52] In addition, the combination of CBL0137 with panobinostat was found to recover H3K27Me3 and increase H3K27Ac, further supporting its therapeutic potential.

These encouraging preclinical results led to a phase I/II clinical trial for pediatric solid and CNS tumors, with an expansion cohort for DMG patients. This trial is currently recruiting (NCT04870944). The recent trial of CBL0137 in adults with advanced solid tumors showed it was well tolerated up to 700 mg/m$^2$ and led to prolonged stable disease in 5 of 83 patients at higher doses (NCT01905228).[55] The continued exploration of FACT inhibitors like CBL0137 represents a promising avenue for targeting chromatin-related pathways in DMG therapy.

### B Cell-Specific Moloney Murine Leukemia Virus Integration Site 1 (BMI-1) Inhibition

B cell-specific Moloney murine leukemia virus integration site 1 (BMI-1), is a key component of the polycomb repressive complex 1 (PRC1) which also includes the RING1A/B, polycomb group RING finger (PCGF), chromobox (CBX), and a polyhomeotic subunit (PHC). This complex plays a crucial role in chromatin remodeling by interpreting repressive H3K27Me3 marks and compacting chromatin to restrict transcription factor access. BMI-1 is implicated as an oncogene by driving cancer initiation and maintaining stem-like properties in cells and is highly expressed in several cancers, including B-cell lymphoma and melanoma.[56,57]

In DMG primary cultures, BMI-1 is significantly overexpressed. Targeting this with *PTC-209*, a BMI-1 inhibitor, inhibits DMG cell growth in a dose-dependent manner and enhances the efficacy of bleomycin.[58] BMI-1 overexpression is driven by the H3K27M mutation, which increases H3K27Ac marks and reduces H3K27Me3 at the BMI1 promoter.[59] Inhibition of BMI-1 led to increased senescence and a loss of self-renewal. In mouse DMG models, combining BMI-1 inhibition with the senolytic agent, obatoclax, resulted in a 50% increase in median survival,[59] highlighting BMI-1 suppression as a potential therapeutic strategy.

*Unesbulin* (PTC596) is an orally administered, BBB-penetrable BMI-1 inhibitor BMI-1 inhibitor that showed promising efficacy in combination with dacarbazine in adult unresectable or metastatic leiomyosarcoma, with a disease control rate over 50% in the highest dose groups.[60] A phase I clinical trial in DMG (NCT03605550) is currently underway evaluating unesbulin in combination with radiotherapy for DMG. Preliminary analyses showed that it is well-tolerated, with effective penetration into the tumor.[61,62] These findings underscore the potential of BMI-1 inhibition as a viable therapeutic approach for DMG and other malignancies.

## Targeting Polyamine Metabolism

Putrescine, spermidine, and spermine are polyamines crucial for cancer cell proliferation and are often elevated in tumors due to increased biosynthesis, uptake, and reduced catabolism.[63] In models of prostate cancer, pancreatic cancer, neuroblastoma, and DMG, targeting the polyamine biosynthetic pathway by inhibiting ornithine decarboxylase (ODC1), which converts ornithine to putrescine, has demonstrated efficacy both in vitro and in vivo.[64–66] Increased expression of *ODC1* and other biosynthetic genes of the polyamine pathway, such as spermidine/spermine N1-acetyltransferase 1a (*SAT1A*), compared to normal brain tissue has recently been observed in DMGs.[67]

DMGs exhibit significantly increased expression of *ODC1* and other biosynthetic genes of the polyamine pathway, such as *SAT1A*, compared to normal brain tissue.[67] Inhibition of ODC1 with difluoromethylornithine (*DFMO*) in conjunction with *AMXT1501*, a solute carrier family 3 member 2 (SLC3A2) polyamine transport inhibitor, has been shown to markedly deplete polyamine levels both in vitro and in vivo,[67] and significantly and potently extended survival in 3 orthotopic mouse models of DMGs.

A phase Ib/IIa clinical trial evaluating the combination of DFMO and AMXT1501 in patients with advanced adult solid tumors and DMG has recently ended recruitment (NCT05500508). Unexpected episodes of cardiac toxicity are under evaluation.

## Receptor Tyrosine Kinase (RTKs) Inhibition

Receptor tyrosine kinases (RTKs) are frequently dysregulated in DMGs contributing to their aggressive nature. They are a family of cell surface membrane receptors involved in regulating important cellular processes such as cell growth, metabolism, differentiation, and migration.[68] A genomic analysis of high-grade gliomas revealed that approximately 69% of DMGs harbored different alterations within the RTK and downstream phosphoinositide 3-kinase/Ak strain transforming/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway.[69]

