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Therapeutic avenues for targeting treatment challenges of diffuse midline gliomas

Aleeha Noon [a], Stefanie Galban [b,c,d,*]

[a] College of Medicine, California Northstate University, 9700 W Taron Drive, Elk Grove, CA 95757, USA
[b] Center for Molecular Imaging, The University of Michigan Medical School, BSRB A502, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA
[c] Department of Radiology, The University of Michigan Medical School, BSRB A502, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA
[d] Rogel Cancer Center, The University of Michigan Medical School, 1500 E Medical Center Drive, Ann Arbor, MI 48109, USA

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ABSTRACT

Diffuse midline glioma (DMG) is the leading cause of brain tumor-related deaths in children. DMG typically presents with variable neurologic symptoms between ages 3 and 10. Currently, radiation remains the standard therapy for DMG to halt progression and reduce tumor bulk to minimize symptoms. However, tumors recur in almost 100% of patients and thus, DMG is still considered an incurable cancer with a median survival of 9-12 months. Surgery is generally contraindicated due to the delicate organization of the brainstem, where DMG is located. Despite extensive research efforts, no chemotherapeutic agents, immune therapies, or molecularly targeted therapies have been approved to provide survival benefit. Furthermore, the efficacy of therapies is limited by poor blood-brain barrier penetration and inherent resistance mechanisms of the tumor. However, novel drug delivery approaches, along with recent advances in molecularly targeted therapies and immunotherapies, have advanced to clinical trials and may provide viable future treatment options for DMG patients. This review seeks to evaluate current therapeutics at the preclinical stage and those that have advanced to clinical trials and to discuss the challenges of drug delivery and inherent resistance to these therapies.

Introduction

Diffuse midline glioma H3 K27-altered (DMG) is a rare pediatric high-grade glioma (HGG) arising most commonly in the pons, thalamus, or spinal cord [1–3]. DMG affects 1-2 per 100,000 children annually in the United States [4] and has a notoriously poor prognosis, with median patient survival of less than a year [5,6]. DMGs are aggressive and generally diagnosed in children between the ages of 3 and 10 [7]. The age of presentation is thought to correlate with more rapid pontine development in this group compared to older-aged children, in whom cerebral tissue proliferation rate begins to decline [4,8].

When the World Health Organization reclassified central nervous system tumors in 2016, partly in an effort to distinguish pediatric from adult tumors [3], DMG was called *diffuse midline glioma H3 K27-mutant* and was defined as a tumor located in the midline structures of the brain with a diffuse growth pattern and K27M mutation in the histone H3 genes *H3F3A* or *HIST1H3B* [3]. WHO updated nomenclature for DMG in 2021 to *diffuse midline glioma H3 K27-altered* [2], reflecting a broader molecular understanding of DMGs that recognizes alterations through other pathogenic mechanisms, including *TP53, ACVR1, PDGFRA, EGFR,* and EZHIP [2]. Throughout this review we will use 'DMG' as we discuss diffuse midline glioma H3 K27-altered and 'diffuse intrinsic pontine glioma (DIPG)' to refer to the subtype of DMG that arises from the pons [1], a distinction made because DMG is a histologic diagnosis made once tissue is obtained, whereas DIPG is a radiographic diagnosis based on the presence of a tumor centered in the pons that occupies more than 50% of the structure at its greatest diameter [2,9–13]. Once tissue is obtained that confirms DMG histologically and molecularly, this nomenclature is used over DIPG. For example, the tumor of a patient who has never had a biopsy would be called DIPG based on the radiographic diagnosis.

Multiple factors contribute to the dismal prognosis for DMG, including the inability to resect these tumors, inherent resistance to treatment, and the inability of some drugs to permeate the blood-brain barrier (BBB). Molecularly characterizing these tumors was long impeded by the inability to surgically resect them, which left researchers with a dearth of DMG tissue for analysis. An increasingly frequent use of stereotactic biopsy, now a recommended standard of care in both the United States and globally in the care of DMG patients [14], has expanded our knowledge about the molecular profile and mutational status of DMG. Molecular characterization has informed treatment decisions and the de-

* Corresponding author at: Center for Molecular Imaging, The University of Michigan Medical School, 109 Zina Pitcher Place, BSRB A502, Ann Arbor, MI 48109-2200, USA.
E-mail address: sgalban@umich.edu (S. Galban).

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velopment of new therapies [14–17]. Furthermore, stereotactic biopsy enables patient stratification based on the biological characteristics of the tumor. Tumor tissue thereby informs therapeutic regimens of drugs being trialed [17] and thus, has become a requisite for admission into many clinical trials for DMGs [14].

While it is considered safe, stereotactic biopsy remains an invasive procedure. Thus, "liquid biopsy" for diagnosis and monitoring treatment response is being explored [18]. This procedure is performed by recovering tumor cells or tumor cell DNA and RNA from cerebrospinal fluid (CSF), blood, or both for sequencing or PCR to evaluate spread of tumoral DNA [15] and detect tumor-associated mutations [19]. Furthermore, liquid biopsies may become useful in the future to predict therapeutic responses.

One of the most significant contributions to HGG research was the identification of the histone H3 gene mutation H3K27M that is present in more than 80% of DIPG [14]. With the WHO reclassifications in 2016 and 2021, H3-mutant DIPGs were reclassified as DMG H3 K27M-altered, while the approximately 20% of DIPGs that are wildtype for H3 are now classified as *diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype* [3]. In H3 K27M-altered tumors, variants in the genes for histone 3 substitute methionine for lysine at position 27 (H3 K27M) [20,21]. The mutations in histone H3 K27M ultimately impact epigenetic modifications, resulting in an overall decrease in bi- and trimethylation and increased acetylation of H3 K27M, which predisposes cells to global oncogenic changes in gene expression [20,22–24]. The ongoing characterization of the genomic landscape of DMG has led to identification of mutations in addition to those in histone H3.1 and H3.3. Co-occurring mutations in Activin A receptor type I (ACVR1) (20–32%), TP53 (22–40%), PDGFRA (32%), PI3KR1/PIK3CA (15%) [25,26], as well as amplification of PDGFR, have been identified. Additionally, receptor tyrosine kinase (RTK)/phosphatidylinositol 3-kinase (PI3K)/mitogen-activated protein kinase (MAPK) pathway activation has been identified in 60% of DMG tumors in any given node [26]. Mutations regulating mammalian target of rapamycin (mTOR) in genes including *TSC2, RPTOR* and *MTOR* have also been identified [26]. A significant fraction of DMG tumors is associated with alterations in cell cycle regulatory genes, namely in the amplification of D-type cyclins and CDK4/6 (Table 1) [27,28]. Platelet-derived growth factor receptor A (PDGF-A) has also been identified as an amplification and/or mutation in DMG (Table 1) [29,30]. Rarer targets include recurrent telomerizing mutations in the gene encoding protein phosphatase 1D, PPM1D (Table 1) [31]. In addition, an inactivating mutation in H4K20 histone methyltransferase KMT5B (SUV420H1) drives inappropriate DNA repair and increased invasion into neighboring DMG cells [32].

The characterization of the genomic landscape of DMG has also shed light on its cell of origin, which has been linked to oligodendroglial precursor cells that are enriched in the pons when these tumors are diagnosed, usually between ages 3 and 10 [33]. These cells are activated at both epigenetic and transcriptional levels [1,20,34]. Further, stem-like cells in DMG resemble these precursor cells, making them an oligodendrocyte precursor (OPC)-like, self-renewing population that drives tumor initiation [1,35]. Studies of the role H3 K27M mutations play in malignant growth in precursor cells show that H3.1 K27M tumors arise in the pons, while H3.3 K27M mutations may give rise to tumors either in the pons (i.e., DIPG) or in other midline structures [1,36,37]. Haag et al. have further shown in a study with human induced neuronal stem cells (NSCs) and OPCs expressing H3.3-K27M that DMG also shares similarities with neuronal progenitor cells (NPCs) [38]. Expression of H3.3-K27M in NSCs results in sustained expression of stemness and OPC genes, which together initiate tumor growth [38].

The complexity of DMG makes it difficult to develop effective treatment options. Regarding the role of immunotherapy, for example, it has been shown that the microenvironment of DMG is neither highly immunosuppressive nor inflammatory: these tumors have neither increased macrophage nor T-cell infiltration relative to non-tumor control and they do not overexpress immunosuppressive factors like programmed death ligand 1 and/or transforming growth factor β1 [39]. Further, H3.3-K27M DMG cells do not repolarize macrophages and are not effectively targeted by activated allogeneic T cells. Thus, the development of future immunotherapies will have to consider recruitment, activation, and retention of tumor-specific effector cells [40].

In this review we first discuss the greatest obstacles to effectively treating DMG, including its unresectable character, effective drug delivery, and complex forms of inherent resistance. Then, we highlight current preclinical and clinical directions in molecularly targeted and immune therapies and drug delivery methods for overcoming these obstacles, in the shared effort to improve survival for children with DMG.

Obstacles to treating DMG

The elaborate molecular pathogenesis, inherent therapeutic resistance, strict BBB regulation, and location that precludes surgical resection have all contributed to failures to improve prognosis for DMG [1]. Due to these challenges, radiotherapy has remained the standard of care. In this section, we will discuss these obstacles to treating DMG, along with modified radiotherapy paradigms that are being explored to improve survival outcomes.

Location and diffuse characteristics of DMG preclude surgery

DMG precludes surgical resection due to the tumor's infiltrative nature and delicate location [14]. DMG and other pediatric HGGs migrate away from the primary tumor mass into the subventricular zone (SVZ), where adult neurogenesis occurs. Moreover, since DMG H3 K27M-altered tumors originate in the pons, which controls vital functions including breathing, blood pressure, and heart rate, surgery risks significant neurological damage [40–42].

Blood-brain barrier permeability and drug delivery

While the inability to perform resection makes therapeutic development for DMG all the more pressing, drug delivery to tumor cells across the BBB is one of the most significant impediments to treating any brain tumor. This cellular structure, which blocks dangerous agents from the brain, also prevents some drugs from reaching it. BBB impermeability is a persistent concern in the development of new therapies, since even those that show promising results in cell and preclinical animal models may fail clinically if they cannot penetrate the BBB.

Drug delivery is a complex obstacle to effective treatment of DMG because it is thought that treatment failure is not due to direct inherent resistance mechanisms to the therapeutic agent, but is instead the result of the agent's inability to penetrate the BBB in sufficient concentrations, or its susceptibility to clearing by efflux transporters [43]. Among mechanisms contributing to BBB impermeability is the presence of efflux transporters within the membrane. For example, palbociclib does not readily cross the BBB or remain in the central nervous system before being cleared by drug efflux transporters. One study found that brain penetration by palbociclib is restricted by P-glycoprotein and other drug efflux transporters (i.e., MRP1, BCRP), which in turn decrease its efficacy against DMG and other HGGs [28]. One question with DMG specifically is whether the tumor itself augments the BBB endothelial cells in an effort to strengthen it. However, Deligne et al. found that DMG cells provided no enhancements that increase the impermeability of the patient's own BBB to render it more resistant to treatment [44].

Therapeutic Resistance

More than 250 clinical trials of chemotherapeutics and novel agents for treating DMG have failed [25,45,46], likely stemming from challenges posed by drug delivery, inherent treatment resistance [47], and acquired mechanisms of resistance [26,48,49]. However, clinical efforts continue as new targets in preclinical studies are identified.

Table 1
Summary of preclinical trials for DMG treatments.

Study	Treatment	Study Model
Biopsy Studies		
Li et al. Standardization of the liquid biopsy for pediatric diffuse midline glioma using ddPCR. *Nature.* 2021.	Liquid biopsy	Human, *in vitro* SF7761, KNS42, and patient tissue derived at autopsy
Molecularly Targeted Treatment Studies		
Anastas et al. LSD1/HDAC Inhibitor Induces Therapeutic Differentiation in DIPG. *Cancer Cell.* 2019	LAQ824, panobinostat	Human, *in vitro* SU-DIPGXIII, SU-DIPGVI, and SU-DIPGIV
Barton et al. PD-0332991 Activity in Brainstem Gliomas. *PLoS One.* 2013.	PD-0332991	Rodent, *in vitro* primary cell cultures
Borsuk et al. Imipridone-Based Combination therapies in DMG. *Am J Cancer Res.* 2021	Imipridone (ONC201, ONC206, and ONC212)	Human, *in vitro* SU-DIPG-IV, SU-DIPG-13, SU-DIPG-25, SU-DIPG-27, SU-DIPG-29, SF8628, SU-DIPG-36
Chang et al. DIPG treatment with CED of PI3K and MEK inhibitors. *Neurooncol Adv.* 2019.	ZSTK474, trametinib	Human, *in vitro* SU-DIPG-IV, SU-DIPG-XIII, and SF8628; Rodent, *in vitro* primary cell culture
Grasso et al. Functionally defined therapeutic targets in DIPG. *Nat Med.* 2015.	panobinostat + GSK-J4	Rodent, *in vivo* & *in vitro* JHH-DIPG-1, SF7761, VU-DIPG-A, VU-DIPG-B, and primary cell cultures
Hashizume et al. Pharmacologic inhibition of histone demethylation as a therapy for pediatric brainstem glioma. *Nat Med.* 2014.	GSKJ4	Human, in vivo SF77618, SF86289, SF9012, SF9402, SF9427, GBM43, and KNS42
Meel et al. MELK Inhibition in Diffuse Intrinsic Pontine Glioma. *Clin Cancer Res.* 2018.	OTSSP167	Human, *in vitro* VUMC-DIPG-A, VUMC-DIPG-F, VUMC-DIPG-08, VUMC-DIPG-10, VUMC-DIPG-11, HSJD-DIPG-07 (34), HSJD-DIPG-08, HSJD-DIPG-12, JHH-DIPG-01, and SF7761
Piunti et. al. BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med.* 2017.	I-BET151 and JQ1	Rodent, *in vivo,* Human, *in vitro* SF8628, SF7761 and SU-DIPG-IV, pcGBM2, SF9402 and SF9427
Tsoli et al. Dual targeting of mitochondrial function and mTOR pathway. *Oncotarget.* 2018	PENAO, temsirolimus	Human, *in vitro* SU-DIPG-IV
Vitanza et al. Optimal HDAC inhibition in DMG. *Neuro Oncol.* 2021.	panobinostat (LBH589), quisinostat (JNJ-26481585), vorinostat (MK0683), entinostat (MS-275), romidepsin (FK228), and CAY10603	Human, *in vitro* PBT-09FH, PBT-22FH, PBT-24FH, PBT-27FH, DRIz-D105, MED-411, HSJD-DIPG007, and SU-DIPG48; Rodent *in vivo*
Wang et al. PARP inhibitor activity in DIPG. *Mol Cancer Res* 2020	Olaparib	Human, *in vitro* SF7761, GBM-002, HSJD-DIPG-012, HSJD-DIPG-013, HSJD-DIPG-007, TT10714, and TT10728
Xu et al., MDM2 antagonist for DIPG. *Acta Neuropathol Commun.* 2021.	RG7388	Human, *in vitro* TT10714, TT10728, TT10630, SF7761, HSJD-DIPG-007, HSJD-DIPG-012, HSJD-DIPG-013, SU-DIPG-VI, SU-DIPG-XIII, SU-DIPG-35, HSJD-DIPG-007-NTC, and HSJD-DIPG-TP53
Chemotherapeutic Treatment Studies		
Bailleul et al. Potentiating effect of HAP drug in treated pediatric gliomas. *Cancers* 2021	Evofosfamide	Human, *in vitro* SF188, KNS42, UW479, HSJD-DIPG007, HSJD-DIPG013, and HSJD-DIPG014
Truffaux et al. Dasatinib alone and in combination for DIPG treatment. *Neuro Oncol.* 2015.	Dasatinib, Cabozantinib	Human, *in vitro* SF188 and novel cell lines
Immunotherapy/Misc. Treatment Studies		
Asby et al. CDK4/6 and mTOR inhibitors synergistically limit growth of DIPG cells. *Cancer Manag Res.* 2018.	Palbociclib + Temsirolimus	Human, *in vitro* SF7761, SF8628, and SU-DIPG IV
Josupeit et al. H1-PV efficacy in eliminating HGG initiating cells. *Viruses.* 2016.	H-1PV	Rodent, *in vivo* & *in vitro* NCH421R, NCH421I, and NCH421k
Majzner et al. CAR T Cells Targeting B7-H3. *Clin Cancer Res.* 2019	B7-H3 CAR T cells	Human *in vitro* MG63.3, K562, EW8, NALM6-GL, DAOY, D283, D425, 293GP, 293T, SU-DIPG VI, and SU-DIPG XVII
Mount et. al. Anti-GD2 CAR T cells in H3-K27M+ diffuse midline gliomas. *Nat Med.* 2018.	CAR T cell Therapy	Human, *in vitro* SU-DIPG-VI and SU-DIPG-XIII
Schumacher et al. A vaccine targeting mutant IDH1 induces antitumour immunity. *Nature.* 2014.	Anti-IDH1(R132H) Vaccine	Rodent, *in vivo* & *in vitro* primary cell cultures