**Table 2.** Receptor Tyrosine Kinase Inhibitors in Clinical Studies or Clinical Development

| Target | Compound | Clinical trial | Clinical trial identifier | Start date | Status |
|---|---|---|---|---|---|
| PDGFRA | Imatinib | Imatinib Mesylate With or Without Radiation Therapy in Treating Young Patients With Newly Diagnosed or Recurrent Glioma | NCT00021229 | 2001-05 | Terminated |
| PDGFRA | Crenolanib | PDGFR Inhibitor Crenolanib in Children/Young Adults With Diffuse Intrinsic Pontine Glioma or Recurrent High-Grade Glioma | NCT01393912 | 2011-07 | Completed |
| KIT/PDGFRA | Avapritinib | A Study of Avapritinib in Pediatric Patients With Solid Tumors Dependent on KIT or PDGFRA Signaling | NCT04773782 | 2022-02-24 | Active, not recruiting |
| EGFR | Gefitinib | Gefitinib and Radiation Therapy in Treating Children With Newly Diagnosed Gliomas | NCT00042991 | 2002-07 | Completed |
| EGFR | Nimotuzumab | Nimotuzumab in Children With Intrinsic Pontine Glioma | NCT00561691 | 2006-04 | Completed |
| EGFR | Nimotuzumab | Phase 2 Study of Nimotuzumab in Pediatric Recurrent Diffuse Intrinsic Pontine Glioma | NCT00600054 | 2007-10 | Completed |
| EGFR | Nimotuzumab | Nimotuzumab in Combined With Chemoradiotherapy to Treat the Newly Diagnosed Diffuse Intrinsic Pontine Glioma in Children | NCT04532229 | 2021-04-03 | Recruiting |
| VEGFR/ EGFR/RET | Vandetanib | Vandetanib and Radiation Therapy in Treating Young Patients With Newly Diagnosed Diffuse Brainstem Glioma | NCT00472017 | 2007-04 | Completed |
| Multi-RTK + VEGFR/ EGFR/RET | Dasatinib + vandetanib | Clinical Trial Evaluating the Combination of Vandetanib and Dasatinib During and After Radiation Therapy (RT) in Children With Newly Diagnosed Diffuse Intrinsic Pontine Glioma (DIPG) | NCT00996723 | 2009-10 | Completed |
| Multi-RTK + ALK/c-MET | Dasatinib + crizotinib | Study of the Combination of Crizotinib and Dasatinib in Pediatric Research Participants With Diffuse Pontine Glioma (DIPG) and High-Grade Glioma (HGG) | NCT01644773 | 2012-11-27 | Completed |
| Multi-RTK + EGFR + mTOR | Dasatinib + erlotinib + everolimus + | Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication (BIOMEDE) | NCT02233049 | 2014-10 | Temporarily closed |
| Multi-RTK + mTOR | Dasatinib + everolimus | Study of Dasatinib in Combination With Everolimus for Children and Young Adults With Gliomas Harboring Platelet-Derived Growth Factor Receptor (PDGFR) Alterations | NCT03352427 | 2017-12-06 | Terminated |

Amplifications of platelet-derived growth factor receptor alpha (PDGFRA) occur in approximately 30% of DMGs.[70] These amplifications are heterogeneous, with some tumor cells co-amplifying multiple RTKs or distinct RTK genes in subclonal populations.[71–73] Several PDGFRA inhibitors have advanced to clinical trials (Table 2) for DMGs initially inspired by preclinical studies in adult glioma models. Among these inhibitors, *imatinib* was the first to show efficacy in inhibiting glioma growth both in vitro and in vivo.[74–76] This initiated a phase I trial that evaluated the combination of imatinib with radiotherapy for children with newly diagnosed or recurrent malignant brainstem gliomas (NCT00021229). Despite limited toxicities, imatinib did not show clinically significant activity and it is likely that there is insufficient BBB penetration.[71]

A phase I clinical trial investigated a more CNS-penetrant, potent, and selective inhibitor of PDGFRA, *crenolanalib*, in DMG and pHGG patients (NCT01393912).[77] Although crenolanib's toxicities were similar to those in adults, no correlation was found between PDGFRA alterations and treatment response, and no objective radiologic responses were observed. This lack of correlation was partly due to limited samples and inconsistent timing of sampling and PDGFRA analysis relative to disease progression, as PDGFRA mutations could be temporally heterogeneous.[70] Biomarker-driven studies are essential for determining the clinical utility of PDGFRA inhibitors like crenolanib in PDGFRA-enriched DMG patients.

Preclinical data demonstrated that *avapritinib*, a BBB-penetrant dual tyrosine-protein kinase KIT (KIT)/PDGFRA inhibitor, significantly extended survival in DMG PDX mouse models.[78] This led to a compassionate use program treating nine DMG patients with avapritinib, which achieved a radiographic response rate of approximately 50%, although tumor progression was eventually observed in most patients.[79] A phase I/II trial of avapritinib in pediatric patients with relapsed or refractory solid tumors, including DMG recently stopped recruitment (NCT04773782). No objective responses were reported.

Epithelial growth factor receptor (EGFR) has a key role in tumorigenesis in adult high-grade gliomas.[80,81] Although it is less frequently a driver in DMG, EGFR amplifications and mutations have also been observed in some DMG autopsy and biopsy samples.[82] Given this potential vulnerability, *gefitinib*, an orally available FDA-approved EGFR inhibitor, was investigated in a phase I/II clinical trial for children with

**Table 3.** PI3K/AKT/mTOR Targeting Agents and Imipridones in Clinical Studies or Clinical Development

| Target | Compound | Clinical trial | Clinical trial identifier | Start date | Status |
|---|---|---|---|---|---|
| AKT | Perifosine | Study of Single Agent Perifosine for Recurrent Pediatric Solid Tumors | NCT00776867 | 2008-10 | Completed |
| AKT + mTOR | Perifosine + temsirolimus | Perifosine With Temsirolimus for Recurrent Pediatric Solid Tumors | NCT01049841 | 2010-01 | Completed |
| mTOR + CDK4/6 | Everolimus + ribociclib | A Study of Ribociclib and Everolimus Following Radiation Therapy in Children With Newly Diagnosed Non-biopsied Diffuse Pontine Gliomas (DIPG) and RB+ Biopsied DIPG and High-Grade Gliomas (HGG) | NCT03355794 | 2017-11-14 | Completed |
| mTOR + CDK4/6 | Everolimus + ribociclib | Ribociclib and Everolimus in Treating Children With Recurrent or Refractory Malignant Brain Tumors | NCT03387020 | 2018-01-13 | Completed |
| mTOR + CDK4/6 | Ribociclib + everolimus | Study of Ribociclib and Everolimus in HGG and DIPG | NCT05843253 | 2024-08-28 | Recruiting |
| PI3K/mTOR | Paxalisib | Study of GDC-0084 in Pediatric Patients With Newly Diagnosed Diffuse Intrinsic Pontine Glioma or Diffuse Midline Gliomas | NCT03696355 | 2018-11-19 | Completed |
| PI3K/mTOR + DRD2/ClpP | Paxalisib + ONC201 | Combination Therapy for the Treatment of Diffuse Midline Gliomas | NCT05009992 | 2021-10-20 | Recruiting |
| DRD2/ClpP | ONC201 | Oral ONC201 in Recurrent GBM, H3 K27M Glioma, and Midline Glioma | NCT02525692 | 2016-01 | Active, not recruiting |
| DRD2/ClpP | ONC201 (dordaviprone) | ONC201 in Pediatric H3 K27M Gliomas | NCT03416530 | 2018-01-29 | Active, not recruiting |
| DRD2/ClpP | ONC201 | ONC201 in H3 K27M-mutant Diffuse Glioma Following Radiotherapy (the ACTION Study) (ACTION) | NCT05580562 | 2023-01-23 | Recruiting |
| DRD2/ClpP + CDK4/6 | ONC201 + everolimus (monotherapies) | Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication 2.0 (BIOMEDE 2) | NCT05476939 | 2022-09-29 | Recruiting |
| DRD2/ClpP | ONC206 | ONC206 for Treatment of Newly Diagnosed, Recurrent Diffuse Midline Gliomas, and Other Recurrent Malignant CNS Tumors (PNOC023) | NCT04732065 | 2021-08-23 | Recruiting |