DMG has also shown itself to be inherently unresponsive to chemotherapeutics and combinations of radiation therapy plus neoadjuvant, concurrent, or adjuvant chemotherapy, which have failed to significantly extend survival [25,45,46]. In general, chemotherapeutic and radiotherapeutic resistance is thought to be mediated by the emergence of cancer stem cells as an acquired mechanism [26,48,49], as well as through inherent resistance by mutations in p53, which co-occur in many malignancies including DMG [47]. Numerous other co-occuring mutations may also contribute to mechanisms of inherent drug and radioresistance [26]. Chemotherapies in combination with radiation have been studied in recent clinical trials and several completed studies offer valuable data for various agents (Supplemental Table 1) [50]. A common sentiment with chemotherapy use for DMG is that overall, the risk vs. benefit does not prove to be significantly favorable. For example, a phase 2 study (NCT00879437) testing valproic acid, radiation, and bevacizumab showed minimal improvement, either in event-free survival or overall survival, thus minimizing the benefit to the patient enduring the therapy [51] (Supplemental Table 1). This is in addition to historic evidence of several chemotherapeutic agents resulting in no clinical improvement. Currently, there are several ongoing clinical trials studying both chemotherapy and combination therapies for DMG (Supplemental Table 1). Of these, the chemotherapeutic agent temozolomide is being studied the most. Trials for temozolomide include a phase 1/phase 2 study (NCT04238819) that is evaluating the combination therapy of abemaciclib, temozolomide, and irinotecan. Four studies (NCT03709680, NCT01837862, NCT04049669, NCT04239092) are also actively recruiting or have completed recruiting to evaluate temozolomide combination therapy. Of these, trial NCT04049669 is studying temozolomide and other chemotherapies with concurrent radiotherapy (Supplemental Table 1).

For DMG, resistance mechanisms are diverse and complex, often stemming from metabolic alterations with tumor cells. Surowiec et al. identified aldehyde dehydrogenase-positive (ALDH+) cancer stem cells as drivers of tumor growth that likely cause resistance to standard of care radiotherapy [48]. Surowiec's group has shown that DMG exhibits heterogeneity in the expression of ALDH, likely potentiating a kind of metabolic reprogramming to enhance the survival of oncogenic cells. These ALDH+ cells were found to concomitantly express increased tran-

Table 2
Methods to improve drug delivery.

NCT Number	Title	Status	Intervention	Phase	Location
NCT03086616	CED With Irinotecan Liposome Injection Using Real Time Imaging in Children With Diffuse Intrinsic Pontine Glioma (DIPG) (PNOC 009)	Completed	Convection Enhanced Delivery (CED) of Nanoliposomal irinotecan (nal-IRI)	Phase 1	USA
NCT03566199	MTX110 by Convection-Enhanced Delivery in Treating Participants With Newly-Diagnosed Diffuse Intrinsic Pontine Glioma	Completed	panobinostat Nanoparticle Formulation MTX110 via Convection-Enhanced Delivery (CED)	Phase 1/Phase 2	USA
NCT05123534	A Phase 1/2 Study of Sonodynamic Therapy Using SONALA-001 and Exablate 4000 Type 2 in Patients With DIPG	Recruiting	SONALA-001 (ALA) & MR-Guided Focused Ultrasound device (MRgFUS)	Phase 2	USA
NCT04264143	CED of MTX110 Newly Diagnosed Diffuse Midline Gliomas	Recruiting	Infusate with MTX110 and gadolinium via Convection-Enhanced Delivery (CED)	Phase 1	USA
NCT05063357	131I-omburtamab Delivered by Convection-Enhanced Delivery in Patients With Diffuse Intrinsic Pontine Glioma	Not yet recruiting	131I-omburtamab via Convention Enhanced Delivery	Phase 1	Not provided
NCT04804709	Non-Invasive Focused Ultrasound (FUS) With Oral panobinostat in Children With Progressive Diffuse Midline Glioma (DMG)	Active, not recruiting	panobinostat via Focused Ultrasound with neuro-navigator-controlled sonication	Phase 1	USA
NCT01502917	Convection-Enhanced Delivery of 124I-omburtamab for Patients With Non-Progressive Diffuse Pontine Gliomas Previously Treated With External Beam Radiation Therapy	Completed	Radioactive iodine-labeled monoclonal antibody omburtamab, External Beam Radiotherapy	Phase 1	USA

Table 3
New radiotherapy trials.

NCT Number	Title	Status	Intervention	Phase	Location
NCT01777633	Palliative Re-irradiation for Progressive Diffuse Intrinsic Pontine Glioma (DIPG) in Children	Completed	Palliative re-irradiation for progressive DIPG in children	Phase 1/Phase 2	Israel
NCT03841435	Hypofractionated Radiotherapy for Recurrent DIPG	Enrolling by invitation	Hypofractionated Radiotherapy	N/A	USA
NCT03126266	Re-Irradiation of Progressive or Recurrent DIPG	Recruiting	Re-irradiation	N/A	Canada, Australia, New Zealand
NCT01469247	Diffuse Intrinsic Pontine Glioma (DIPG) Reirradiation (ReRT)	Completed	Radiation Therapy	Phase 1/Phase 2	USA
NCT04670016	HRQL and Symptom Assessment for Patients With DIPG or Recurrent and Re-irradiated Brain Tumours and Their Caregivers	Recruiting	Radiotherapy	N/A	Canada
NCT01878266	Prospective Trial of Two Hypofractionated Radiotherapy Regimens Versus Conventional Radiotherapy in Diffuse Brainstem Glioma in Children	Completed	Hypofractionated Arm vs Conventional Arm Radiation	N/A	Egypt
NCT01445288	Exploratory Study of Effects of Radiation Therapy in Pediatric Patients With Central Nervous System Tumors	Completed	Radiotherapy	N/A	USA

scription of MYC, DNA-damage repair genes, E2F, genes related to glycolytic metabolism, and mTOR in comparison to ALDH-negative cells.

Radiotherapy remains the standard of care for DMG

Due to obstacles posed by drug delivery and resistance and the inability to resect DMGs, focal radiation remains the standard of care for this disease, though it is considered palliative. Radiation is used to halt tumor progression and increases overall survival by about three months; without radiotherapy, overall survival is about 5 months [15]. Radiation fields are restricted to the tumor and 1-2 cm of adjacent brainstem tissue [52]. The standard dose is 1.8 Gy per day, 5 days per week, ultimately yielding a total dose of 54-59.4 Gy [50].

However, radioresistance ultimately arises in all DMG, resulting in tumor recurrence in ~100% of patients. A contributor to radioresistance in gliomas is thought to be tumor hypoxia, as adequate oxygenation is necessary for radiotherapy to remain effective. When hypoxia occurs, cells upregulate hypoxia-inducible factors (HIFs), which in turn stimulate enzymes responsible for cancer survival under hypoxic stress. Current literature offers compelling evidence that drugs seeking to inhibit tumor hypoxia, such as quinacrine, atovaquone, proguanil, ivermectin, and mefloquine, may prove to be effective against DMG and other HGGs by enhancing radiosensitivity [53]. Other mechanisms of radioresistance in DMG may be related to alterations in the TP53 pathway [47]. Investigations of these mechanisms have led to successful preclinical trials studying p53 pathway inhibitors such as RG7388, a MDM2 inhibitor [54], and GSK2830371 [31] (Table 1).

In an effort to improve survival outcomes for children with DMG and tackle mechanisms of radioresistance, new innovative radiotherapeutic paradigms are being explored (Table 3). For example, re-irradiation using a higher loading dosage than the initial radiation for children with markedly progressive tumors, or for whom initial radiotherapy fails, are being trialed (NCT01777633, NCT03126266, NCT01469247; Table 3). Re-irradiation has been found to improve survival time by several weeks and lead to clinical improvement in up to 77% of patients [55]. In addition, the benefits of hypofractionated radiation therapy (RT), which shortens treatment periods, are also being evaluated (NCT03841435, NCT01878266; Table 3). The shorter treatment period of hypofractionated RT decreases the length of time patients must undergo debilitating radiation and thus, potentially reduces treatment costs. However, to ensure adequate dosing, a larger radiation dose is administered at each session, which can both increase side effects and decrease tolerability [56]. Prior studies have found that hypofractionated and conventional radiotherapy for DMG produce similar survival outcomes [56]. If ongoing trials yield similar results, it will be interesting to see how hypofractionated RT is incorporated into treatment plans. Shorter treatment sessions may be especially desired in DMG due to the younger patient population and average expected survival of < 1 year.

In summary, the understanding of the complex molecular pathobiology of DMG and impediments to effectively treating it have led to the identification of new treatment targets in preclinical studies. In addition, new targets have been important to modifying radiotherapy approaches. The resulting clinical trials for new regimens allow some optimism for future patients [57,58].

Developments of new therapeutics and treatment paradigms

Breakthroughs have advanced our understanding of the molecular profiles of DMG through cell lines obtained from autopsy tissue sampling and stereotactic biopsy. The identification of factors that contribute to radio- and chemoresistance continues to drive efforts to find new drug targets. In this section, we focus on two broad therapeutic areas of molecularly targeted therapies, epigenetic modifiers and immune therapies, as we discuss promising preclinical investigations and clinical trials of potential treatments for DMG.

Molecularly targeted therapies

The advances made in understanding the pathobiology of DMG have led to the development of new therapeutics and therapeutic paradigms targeting aberrant molecular pathways in this disease. These include promising targeted therapies with imipridone (ONC201, Chimerix), a small molecule known to induce apoptosis in cancer through the upregulation of the TNF-related apoptosis inducing ligand (TRAIL) pathway and its respective pro-apoptotic receptor DR5 (Table 4). ONC201 works as a selective antagonist at the dopamine receptor D2 (DRD2), leading to activation of the integrated stress response (ISR) within DMG tumor cells that ultimately leads to the upregulation of several apoptosis-related genes such as the TRAIL-receptor DR5 [59]. It is also a potent agonist of the mitochondrial Caseinolytic protease P (ClpP) [60,61], which drives degradation of mitochondrial respiratory chain enzymes, leading to p53-independent apoptosis and cancer-selective cell death [60].

ONC201 was initially studied in vitro and in mouse models with glioblastoma, and was found to cross the BBB and inhibit tumor growth [59]. Subsequent preclinical studies have found that ONC201 exhibits significant anti-proliferative and pro-apoptotic effects against a vast range of tumor cells but not normal cells, suggesting it may hold promise as a therapy for DMG [62]. Furthermore, preclinical trials for this drug suggest that while H3K27M-mutant DMG cells demonstrate an apoptotic response to ONC201, they show increased sensitivity to other imipridone analogs (i.e., ONC206 and ONC212) (Table 1) [63,64]. Thus, combination therapies using imipridone or improved imipridone analogs with radiotherapy, chemotherapeutics or other molecularly targeted therapeutics such as epigenetic modifiers are currently being explored in preclinical testing and some have advanced to clinical trials (NCT03416530; Table 4). ONC201 has previously been studied as part of a phase 2 trial for adult glioblastoma with positive results (NCT02525692), and thus, it is no surprise that its efficacy is now tested for DMGs in several ongoing clinical trials (Table 4). These include the evaluation of the efficiency and safety of oral ONC201 and its effects following other lines of therapy, including RT, in patients with both new and recurrent/refractory H3K27M-positive gliomas (NCT03416530, NCT05009992; Table 4). A trial of ONC201 for treating H3K27M-positive DMG is currently in phase 1 (NCT03416530). A trial assessing different combinations of ONC201, radiation, and the BBB-penetrant small molecule PI3K/mTOR inhibitor paxalisib for treating children and young adults with newly-diagnosed and recurrent DMG, as well as those previously treated with radiotherapy, is currently in phase 2 (NCT05009992; Table 4).

Notably, access to ONC201 for treating DMG has been expanded beyond the United States and Japan, the sites of most clinical trials, to other parts of the world through compassionate care programs in Germany, France, and the United Kingdom [60,65]. Preclinical testing of a formulation of ONC201 synthesized in Germany showed results similar to that being tested in clinical trials [60,65]. Duchatel et al. showed median overall survival of 18 months, both in patients treated with the German formulation of ONC201 in combination with other therapies, and in those who received the treatment following radiation therapy, but whose cancer had not recurred [60,65]. The median time from diagnosis to first administration of this formulation of ONC201 was 7.5 months [60].

A significant number of DMG exhibit alterations in cell cycle regulatory genes, namely in the amplification of D-type cyclins and CDK4/6 [27,28] and thus present therapeutic targets of interest (Table 1). The Barton group explored this possibility by using the CDK4/6 inhibitor PD-033299, also known as palbociclib, in a platelet-derived growth factor B (PDGFB)-driven brainstem glioma mouse model. They found that PD-033299 induced cell-cycle arrest, but interestingly, when used after a chemoradiotherapeutic regimen, it resulted in a survival benefit of 19% (Table 1) [27,28]. This and other studies support the use of CDK4/6 inhibitors like palbociclib for treating DMG patients, whose tumors are deficient in the INK4-ARF locus (formally designated CDKN2A and CDKN2B), which includes the three intimately linked tumor suppressor genes (INK4A, INK4B, and ARF) and encodes for the proteins p16, p15 and p14, respectively, that regulate CDK4/6 [27]. These and other findings have resulted in the advancement of palbociclib to clinical trials for DMG patients (NCT02255461; Supplemental Table 2) [66]. However, resistance to CDK4/6 inhibition has been described and is a concern for utilizing palbociclib clinically. One of the mechanisms of acquired resistance observed in mouse models treated with CDK4/6 inhibitors are the alterations observed in ABCB1 transporters [67]. Thus, other CDK4/6 inhibitors, such as ribociclib, have been developed and are being tested. Ribociclib showed synergistc effects when combined with everolimus in mice [68], and has advanced to clinical trials (NCT03387020, NCT03355794; Table 4). Phase 1 results have shown that therapeutic concentrations of ribociclib could potentially be achieved in both CSF and tumor tissue [68]. Furthermore, the steady state plasma and CSF concentration remained higher in patients receiving dual therapy without impacting the pharmacokinetics of either drug, further supporting the use of both medications together (NCT03387020; Table 4) [68]. Another active study is investigating the combination of ribociclib and everolimus, with the addition of radiotherapy in Rb+ DMGs (NCT03355794; Table 4).