DMG (NCT00042991). It was well tolerated, with manageable toxicity; however, gefitinib did not significantly improve survival.[83] Possible reasons for this include a lack of BBB penetrability or drug efflux, which is thought to be the reason for treatment failure in adult gliomas.[84,85]

*Nimotuzumab*, a monoclonal antibody targeting EGFR, has demonstrated modest efficacy in a small subset of patients across both phase II and III clinical trials (NCT00561691, NCT00600054).[86,87] These results highlight the need for molecular profiling and biopsy before treatment to identify potential responders, as earlier studies did not incorporate these analyses. Nimotuzumab is currently being evaluated in an additional phase III clinical trial (NCT04532229).

Other RTKs, such as vascular endothelial growth factor receptor 2 (VEGFR2), have been targeted, given their role in proangiogenic pathways identified in adult glioblastoma (GBM).[88] *Vandetanib*, an inhibitor of VEGFR/EGFR/Proto-oncogene tyrosine-protein kinase receptor Ret (RET) was tested in conjunction with radiotherapy in a phase I clinical trial for newly diagnosed DMG patients (NCT00472017). However, while the toxicity profile was comparable to that observed in adult cohorts, the treatment did not significantly extend to survival with a median OS of only 7 months.[89]

Failure of single-agent therapies has since driven the exploration of combination therapies, including those incorporating *dasatinib*, a multi-RTK inhibitor. Combination therapy with dasatinib and vandetanib was found to be tolerable (NCT00996723).[90] In contrast, the combination of *crizotinib*, a dual anaplastic lymphoma kinase(ALK)/mesenchymal-epithelial transition factor (c-MET) inhibitor, with dasatinib caused unfavorable pharmacokinetic interactions that impaired the drug absorption rate and clearance (NCT01644773).[91] Neither combination was observed to extend the survival of patients. The BIOMEDE 1 trial (NCT02233049), a large, randomized biomarker-driven study for DMG, stratified patients into treatment cohorts based on their biopsy sequencing and mutational profiles targeting EGFR (*erlotinib*), PDGFRA (dasatinib), or mTOR (*everolimus*) inhibition. Although the trial demonstrated that ambitious, randomized, biomarker-driven adaptive studies like these are feasible, no significant improvement in survival was observed in any of the treatment arms.[92] Similarly, another trial assessing the efficacy of dasatinib combined with everolimus in pediatric glioma patients, including those with DMG, failed to improve survival outcomes (NCT03352427).[93]

## PI3K/AKT/mTOR Inhibitors and Imipridones

RTK activation promotes cell proliferation and survival via the PI3K/AKT/mTOR pathway. Over 60% of DMGs have alterations in this signaling cascade, making these proteins

compelling therapeutic targets.[94] To date, 5 agents targeting this pathway have reported clinical trial results: *perifosine* (AKT inhibitor), *temsirolimus* (mTOR inhibitor), *everolimus* (mTOR inhibitor), and *paxalisib* (PI3K inhibitor) (Table 3).

Perifosine, the first to be clinically investigated, is a third-generation alkyl phospholipid that inhibits AKT membrane localization and activation.[95] This agent demonstrated activity in other preclinical cancer models including adult GBM, medulloblastoma, and neuroblastoma.[96–98] Although perifosine was well tolerated in children with solid tumors, only 1 of 3 DMG patients achieved stable disease for 2 months, and no objective radiological responses were observed (NCT00776867).[99]

Efforts to discover rational combinations in pediatric GBM models with perifosine revealed its synergy with temsirolimus, which targets downstream mTOR.[95] Temsirolimus monotherapy prolonged stable disease in some glioma, neuroblastoma, and rhabdomyosarcoma patients.[100] Perifosine and temsirolimus combination therapy was evaluated in a phase I clinical trial in children with recurrent solid tumors, including DMG (NCT01049841). Unfortunately, no objective responses were observed, but 5/8 DMG patients experienced stable disease.[101] The authors noted that poor BBB penetrability of the drugs might be a factor, citing a study showing limited CSF availability of perifosine in rhesus macaques.[102]

Everolimus, an FDA-approved drug for breast and renal cancers, inhibits the interaction between Raptor and mTOR, preventing phosphorylation and tumor cell proliferation.[103,104] This drug has shown preliminary clinical efficacy in other malignancies, such as adult sarcomas and breast cancer,[105,106] and is capable of crossing the BBB.[107] Two phase I trials have investigated the safety and tolerability of everolimus and ribociclib, a CDK4/6 inhibitor, combination therapy in DMGs. The first study enrolled patients with newly diagnosed DMG and pHGG after radiotherapy and showed that the combination therapy was well tolerated (NCT03355794).[108] Impressively, the OS at 36 months was 38.9%, compared to under 10% in historical controls surviving 2 or more years. However, 2 of these patients were under 3 years old, and long-term survival is associated with diagnosis at age extremes.[8]