Other pertinent mutations occurring in DMG are found in the tumor suppressor p53, prompting development of new therapeutic options [47]. As many DMGs exhibit co-occurring mutations in the tumor suppressor p53 or show PPM1D gain-of-function mutations that ultimately suppress p53, targeted therapies against this commonly dysregulated protein in oncogenesis are being tested for DMG (Table 1) [54]. Furthermore, pathogenic variants of p53 found in tumors may represent promising targets during radiotherapy, because a recent study suggested that gene deletion of ataxia telangiectasia (ATM) with p53 mutations resulted in improved outcome of radiotherapy [69]. MDM2 is a negative regulator of p53 and thus respresents a promising protein to target for a subset of DMGs that is wildtype for p53. RG7388, a recently developed MDM2 inhibitor, showed significant therapeutic efficacy in patient-derived cell lines (Tables 1, 4) [54].

Several signaling pathways have also been identified as targets for DMG therapy. Adjuvant treatment combining trametinib with the PI3K inhibitor ZSTK474 have shown synergy and inhibited growth of DMG without significant neurotoxicity (Table 1) [70]. A clinical study (NCT05009992; Table 4) is now investigating the combination of the PI3K inhibitor paxalisib with ONC201 and radiotherapy. PARP and PPM1D inhibitors have also shown a synergistic effect in DMG, though this has only been studied in PPM1D-mutant DMG tumor cells (Table 1) [31]. Truffaux et al. suggested that the c-Met signaling pathway may provide another therapeutic target, in a preclinical study showing that the anti-tumor effect of dasatinib on DMG may be increased by combining it with other RTK inhibitors like cabozantinib, which acts specifically as a c-Met inhibitor (Table 1) [30]. Several studies are currently evaluating dasantinib, including one studying dasatinib with vandetanib (NCT00996723; Supplemental Table 1) and another studying dasatinib with crizotinib (NCT01644773; Supplemental Table 1).

STAT3 is another pathway actively studied in DMG and other HGGs, and one study found pyrazole-based STAT3 pathway inhibitors selectively induced apoptosis to decrease cell survival in HGGs without imposing significant toxicity on host astrocytes and other healthy neigh-

Table 4
Molecularly targeted and immune therapies.

NCT Number	Title	Status	Intervention	Phase	Location
Molecularly Targeted Treatment Studies					
NCT02233049	Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication	Unknown status	Erlotinib, Everolimus, Dasatinib	Phase 2	France
NCT01393912	PDGFR Inhibitor Crenolanib in Children/Young Adults With Diffuse Intrinsic Pontine Glioma or Recurrent High-Grade Glioma	Completed	Crenolanib	Phase 1	USA
NCT03416530	ONC201 in Pediatric H3 K27M Gliomas	Active, not recruiting	ONC201	Phase 1	USA
NCT00600054	Phase 2 Study of Nimotuzumab in Pediatric Recurrent Diffuse Intrinsic Pontine Glioma	Completed	nimotuzumab (anti EGFR humanized monoclonal antibody)	Phase 2	USA, Canada, Israel
NCT02359565	Pembrolizumab in Treating Younger Patients With Recurrent, Progressive, or Refractory High-Grade Gliomas, Diffuse Intrinsic Pontine Gliomas, Hypermutated Brain Tumors, Ependymoma or Medulloblastoma	Recruiting	Pembrolizumab	Phase 1	Canada, USA
NCT03355794	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)	Completed	ribociclib, everolimus	Phase 1	USA
NCT02717455	Trial of Panobinostat in Children with Diffuse Intrinsic Pontine Glioma (PBTC-047)	Active, not recruiting	panobinostat	Phase 1	USA
NCT04341311	Phase I Study of Marizomib + panobinostat for Children With DIPG	Active, not recruiting	Marizomib, panobinostat	Phase 1	USA
NCT03893487	Fimepinostat in Treating Brain Tumors in Children and Young Adults	Active, not recruiting	Fimepinostat	Early Phase 1	USA, Switzerland
NCT03387020	Ribociclib and Everolimus in Treating Children With Recurrent or Refractory Malignant Brain Tumors	Completed	Everolimus, Ribociclib	Phase 1	USA
NCT03389802	Phase I Study of APX005M in Pediatric CNS Tumors	Recruiting	APX005M	Phase 1	USA
NCT00561691	Nimotuzumab in Children With Intrinsic Pontine Glioma	Completed	nimotuzumab	Phase 3	Germany
NCT02644291	Phase I Study of Mebendazole Therapy for Recurrent/Progressive Pediatric Brain Tumors	Completed	Mebendazole	Phase 1	USA
NCT03478462	Dose Escalation Study of CLR 131 in Children, Adolescents, and Young Adults With Relapsed or Refractory Malignant Tumors Including But Not Limited to Neuroblastoma, Rhabdomyosarcoma, Ewings Sarcoma, and Osteosarcoma	Recruiting	CLR 131	Phase 1	USA, Australia, Canada
Molecularly Targeted Treatment + Radiotherapy					
NCT02420613	Vorinostat and Temsirolimus With or Without Radiation Therapy in Treating Younger Patients With Newly Diagnosed or Progressive Diffuse Intrinsic Pontine Glioma	Active, not recruiting	Radiation Therapy, Temsirolimus, Vorinostat	Phase 1	USA
NCT04758533	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	Recruiting	AloCELYVIR	Phase 1/Phase 2	Spain
NCT03605550	A Phase 1b Study of PTC596 in Children With Newly Diagnosed Diffuse Intrinsic Pontine Glioma and High Grade Glioma	Recruiting	PTC596 + Radiotherapy	Phase 1	USA
NCT04532229	Nimotuzumab in Combined With Concurrent Radiochemotherapy in the Treatment of Newly Diagnosed Diffuse Intrinsic Pontine Glioma (DIPG) in Children	Recruiting	Nimotuzumab+CRT(concurrent IMRT and TMZ)	Phase 3	China
NCT03620032	Study of Re-irradiation at Relapse Versus RT and Multiple Elective rt Courses	Active, not recruiting	Nimotuzumab, Vinorelbine, Radiotherapy	Phase 2	Italy
NCT01189266	Vorinostat and Radiation Therapy Followed by Maintenance Therapy With Vorinostat in Treating Younger Patients With Newly Diagnosed Diffuse Intrinsic Pontine Glioma	Completed	3-Dimensional Conformal Radiation Therapy, Vorinostat	Phase 1/Phase 2	Australia, Canada, USA
NCT01922076	Adavosertib and Local Radiation Therapy in Treating Children With Newly Diagnosed Diffuse Intrinsic Pontine Gliomas	Completed	Adavosertib, Radiation Therapy	Phase 1	Canada, USA
NCT03696355	Study of GDC-0084 in Pediatric Patients With Newly Diagnosed Diffuse Intrinsic Pontine Glioma or Diffuse Midline Gliomas	Completed	GDC-0084, radiation therapy	Phase 1	USA
NCT05009992	Combination Therapy for the Treatment of Diffuse Midline Gliomas	Recruiting	ONC201, Radiation Therapy, paxalisib	Phase 2	Australia, Israel, Netherlands, New Zealand, Switzerland, USA
NCT01222754	Lenalidomide and Radiation Therapy in High Grade Gliomas or Diffuse Intrinsic Pontine Gliomas	Completed	Lenalidomide, Radiation 54-59.4 Gy	Phase 1	USA
NCT04250064	A Study of Low Dose Bevacizumab With Conventional Radiotherapy Alone in Diffuse Intrinsic Pontine Glioma	Recruiting	Bevacizumab Injection, ultra-low-dose RT	Phase 2	India
NCT05099003	A Study of the Drug Selinexor With Radiation Therapy in Patients With Newly-Diagnosed Diffuse Intrinsic Pontine (DIPG) Glioma and High-Grade Glioma (HGG)	Recruiting	Radiation Therapy, Selinexor	Phase 1/Phase 2	Canada, USA
Immunotherapy/Misc. Treatment Studies					
NCT04911621	Adjuvant Dendritic Cell Immunotherapy for Pediatric Patients With High-grade Glioma or Diffuse Intrinsic Pontine Glioma	Active, not recruiting	Dendritic cell vaccination + temozolomide-based chemoradiation	Phase 1/Phase 2	Belgium
NCT04749641	Neoantigen Vaccine Therapy Against H3.3-K27M Diffuse Intrinsic Pontine Glioma	Recruiting	Histone H3.3-K27M Neoantigen Vaccine Therapy	Phase 1	China
NCT02840123	Safety Study of DIPG Treatment With Autologous Dendritic Cells Pulsed With Lysated Allegenic Tumor Lines	Unknown status	Autologous dendritic cells	Phase 1	Spain
NCT03914768	Immune Modulatory DC Vaccine Against Brain Tumor	Unknown status	Immunomodulatory DC vaccine to target DIPG & GBM	Phase 1	China

(*continued on next page*)

Table 4 (*continued*)

NCT Number	Title	Status	Intervention	Phase	Location
NCT04943848	rHSC-DIPGVax Plus Checkpoint Blockade for the Treatment of Newly Diagnosed DIPG and DMG	Recruiting	rHSC-DIPGVax, Balstilimab, Zalifrelimab	Phase 1	USA
NCT03178032	Oncolytic Adenovirus, DNX-2401, for Naive Diffuse Intrinsic Pontine Gliomas	Unknown status	DNX-2401	Phase 1	Spain
NCT04837547	PEACH TRIAL- Precision Medicine and Adoptive Cellular Therapy	Recruiting	Tumor-specific ex vivo expanded autologous lymphocyte transfer (TTRNA-xALT)	Phase 1	USA
NCT00036569	A Phase II Study of Pegylated Interferon Alfa 2b (PEG-Intron(Trademark)) in Children With Diffuse Pontine Gliomas	Completed	Adjuvant therapy with pegylated interferon alfa	Phase 2	USA
NCT02274987	Molecular Profiling for Individualized Treatment Plan for DIPG	Completed	Specialized tumor board recommendation (Standard radiation therapy for all patients)	N/A	USA
NCT05096481	PEP-CMV Vaccine Targeting CMV Antigen to Treat Newly Diagnosed Pediatric HGG and DIPG and Recurrent Medulloblastoma	Not yet recruiting	PEP-CMV, Temozolomide, Tetanus Diphtheria Vaccine	Phase 2	USA
NCT04185038	Study of B7-H3-Specific CAR T Cell Locoregional Immunotherapy for Diffuse Intrinsic Pontine Glioma/Diffuse Midline Glioma and Recurrent or Refractory Pediatric Central Nervous System Tumors	Recruiting	SCRI-CARB7H3(s); B7H3-specific chimeric antigen receptor (CAR) T cell	Phase 1	USA
NCT03396575	Brain Stem Gliomas Treated With Adoptive Cellular Therapy During Focal Radiotherapy Recovery Alone or With Dose-intensified Temozolomide (Phase I)	Recruiting	TTRNA-DC vaccines with GM-CSF, Cyclophosphamide + Fludarabine Lymphodepletive Conditioning, Dose-Intensified TMZ, Td vaccine, Autologous Hematopoietic Stem Cells (HSC)	Phase 1	USA
NCT04978727	A Pilot Study of SurVaxM in Children Progressive or Relapsed Medulloblastoma, High Grade Glioma, Ependymoma and Newly Diagnosed Diffuse Intrinsic Pontine Glioma	Recruiting	SurVaxM	Phase 1	USA
NCT02750891	A Study of DSP-7888 in Pediatric Patients With Relapsed or Refractory High Grade Gliomas	Completed	DSP-7888	Phase 1/Phase 2	Japan
NCT02960230	H3.3K27M Peptide Vaccine With Nivolumab for Children With Newly Diagnosed DIPG and Other Gliomas	Active, not recruiting	K27M peptide, Nivolumab	Phase 1/Phase 2	USA, Switzerland
NCT01952769	Anti PD1 Antibody in Diffuse Intrinsic Pontine Glioma	Unknown status	MDV9300	Phase 1/Phase 2	Israel
NCT04196413	GD2 CAR T Cells in Diffuse Intrinsic Pontine Gliomas(DIPG) & Spinal Diffuse Midline Glioma(DMG)	Recruiting	GD2 CAR T cells, Fludarabine, Cyclophosphamide	Phase 1	USA
NCT04808245	A MultIceNTER Phase I Peptide VaCcine Trial for the Treatment of H3-Mutated Gliomas (INTERCEPT-H3)	Recruiting	tecentriq, H3K27M peptide vaccine, imiquimod	Phase 1	Germany
NCT03739372	Clinical Benefit of Using Molecular Profiling to Determine an Individualized Treatment Plan for Patients With High Grade Glioma	Active, not recruiting	Specialized tumor board recommendation	N/A	USA
NCT03690869	REGN2810 in Pediatric Patients With Relapsed, Refractory Solid, or Central Nervous System (CNS) Tumors and Safety and Efficacy of REGN2810 in Combination With Radiotherapy in Pediatric Patients With Newly Diagnosed or Recurrent Glioma	Recruiting	cemiplimab	Phase 1/Phase 2	USA
NCT04099797	C7R-GD2.CAR T Cells for Patients With GD2-expressing Brain Tumors (GAIL-B)	Recruiting	(C7R)-GD2.CART cells, Cyclophosphamide, Fludarabine	Phase 1	USA
NCT02444546	Wild-Type Reovirus in Combination With Sargramostim in Treating Younger Patients With High-Grade Relapsed or Refractory Brain Tumors	Active, not recruiting	Sargramostim, Wild-type Reovirus	Phase 1	USA
NCT05298995	GD2-CAR T Cells for Pediatric Brain Tumours	Not yet recruiting	GD2-CART01 (iC9-GD2-CAR T-cells)	Phase 1	USA

boring cells [71]. Researchers have also identified the MAPK pathway as a potential therapeutic target for DMG, with the mitogen-activated protein kinase (MEK) 1/2 inhibitor, trametinib, and the tyrosine kinase inhibitor, dasatinib, showing synergistic effects in DMG apoptosis, even with trametinib-resistant DMG cells [72]. DMG has also been associated with amplifications in the PI3K pathway alongside the activation of the MEK pathway, which may provide an avenue for clinical treatment [48].

A study evaluating inhibition of the maternal embryonic leucine zipper kinase (MELK) in the treatment of DMG found this kinase highly expressed in DMG cells, and its inhibition with OTSSP167 resulted in reduced inhibitory phosphorylation of PPARγ. This caused an increase in nuclear translocation and consequently, transcriptional activity, ultimately decreasing the proliferation of DMG cells in mouse models despite limited BBB penetration. As a result, this study identified the MELK-PPARγ signaling axis as an additional pertinent therapeutic target for improving survival for DMG (Table 1) [73]. However, there are currently no ongoing clinical trials targeting MELK specifically. This enhanced signaling within the MAPK/PI3K/mTOR pathways could provide a therapeutic target by taking advantage of the tumor's inherent need for increased metabolic activity [48]. DMG has also been found to express high levels of adenine nucleotide translocase 2 (ANT2). Therapeutically, this metabolic change sensitizes the tumor to the mitochondrial inhibitor PENAO by inducing oxidative stress. Furthermore, combining PENAO with the mTOR inhibitor temsirolimus enhances the effects of oxidative stress. While PENAO is thought to not readily penetrate the BBB, future testing in conjunction with an agent such as rapamycin, which slows drug efflux, may improve efficacy [74]. Finally, success with potentiating the hypoxic nature of DMG with evofosfamide (Evo), a second-generation hypoxia-activated prodrug (HAP) has been reported. Evo was found to inhibit the growth of all cells tested, both alone or in combination with doxorubicin, etoposide, and SN38 (i.e., the active metabolite of irinotecan). In fact, when combination therapies were tested, significant synergism was noted (Table 1) [75].