The second study focused on pediatric patients with recurrent or refractory malignant brain tumors, including DMGs, and aimed to assess the exposure of both drugs in tumor tissues (NCT03387020). The treatment was well-tolerated in children with ribociclib reaching adequate concentrations in CSF and tumor tissues which was above the in vitro $IC_{50}$ of 0.01–0.039 μM.[109] Furthermore, steady-state everolimus exposure was 2.5 times greater in combination with ribociclib, compared to everolimus alone. This suggested a potential synergistic drug-drug pharmacokinetic interaction. However, no significant efficacy was observed. Despite these findings, the combination therapy shows promise, prompting a phase II study that is currently recruiting DMG patients with alterations in the cell cycle or PI3K/AKT/mTOR pathways (NCT05843253).

Lastly, paxalisib, (formerly known as GDC-0084) is a BBB-penetrant pan-PI3K inhibitor that has demonstrated efficacy in inhibiting the growth of GBM PDXs.[99] A phase I clinical trial for recurrent adult glioma showed that paxalisib significantly penetrated tumors.[110] The drug was well tolerated in children with newly diagnosed DMG, with a maximum tolerated dose of 27 mg/m$^2$, comparable to adults and other drugs of this class; however, survival analyses are pending (NCT03696355).[111]

Preclinical studies have further revealed that paxalisib synergizes with *ONC201*, an imipridone that acts as a novel selective dopamine receptor D2 (DRD2) antagonist,[112] and an agonist of ATP-dependent Clp protease (ClpP), a mitochondrial protein that degrades respiratory chain proteins.[113] Analyses from the Pediatric Brain Tumor Atlas (PBTA) and Clinical Proteomic Tumor Analysis Consortium (CPTAC) found that higher ClpP expression correlated with more aggressive DMG characteristics, such as higher tumor grade and poorer overall survival.[114] The authors also discovered that DMGs were sensitive to ONC201 treatment, as well as *ONC206*, a more potent structural analog. Recent clinical case studies and expanded access programs showed promising results with ONC201 monotherapy in DMG patients, including extended survival and a complete response in one patient.[115–117] There is substantial interest in this therapeutic approach, leading to multiple ongoing DMG clinical trials with imipridones, including the successor BIOMEDE 2 trial and where ONC201 will be randomized against everolimus in the control arm (NCT05009992, NCT02525692, NCT03416530, NCT05580562, NCT05476939, and NCT04732065). An integrated analysis of efficacy data from patients treated with ONC201 monotherapy revealed a robust overall response rate of 20% with a median OS of 21.7 months.[118] However, these data represent a highly selected cohort extracted from 5 separate trials, several of which have not yet been reported in full.[119] The results of the multiple ongoing ONC201 trials are awaited to determine whether the early signal of activity can be validated.

## TRK Inhibition

Fusions of neurotrophic receptor tyrosine kinase (NTRK) have been observed in pHGGs,[120] and have been identified to co-occur with H3.3K27M mutations in a subset of DMGs.[121] Chromosomal translocations of the *NTRK* genes generate constitutively active TRK receptors capable of signaling through the same pathways as full-length TRK proteins.[122] An investigation of TRK inhibitors, *larotrectinib* and *entrectinib*, in ex vivo DMG models showed that *NTRK* fusion-positive DMG cells were sensitive to TRK inhibition, while *NTRK* fusion-negative DMG cells were not.[121] Larotrectinib has induced rapid and robust responses in other *NTRK* fusion-driven pediatric cancers,[123,124] and has been shown to be potent as a single agent in TRK fusion-driven low-grade and high-grade gliomas. Larotrectinib is currently being evaluated as a first-line therapy, with or without chemotherapy in infants with TRK fusion-driven pHGGs (NCT04655404).

## Targeting the Cell Cycle

Approximately 30% of DMGs exhibit cell cycle gene aberrations, such as cyclin-dependent kinase 4/6 (CDK4/6) amplification, cyclin D overexpression, and p16 underexpression,

**Table 4.** Cell Cycle Targeting Agents in Clinical Studies or Clinical Development

| Target | Compound | Clinical trial | Clinical trial identifier | Start date | Status |
|---|---|---|---|---|---|
| CDK4/6 | Abemaciclib | Abemaciclib in Children With DIPG or Recurrent/Refractory Solid Tumors (AflacST1501) | NCT02644460 | 2016-02 | Completed |
| CDK4/6 | Abemaciclib + irinotecan + temozolomide + dinutuximab + GM-CSF | A Study of Abemaciclib (LY2835219) in Combination With Other Anti-Cancer Treatments in Children and Young Adult Participants With Solid Tumors, Including Neuroblastoma | NCT04238819 | 2020-11-09 | Active, not recruiting |
| CDK4/6 | Abemaciclib | Abemaciclib Neuropharmacokinetics of Diffuse Midline Glioma Using Intratumoral Microdialysis | NCT05413304 | 2023-04-07 | Recruiting |
| CDK4/6 | Ribociclib | A Phase I/II Study of Ribociclib,a CDK4/6 Inhibitor, Following Radiation Therapy | NCT02607124 | 2016-04 | Terminated |
| CDK4/6 | Palbociclib | Palbociclib Isethionate in Treating Younger Patients With Recurrent, Progressive, or Refractory Central Nervous System Tumors | NCT02255461 | 2014-12-08 | Terminated |
| CDK4/6 | Palbociclib | Study Of Palbociclib Combined With Chemotherapy In Pediatric Patients With Recurrent/Refractory Solid Tumors | NCT03709680 | 2019-05-24 | Active, not recruiting |
| WEE1 | Adavosertib | Adavosertib and Local Radiation Therapy in Treating Children With Newly Diagnosed Diffuse Intrinsic Pontine Gliomas | NCT01922076 | 2013-09-03 | Completed |

which promote tumorigenesis by advancing cell cycle progression.[70] Cyclin D binds to CDK4/6, activating these kinases to phosphorylate the retinoblastoma (RB) protein. This releases E2F transcription factors, driving the G1 to S phase transition.[125] Consequently, targeting this pathway is an attractive therapeutic strategy. Several FDA-approved cell cycle inhibitors have been explored for DMGs, including *ribociclib*, *palbociclib*, and *abemaciclib* (Table 4). These agents function by stabilizing RB in a hypophosphorylated state thereby preserving its transcriptional repression and inducing G1 cell cycle arrest.[126]