Epigenetic therapies

H3 K27M alterations found in DMG regulate the epigenetic landscape by affecting methylation and acetylation of histones, thereby regulating gene expression in these tumors. While epigenetic modifier therapies are being developed and tested in DMGs, they usually are systemically toxic, and, as with many molecular therapeutics, BBB penetrance and development of therapeutic resistance are major issues that will need to be overcome for the successful implementation of these therapies and therapeutic paradigms in the clinic. We will highlight the

mechanisms and targets for these therapies, as well as difficulties in implementing them in the clinical setting.

Several therapies targeting epigenetic modifiers are currently being investigated in preclinical studies and clinical trials, including inhibitors of histone deactylase (HDAC), bromodomain and extraterminal (BET) proteins, and histone demethylase [76]. HDAC inhibitors are being explored for treatment of DMG H3 K27M-altered (Table 1) [77,78]. These anti-cancer agents regulate epigenetics [78] and non-epigenetic processes such as DNA repair [79], and induce cell death and cell cycle arrest in cancer cells [80]. HDACs remove the acetyl groups from the lysine residues of histones, generally leading to the formation of a condensed and transcriptionally silenced chromatin. HDAC inhibition was identified in drug screens as a target for treating DMG [81], and has since been explored in clinical trials using the HDAC inhibitor panobinostat in children with DMG (NCT02717455; Table 4). While the use of HDAC inhibitors for DMG seems somewhat counterintuitive since it would result in transcriptional activation rather than silencing, their efficacy in cells and preclinical mouse models has been overall very impressive for targeting pediatric brain tumors [82]. One hypothesis is that use of HDAC inhibitors may increase the acetylation of histone marks located at tumor suppressors, thereby increasing their expression [29]. Secondly, HDAC inhibitors have been shown to decrease the expression of proteins involved in homologous recombination like Rad51, the main DNA damage response (DDR) gene, and thus synergize with radiotherapy [79]. However, the excitement about HDAC inhibitors and their therapeutic benefit for DMG has already been dampened by studies that indicate the emergence of therapeutic resistance [20]. In addition to acquired resistance to HDAC inhibition, inherent resistance to these inhibitors is a concern for DMG treatment (Table 1) [78].

Another emerging epigenetic treatment for DMG, BET inhibitors, work by preventing the interaction of the epigenetic regulator BRD4 with acetylated lysines on histones, leading to the repression of BRD4 transcriptional targets and thereby limiting tumor growth [76]. Examples of these inhibitors include JQ-1, I-BET762, I-BET151, and OTX-015 [76]. Within DMG specifically, preclinical studies have found JQ1 and I-BET151 to promote antitumor activity by impairing proliferation and stimulating differentiation of H3K27M-positive DMG cells [83]. Currently, a few clinical trials are studying a variety of BET inhibitors for adult tumors, including GBM. These studies show promise for future trials investigating BET inhibitors, though as of now, there are no ongoing trials for DMGs.

Histone demethylase inhibitors are another variety of epigenetic modifiers being studied for CNS tumors like DMG. It is known that a global reduction of H3K27 methylation is a key epigenetic event in H3K27M-altered DMG [76]. Therefore, it is intuitive to consider restoration of methylation as a therapeutic strategy for DMG. This may be accomplished by either enhancing PRC2 methyltransferase activity or by inhibiting demethylase activity for the 27-lysine residue [84]. One molecule, GSKJ4, and its prodrug, GSKJ1, have been found to increase cellular H3K27 methylation by directly targeting the 27-lysine demethylase JMJD3 [85]. This inhibitor has also been found to successfully treat pediatric brainstem gliomas, both *in vivo* and *in vitro*, by increasing methylation and subsequently decreasing DMG tumor cell growth [85]. We are currently awaiting the implementation of clinical trials for such histone demethylase inhibitors for DMG.

Inherent resistance to HDAC inhibitors and other epigenetic therapies is a concern for the treatment of DMG [78]. Strategies to overcome these are desperately needed and often include the search for combination therapies that target the upregulation of compensatory pathways as the source of resistance. To overcome this resistance, Grasso et al. simultaneously used multiple inhibitors that target the epigenetically-induced changes in DMG chromatin as a result of the histone 3 mutation (Table 1). In this study, 83 drugs were screened, including small molecule compounds or traditional chemotherapeutics. Among the 14 agents that demonstrated sensitivity in a DMG culture panel, the HDAC inhibitor panobinostat, in combination with the histone lysine demethylase inhibitor GSK-J4, showed significant synergy in the eradication of patient-derived DMG cells *in vitro* and in *in vivo* murine models (Table 1) [78]. It is important to note that while panobinostat stands out among ongoing molecularly targeted therapy trials (NCT02717455, NCT04341311; Table 4), it was withdrawn from the United States market in 2021 [86]. Thus, many trials of panobinostat, including one completed phase 1/phase 2 study (NCT03566199; Table 2), may not continue to subsequent phases.

Another study aimed to overcome HDAC resistance with a CDK7 inhibitor, THZ1, which targets transcriptional activity. This was tested in panobinostat-resistant cells and resulted in re-sensitization to HDAC inhibitors [20]. These findings suggest there may be more transcriptional vulnerabilities within the pathology of DMG [20]. Despite the challenges in implementing HDAC-inhibitor therapy as treatment, the premise of the interaction between the DMG-specific histone mutation and HDAC inhibitors in altering anti-tumor gene expression provides a platform for future studies in epigenetic-targeted therapy for DMG. Overall, understanding and identifying these H3K27M-associated epigenetic vulnerabilities of DMG are impetus to develop and test new tumor-specific targeted therapies that have the potential to evade inherent resistance [87].

Epigenetic reprogramming also involves the action of histone acetyltransferases (HATs), such as the p300/CREB-binding protein (CBP) [76]. Inhibition of HAT p300/CBP with HAT inhibitor II or ICG-001 are being explored for DMGs and other brain tumors and show efficacy when combined with BET inhibitors by reversing the inadvertent activation of detrimental super enhancer programs [88,89]. This study and others are promising for future epigenetic targeted therapies for DMGs and will likely be explored in preclinical and clinical settings.

Immune therapies

Immunotherapy is at the forefront of therapeutic development for DMG. DMG is considered an immune cold tumor [39], confirmed by recent analysis of the tumor and tumor microenvironment, where limited immune infiltration of myeloid cells and activated T cells were observed, indicative of minimal immune recognition [40,90–92]. Furthermore, expression of immune checkpoint markers was found to be low, likely indicating an overall lack of response to immune checkpoint therapies as discussed below [90,39]. We will discuss some new immunotherapeutic options that have advanced to clinical trials as single agent therapies or in combination with standard of care, along with factors that may enhance immune recognition in DMG.

CAR T-cell therapy

In chimeric antigen receptor (CAR) T-cell therapy, patient T cells are genetically altered so they may attack cancer cells more effectively. CAR T-cell therapy has been utilized in treating hematologic cancers, such as acute lymphoblastic leukemia and diffuse large B-cell lymphoma [93]. Several preclinical studies have also shown CAR T-cell therapy to markedly reduce tumor growth in several CNS tumors (GBM, DMG) using a variety of tumor antigens, including EGFRvIII and HER2 [94]. An exciting use of CAR T-cell therapy for DMG involves developing T cells against prominent glycans, including an investigation of the glycolipids GD2, a disialoganglioside highly expressed on H3 K27M-mutant DMG cells (Table 1) [95,96]. GD2 has shown the unique tendency of differential overexpression in solid tumor cells compared to surrounding healthy tissue in DMG, based on patient-derived cells [97]. Previously, anti-GD2 therapies have been studied in pediatric neuroblastoma and have shown good safety profiles that support potential evaluation in DMG [98]. CAR T-cell therapy against GD2 is currently underway (NCT05298995, NCT04099797, NCT04196413; Table 4). Another CAR T target that has been explored experimentally in preclinical studies is the immune checkpoint protein B7H3 (CD276) [99]. B7H3-CAR-T

cells showed efficacy in reducing tumor growth in mouse xenograft models of pediatric solid Ewing sarcoma, osteosarcoma, and brain tumors (medulloblastomas) and were highly effective in inhibiting tumor growth in a clinical study of glioblastoma (Table 1) [100,101]. Not surprisingly, targeting of checkpoint protein B7H3 using CAR T-cells has advanced to a phase 1 clinical trial that is currently recruiting to investigate safety in patients with DMG and other refractory pediatric CNS tumors (NCT04185038; Table 4).