Abemaciclib, an FDA-approved CDK4/6 inhibitor for metastatic breast cancer, was the first to be evaluated in clinical trials for DMGs. It has demonstrated BBB penetration with efficacy in PDX models[127] and has achieved disease stabilization in some GBM patients.[128] Although specific preclinical data for DMG models are lacking, altered CDK4/6 activity in DMGs and efficacy in adult trials warrant its evaluation. Consequently, abemaciclib has been included in a phase I clinical trial designed to assess its safety and maximum tolerated dose in combination with radiotherapy for DMGs and other recurrent or refractory solid tumors (NCT02644460). This trial has been completed, but results are pending.

Two additional clinical studies are currently underway. The first is a phase Ib/II study, assessing the combination of abemaciclib with numerous antineoplastic agents, including: irinotecan, temozolomide, dinutuximab, and GM-CSF (NCT04238819). The second phase I trial examines the neuropharmacokinetics of abemaciclib and temozolomide, using intratumoral microdialysis to monitor real-time pharmacokinetics and BBB penetration in DMG (NCT05413304).[129] To date, results from these clinical trials have not been reported.

Ribociclib was the second CDK4/6 inhibitor trialed in DMG and is FDA-approved for breast cancer. Prior to its investigation in combination with everolimus,[108,109] ribociclib was investigated as a monotherapy in phase I/II trial in post-radiotherapy DMG patients (NCT02607124).[130] This trial showed promise, with a median OS of 16.1 months, and 2 patients surviving 28 and 30 months. However, these patients were diagnosed at age extremes: 1 patient under 3 years of age and the other over 15 years of age. The study was terminated early to focus on combining everolimus and ribociclib in subsequent trials (NCT03355794, NCT03387020).

Palbociclib has demonstrated potential efficacy against DMG in preclinical studies, showing nanomolar sensitivity in DMG cells and significantly extending survival in 3 PDX models.[131] However, concerns were raised regarding the drug concentrations used in this study. The murine doses of 150 mg/kg/day, far exceeded the MTD in humans, which equates to approximately 22 mg/kg/day.[132] Consequently, preclinical efficacy was not replicated in children (NCT02255461). While palbociclib was safely administered to children, it likely did not achieve therapeutically active concentrations in vivo, resulting in no therapeutic benefit for children with progressive brain tumors, including DMG.[133] It was recognized that these drugs may be maximally effective when administered in combination with other chemotherapeutics or radiotherapy.

Supporting this, preclinical evidence shows that CDK4/6 inhibitors are more effective when combined with other drugs.[134] This finding is further supported by adult clinical trials demonstrating improved survival outcomes compared to monotherapy.[135] Based on these data, trials are investigating the efficacy of combining palbociclib with *temozolomide*, *irinotecan*, *topotecan*, and *cyclophosphamide* in pediatric patients with recurrent or refractory solid tumors (NCT03709680), and as mentioned earlier ribicoclib and everolimus (NCT05843253).

**Table 5.** Novel Small Molecule Inhibitors in Clinical Studies or Clinical Development

| Target | Compound | Clinical trial title | Clinical trial identifier | Start date | Status |
|---|---|---|---|---|---|
| PARP | Veliparib | Veliparib, Radiation Therapy, and Temozolomide in Treating Younger Patients With Newly Diagnosed Diffuse Pontine Gliomas | NCT01514201 | 2012-02-01 | Completed |
| Telomerase | Imetelstat | Imetelstat Sodium in Treating Younger Patients With Recurrent or Refractory Brain Tumors | NCT01836549 | 2013-03 | Terminated |
| PAI-1 | ACT001 | A study to evaluate the safety, tolerability, and pharmacokinetics of ACT001 in children with advanced brain and solid tumors | ACTRN12618001934235 | 2018-11-28 | Completed |
| ADAM10/17 | INCB7839 | INCB7839 in Treating Children With Recurrent/Progressive High-Grade Gliomas | NCT04295759 | 2020-07-27 | Active, not recruiting |
| Somatostatin | Lutathera | Lutathera for Treatment of Recurrent or Progressive High-Grade CNS Tumors | NCT05278208 | 2022-11-21 | Recruiting |

Instead of inhibiting cell cycle progression, other strategies aim to cause mitotic catastrophe by premature transitions through the cycle. WEE1, a key gatekeeper kinase for $G_2$-M cell cycle progression, was overexpressed in pediatric gliomas, particularly in a patient-derived DMG cell line.[136] WEE1 activation inhibits CDC2, facilitating the $G_2$-M transition. WEE1 inhibition sensitizes cancer cells to radiation by enabling them to progress past the $G_2$ checkpoint despite DNA damage.[137,138] *Adavosertib*, a BBB-penetrable WEE1 inhibitor, has shown efficacy in preclinical glioma and DMG models and radiosensitization was also observed.[136,139,140] It was investigated with radiotherapy in children with DMG in a phase I trial (NCT01922076), showing good tolerance and no MTD was reached. However, median survival did not improve compared to historical controls.[141] The authors conceded that radiosensitization alone is insufficient for long-term tumor control without combination therapies. Furthermore, limited molecular profiling of tumor samples and lack of ongoing maintenance therapy may have restricted its effectiveness.

## Novel Small Molecule Inhibitors

Considering the failures of traditional chemotherapy and the disappointing outcomes of many older targeted therapies, it is evident that treatments with a meaningful impact on survival must be highly innovative and involve a multi-pronged approach. Several novel therapeutic agents have recently entered clinical trials for DMGs, including *veliparib*, *imetelstat*, *ACT001*, *INCB7839*, and *lutathera* (Table 5).

Veliparib is an orally available and BBB-penetrable poly(ADP-ribose) polymerase (PARP) inhibitor. These inhibitors work as trapping agents, by sequestering PARP at sites of DNA damage.[142] It has been shown to radiosensitize tumors in preclinical cancer models.[143,144] This is unsurprising as PARP is involved in both single- and double-stranded DNA damage repair.[145] Given the potential therapeutic benefits of radiosensitisation, veliparib was evaluated in a phase I/II trial in post-radiotherapy-treated DMG patients and in combination with temozolomide (NCT01514201). This combination was not successful at prolonging survival.[146] Despite a decrease in poly(ADP-ribose) (PAR) levels in the majority of patients, there was no associated survival benefit. Further analysis showed increased BRCA2 levels, suggesting possible resistance mechanisms. The investigators noted that PARP responses are likely heterogeneous, and that resistance is common, supporting the exploration of other combinations with veliparib, like HDACi or CDK4/6 inhibitors.

Imetelstat is the only currently FDA-approved telomerase inhibitor. Telomerases are enzymes crucial for maintaining the replicative potential of cancer cells by renovating the telomere tandem repeats (TTAGGG) on chromosomes.[147] *hTERT* and *hTERC* encode for a protein and a functional RNA, respectively, that form the telomerase complex. Although their expression in normal cells is minimal, they are upregulated in human cancers, including 76% of DMGs, where it is associated with heightened telomerase activity.[148] As a competitive inhibitor of the telomerase complex,[149] imetelstat demonstrated promising efficacy in adult myeloproliferative cancers.[150,151] However, in a phase II clinical trial involving children with CNS tumors, including a DMG cohort, patients received 285 mg/m$^2$ (NCT01836549), the maximally tolerated dose established in a separate trial for non-CNS tumors in children.[152] Despite receiving this dose, the trial was terminated prematurely after several children developed intracranial hemorrhages due to imetelstat-induced thrombocytopenia, resulting in 2 fatalities. Attempts to mitigate this risk with dose reduction were unsuccessful. Given these severe toxicities, further exploration of telomerase inhibition for clinical development in DMG has been discontinued.

ACT001, a derivative of the natural product parthenolide, is known to inhibit several oncogenic pathways, including STAT3, NF-κB, and PI3K/AKT/mTOR signaling.[153–155] Preclinical studies in GBM and DMG revealed that ACT001 is BBB penetrable and potently inhibits tumor cell proliferation both in vitro and in vivo.[155,156] These promising results prompted a phase I clinical trial in Australia, enrolling 39 patients with solid and CNS tumors, including DMG (ACTRN12618001934235). This recently completed trial reported no dose-limiting toxicities.[157] 8/14 DMG patients receiving 700 mg/m$^2$ or higher doses experienced either an

objective response or stable disease. These encouraging preliminary signs of efficacy have led to the development of a phase II trial for children with DMG, at the recommended phase II dose of 875 mg/m² that is planned to open in 2025.

INCB7839 is a novel inhibitor of the A disintegrin and metalloprotease (ADAM) 10 and ADAM 17 proteases. These enzymes are responsible for an activity known as "shedding," where membrane-bound proteins are cleaved and released from the membrane. Preclinical studies in HGG and DMG have shown that cleavage and secretion of the synaptic molecule, neuroligin-3, via ADAM10 are critical for tumor growth.[158,159] Furthermore, targeting these enzymes with INCB7839 in GBM PDX inhibited tumor growth and was also blood–brain penetrable.[159] INCB7839 was well tolerated in breast cancer,[160] and is currently being investigated in a multicenter phase I trial for children with pHGG, including DMG (NCT04295759).

Lutathera (Lu-177-Dotatate) is the first FDA-approved peptide receptor radionuclide therapy (PRRT) for neuroendocrine tumors. This drug is a radiolabeled somatostatin analog, which binds to somatostatin receptors. This radiolabeled somatostatin analog binds to overexpressed somatostatin receptors in 70% of neuroendocrine tumors and gliomas, allowing targeted radiation delivery to the tumor site.[161,162] It is in phase I/II trials, recruiting pediatric and adult patients with high-grade CNS tumors, including DMG, based on DOTATATE PET imaging for somatostatin-positive tumors (NCT05278208). Innovative therapies like these show promise for improving DMG outcomes. Future trials should prioritize novel targeted therapies and, when possible, draw from robust preclinical evidence in relevant DMG models to enhance translational success.

## Immunotherapies

While targeted therapies remain a central focus in DMG research, immunotherapies are also emerging as a promising therapeutic strategy (Table 6). DMGs are characterized by an inert immune microenvironment, referring to its neither immunosuppressive nor inflammatory state. This environment exhibits limited immune surveillance, with low T cell and macrophage infiltration, minimal immune checkpoint marker expression, and reduced cytokine production.[163,164] However, this does not imply a complete absence of immune activity. H3.3K27M-specific CD8+ T cells have been detected in the peripheral blood of DMG patients, suggesting that the immune system can generate targeted cellular immunity against DMG cells.[165]

Primary H3K27M-mutant DMG cells uniformly express high levels of the surface marker GD2, while H3-WT tumors do not, suggesting that H3K27M-induced epigenetic alterations drive this overexpression.[166] Anti-GD2 CAR T-cell therapy in DMG PDX mice showed significant survival benefits and near-complete tumor clearance but was also associated with a severe neuroinflammatory response.[166] Given that these toxicities are clinically manageable, as demonstrated in previous trials,[146] anti-GD2 CAR T-cell therapy was trialed in 11 H3K27M-mutant DMG patients (NCT04196413).[167] As anticipated, patients who experienced tumor inflammation-associated neurotoxicity were safely managed. At data cut-off, one sustained response lasting 30 months was observed and remains ongoing, with 4 patients experiencing striking tumor reductions of 52%, 54%, 91%, and 100%. However, the authors caution that the study's selective cohort, limited to patients with better performance status and less severe disease, raises uncertainty about the impact of GD2-CAR T-cell therapy on survival outcomes. This initial clinical study highlights the potential of CAR T-cell therapy, leading to several ongoing anti-GD2 clinical trials for children with DMG which are all currently recruiting (ACTRN12622000675729, NCT04099797, NCT04196413, NCT05544526, and NCT05298995).

CAR T-cells targeting B7H3, an immunoregulatory protein, are also in clinical development for DMGs.[168] It is overexpressed in DMGs, as well as in several other cancers.[169,170] B7-H3 CAR T-cells were highly effective in PDX mice, where all small-spacer and medium-spacer CAR T-cell-treated mice survived to 90 days, versus a median survival of 29 days in the mock-treated group.[168] A spacer is an extracellular structural component of the CAR that optimizes its binding to its epitope. The medium-spacer B7-H3 CAR T-cells induced the greatest specific lysis against DMG cells in vitro and entered phase I clinical trials to assess safety and tolerability through locoregional infusion (NCT04185038). Preliminary results in three patients showed no DLTs, with one achieving significant clinical and radiographic improvement lasting over 12 months.[168] Ongoing phase I trials for B7H3 in DMG include one studying CAR-T cells against HER2, EGFR, and IL13-zetakine (NCT05768880), and another evaluating IL-7Ra CAR-T cells designed to improve T-cell survival via IL-7 signaling (NCT06221553).

Additional immunotherapy strategies being developed include monoclonal antibodies, cancer vaccines, and oncolytic viruses. Nivolumab, an FDA-approved monoclonal antibody that targets the PD-1 receptor is also under clinical investigation. A single-center retrospective study showed that nivolumab plus radiotherapy did not enhance survival over radiotherapy alone, despite being well-tolerated.[171] The subsequent CheckMate 908 trial evaluated nivolumab, either alone or with ipilimumab, for DMG, but found no survival benefit (NCT03130959).[172]

H3.3K27M vaccines are also in development; one trial found a 6-month survival benefit in patients with H3.3K27M-specific CD8+ cells compared to non-responders (NCT02960230).[173] Furthermore, the ENACTING trial (NCT04749641), combining an H3.3K27M vaccine with radiotherapy in 10 DMG patients resulted in 1 complete response and stable disease in 9 others.[174] These data suggest that H3.3K27M vaccines might improve patient outcomes when combined with other therapies. Ongoing trials are also assessing immunomodulatory vaccines targeting neoantigens such as heat shock proteins and bivalent vaccines for H3.1K27M and H3.3K27M, with some also explored in combination therapies. (NCT03396575, NCT03914768, NCT04749641, NCT04808245, NCT04911621, NCT04943848, NCT04978727, NCT05096481, and NCT06305910).

**Table 6.** Immunotherapy-Based Clinical Trials Either Completed or Ongoing

| Target | Therapy | Clinical trial title | Clinical trial identifier | Start date | Status |
|---|---|---|---|---|---|
| GD2 | CART cells | C7R-GD2.CART Cells for Patients With GD2-expressing Brain Tumors (GAIL-B) | NCT04099797 | 2020-02-03 | Recruiting |
| GD2 | CART cells | GD2 CART Cells in Diffuse Intrinsic Pontine Gliomas (DIPG) and Spinal Diffuse Midline Glioma (DMG) | NCT04196413 | 2020-06-04 | Recruiting |
| GD2 | CART cells | Leveraging Chimeric Antigen Receptor-Expressing T Cells for Children with Diffuse Midline Glioma | ACTRN12622000675729 | 2022-05-10 | Recruiting |
| GD2 | CART cells | CART Cells to Target GD2 for DMG (CARMIGO) | NCT05544526 | 2023-08-15 | Recruiting |
| GD2 | CART cells | GD2-CART Cells for Pediatric Brain Tumors | NCT05298995 | 2023-11-09 | Recruiting |
| B7H3 | CART cells | Study of B7-H3-Specific CART Cell Locoregional Immunotherapy for Diffuse Intrinsic Pontine Glioma/Diffuse Midline Glioma and Recurrent or Refractory Pediatric Central Nervous System Tumors | NCT04185038 | 2019-12-11 | Recruiting |
| B7H3/ EGFR/ HER2/ IL13 | CART cells | Study of B7-H3, EGFR806, HER2, and IL13-Zetakine (Quad) CART Cell Locoregional Immunotherapy For Pediatric Diffuse Intrinsic Pontine Glioma, Diffuse Midline Glioma, And Recurrent Or Refractory Central Nervous System Tumors | NCT05768880 | 2023-05-05 | Recruiting |
| B7H3 | CART cells | Safety and Efficacy of Loco-regional B7H3 IL-7Ra CART Cell in DIPG | NCT06221553 | 2024-03-01 | Recruiting |
| PD-1 | Nivolumab + ipilumab | A Study to Evaluate the Safety and Efficacy of Nivolumab Monotherapy and Nivolumab in Combination With Ipilimumab in Pediatric Participants With High-Grade Primary Central Nervous System (CNS) Malignancies (CheckMate 908) | NCT03130959 | 2017-06-12 | Completed |
| PD-1 | Nivolumab + H3.3K27M vaccine | H3.3K27M Peptide Vaccine With Nivolumab for Children With Newly Diagnosed DIPG and Other Gliomas | NCT02960230 | 2016-11-18 | Completed |
| | H3.3K27M vaccine | Neoantigen Vaccine Therapy Against H3.3-K27M Diffuse Intrinsic Pontine Glioma (ENACTING) | NCT04749641 | 2021-03-08 | Recruiting |
| | TTRNA-DC + TTRNA-xALT plus Td vaccines + radiotherapy/ temozolomide | Brain Stem Gliomas Treated With Adoptive Cellular Therapy During Focal Radiotherapy Recovery Alone or With Dose-intensified Temozolomide (Phase I) (BRAVO) | NCT03396575 | 2018-07-17 | Active, not recruiting |
| | DC vaccine | Immune Modulatory DC Vaccine Against Brain Tumor | NCT03914768 | 2019-03-31 | Unknown |
| | H3.3K27M vaccine | Neoantigen Vaccine Therapy Against H3.3-K27M Diffuse Intrinsic Pontine Glioma (ENACTING) | NCT04749641 | 2021-03-08 | Recruiting |
| | H3K27M vaccine + atezolizumab | A MultIceNTER Phase I Peptide VaCcine Trial for the Treatment of H3-Mutated Gliomas (INTERCEPT-H3) | NCT04808245 | 2023-02-15 | Recruiting |
| | DC vaccine + temozolomide-based chemoradiation/conventional next-line treatment | Adjuvant Dendritic Cell Immunotherapy for Pediatric Patients With High-grade Glioma or Diffuse Intrinsic Pontine Glioma (ADDICT-pedGLIO) | NCT04911621 | 2021-09-10 | Active, not recruiting |
| | rHSC-DIPGVax + balstilimab + zalifrelimab | rHSC-DIPGVax Plus Checkpoint Blockade for the Treatment of Newly Diagnosed DIPG and DMG | NCT04943848 | 2022-01-10 | Recruiting |
| | SurVaxM | A Pilot Study of SurVaxM in Children Progressive or Relapsed Medulloblastoma, High-Grade Glioma, Ependymoma and Newly Diagnosed Diffuse Intrinsic Pontine Glioma | NCT04978727 | 2022-07-01 | Recruiting |
| | PEP-CMV | PEP-CMV Vaccine Targeting CMV Antigen to Treat Newly Diagnosed Pediatric HGG and DIPG and Recurrent Medulloblastoma | NCT05096481 | 2024-07-30 | Recruiting |

**Table 6.** Continued

| Target | Therapy | Clinical trial title | Clinical trial identifier | Start date | Status |
|---|---|---|---|---|---|
| | CD200AR + GBM6-AD | CD200AR-L and Allogeneic Tumor Lysate Vaccine Immunotherapy for Recurrent HGG and Newly Diagnosed DMG/DIPG in Children and Young Adults | NCT06305910 | 2024-03-15 | Recruiting |
| | DNX-2401 | Oncolytic Adenovirus, DNX-2401, for Naive Diffuse Intrinsic Pontine Gliomas | NCT03178032 | 2017-05-26 | Completed |
| | AloCELYVIR | Clinical Trial to Assess the Safety and Efficacy of AloCELYVIR With Newly Diagnosed Diffuse Intrinsic Pontine Glioma (DIPG) in Combination With Radiotherapy or Medulloblastoma in Monotherapy (AloCELYVIR) | NCT04758533 | 2021-04-19 | Active, not recruiting |
| | Ad-TD-nsIL12 | Oncolytic Virus Ad-TD-nsIL12 for Primary Pediatric Diffuse Intrinsic Pontine Glioma | NCT05717712 | 2023-01-04 | Recruiting |
| | Ad-TD-nsIL12 | Oncolytic Virus Ad-TD-nsIL12 for Progressive Pediatric Diffuse Intrinsic Pontine Glioma | NCT05717699 | 2023-01-04 | Recruiting |

Oncolytic viruses selectively replicate in tumor cells, causing cell damage and potentially stimulating an immune response.[175] DNX-2401, an oncolytic virus effective in adult GBMs and DMG animal models,[176,177] entered a phase I clinical trial for DMGs (NCT03178032). A total of 12 patients diagnosed with DMG, received intratumoral injections of DNX-2401 in combination with radiotherapy.[176] Radiographic improvement occurred in 9 patients (75%), with 3 patients (25%) showing partial response and a median survival of approximately 18 months. Clinical trials are also evaluating other oncolytic virus therapies, such as AloCELYVIR, which uses mesenchymal stem cells for delivery (NCT04758533), and Ad-TD-nsIL12, an oncolytic adenovirus for primary (NCT05717712) and progressive (NCT05717699) DMGs. While these immunotherapies are promising, they will likely need to be part of a multimodal, personalized approach to tackle the complexities of heterogeneous cancers like DMG.[178]

## Conclusions

Although there has been limited clinical improvement in patients with DMG, this should not discourage ongoing research efforts. The experiences from completed clinical trials have provided valuable insights. (i) Clinical trials should be guided by robust and clinically relevant evidence from DMG in vitro and in vivo cell models, rather than solely relying on experiences from other gliomas and pediatric cancers. Despite identifying effective targets for inhibition, the developed drugs exhibit limited BBB penetration while avoiding significant toxicity. (ii) Monotherapy or a one-size-fits-all approach is unlikely to extend patient survival. Many single-agent preclinical and clinical studies suggest the necessity for developing rational combination strategies. Precision medicine and combinatorial treatment strategies are expected to yield better outcomes given the molecular heterogeneity of DMGs. (iii) Innovative multimodal approaches, such as combining targeted therapies with immunotherapy, may offer the most promise due to the drug-resistant nature of DMGs. Therefore, future studies should prioritize developing novel combination strategies, informed by robust data from clinically relevant DMG models, and incorporate emerging technologies such as immunotherapies and CED.

## Keywords

clinical trials | diffuse midline glioma | diffuse intrinsic pontine glioma | targeted therapies

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## Author contributions

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