In summary, CAR T-cell therapy is a novel therapeutic strategy that certainly will be expanded upon with our ever-growing understanding of the molecular underpinnings and pathobiology of DMGs. Efforts to identify glycoproteins in DMG and other diseases, or other cell surface markers expressed on DMG but not in normal tissue, are underway. ALDH, a cancer stem cell marker highly expressed in a subpopulation of likely therapy resistant DMGs [48], may represent a new target for developing CAR T-cell therapy.

Immune checkpoint therapies

Within the realm of immunotherapy are immune checkpoint inhibitors (ICIs). Just as other immunotherapeutic agents seek to utilize the patient's own immune system to eradicate cancer cells, ICIs essentially augment the host's T-cell response, which ultimately leads to tumor cell death [90]. ICI therapy has shown some success in adult gliomas, which garnered interest in implementing these drugs for pediatric tumors like DMG [102,103]. Several inhibitory immune checkpoints have been identified that are expressed in tumors like DMG, namely, PD-1, PD-L1, and CTLA-4 [39]. However, the expression of these markers appears to be very low in DMG and may not suffice to obtain efficacy in therapeutic strategies with ICIs. As of now, several ICIs are currently being studied in children with DMG, including treatment with the PD-1 inhibitor pembrolizumab (NCT02359565; Table 4) [102]. Another PD-1 inhibitor, nivolumab, has also been studied for DMG. In a retrospective cohort analysis of children with recurrent DMG who received reirradiation therapy with or without nivolumab, Kline et al. reported slightly improved overall survival in patients who received combination therapy versus those who received reirradiation alone [104]. Both the combination therapy and reirradiation were tolerated without acute or late toxicity [104]. However, the overall benefit was found to be minimal, which is not surprising given the low expression of immune checkpoints and the overall lack of T-cell infiltration in DMG [90,39]. Without boosting immune infiltration in DMG, which could be achieved with the MRI guided ultrasound technologies discussed below, ICI therapy is unlikely to be an effective strategy for the treatment of DMG.

Virotherapy

The use of oncolytic viruses is an emerging immuno-oncologic therapeutic approach for various tumors including DMG. This approach, called virotherapy, seeks to eradicate tumor cells through the insertion of a virus directly into these cells while still preserving healthy cells [90]. Virotherapy is an emerging modality for treating brain cancers that aims to attack a tumor's environment and metabolic tendencies. When an oncolytic virus enters the tumor, it targets cancer cells via disruption of cancer-specific cellular regulation and induces a cytotoxic effect to promote cellular lysis [105,106]. This has been observed in glioblastoma multiforme, where the oncolytic parvovirus H-1 (H-1PV) dysregulated several genes within the tumor (Table 1) [105].

Feasibility and success of each oncolytic virus is dependent on adequate viral entry and replication within cancer cells [97]. Glioblastoma has been the target of several oncolytic vaccines in recent years with favorable results, including a polio–rhinovirus chimera and replication-deficient adenoviruses [107]. The oncolytic adenovirus DNX2401 targets Rb with a 24 base-pair deletion in the early region 1A gene (E1A), a mutation that prevents formation of the E1A-Rb protein complex. When injected intratumorally, DNX2401 can only replicate within cells expressing Rb dysfunction, i.e., cancer cells, thereby sparing healthy cells. In DMG, DNX2401 demonstrated antagonism towards in vitro mouse model DMG cells via destruction of the NP53 and XFM DMG cell lines, a result that suggests its utility as a potential treatment for children with DMG [106]. A clinical trial stemming from these findings is now in its early phases (NCT03178032; Table 4) [106].

Historically, several oncolytic viruses have been trialed for glioblastoma. These studies of the efficacy of virotherapy in treating this disease have revealed viral markers, such as CD111 (used for HSV viral entry), that are more prolific in pediatric than adult brain tumors. These are currently being studied (NCT02457845) with promising safety results [108]. Several studies of the efficacy of oncolytic adenoviruses are also ongoing, notably NCT03178032, which is testing the virus DNX-2401 (Table 4). A wildtype reovirus is also being investigated (NCT02444546; Table 4). Reovirus has previously been shown to naturally replicate within cancer cells, in particular with rhabdomyosarcoma and osteosarcoma [109].

In summary, oncolytic viruses show great promise for the future of DMG therapy, based on a history of successful preclinical studies and early phases of clinical trials. Given the known dismal prognosis of DMG, such advancements in immunotherapy provide hope to patients in the future.

Vaccine therapies

Vaccine therapy is another emerging modality being trialed for DMG treatment. Historically, tumor vaccines have been used to stimulate a T-cell mediated response to the tumor-specific antigens native to the cancer [97]. Here, we discuss vaccine therapies currently being studied for DMG. In vaccine therapy, a vaccine is used to target a unique antigen found within DMG to induce a natural immune response within the patient to the cancer [97]. Targets for vaccines have been established over years of preclinical research to find appropriate neoantigens. One notable preclinical study evaluated a vaccine targeting mutant isocitrate dehydrogenase 1 (IDH1) in mouse models and showed adequate tumor targeting and inhibition of tumor progression (Table 1) [110]. Importantly, the success of vaccine therapy depends on the extent of homogeneity within the tumor for the vaccine to best target. In other words, if a tumor were to express several mutations heterogeneously, a vaccine targeting one specific neoantigen may not be able to efficiently eradicate this particular tumor [97]. Current clinical trials are studying vaccine therapy as an adjuvant with other agents. These are primarily in very early phases and include a H3K27M peptide vaccine with imiquimod (NCT04808245; Table 4) [111] and a neoantigen vaccine for H3K27M-mutant DMG (NCT04749641; Table 4) [112]. The PEP-CMV vaccine as treatment for DMG is unique, as the CMV virus shares a segment of the protein pp65 with the tumor cells (NCT05096481; Table 4). Another trial is studying the rHSC-DMGVax vaccine, which is targeting the specific histone mutations unique to DMG in an effort to halt progression (NCT04943848; Table 4) [97]. Four studies (NCT04911621, NCT03914768, NCT02840123, NCT03396575) are investigating dendritic cell vaccines in HGG/DMG, DMG/GBM, DMG, and DMG and brainstem glioma, respectively (Table 4). The trial for DMG patients (NCT02840123) has shown that autologous dendritic cell vaccines generate an immune response in H3K27M-mutant DMG [113]. However, in glioblastoma studies, there have been some inconsistent results with this treatment modality [114].

In summary, development of vaccine therapies show promise for DMG treatment in preclinical models, with some now being tested in clinical trials as adjuvants with other agents. Though there may still be difficulty in fully implementing this therapy, given the possibility of tumor antigen heterogeneity yielding variable results, we look forward to seeing how vaccine efficiency may be improved through greater specificity in targeting tumor antigens or making improvements to combination therapy.

Improving drug delivery

DMG remains a devastating disease and although promising new drugs and therapeutic strategies have been developed, the inherent nature and location of brain tumors remain a challenge for drug delivery, which in turn poses difficulties for improving drug efficacy. Below, several innovative methods to improve delivery are discussed (Table 2).

Ultrasound-targeted drug delivery methods offer a powerful and innovative approach to increasing therapeutic efficacy by facilitating delivery. Whether this includes traditional chemotherapeutics, molecularly targeted therapies such as epigentic modifiers, checkpoint inhibitors or CAR T-cells, the use of focused ultrasound (FUS) increases drug delivery. Thus, opening the BBB with FUS techniques such as low-intensity pulsed ultrasound (LIPU) has emerged over the past 20 years as a promising technique for enhanced treatment delivery [115]. The initial phases of this research proved effective in delivering various therapeutic agents across the BBB, including antibodies, immune cells, and low-molecular-weight drugs. Success in controlling tumor expansion and increasing survival have led to testing LIPU-based extra- and intracranial devices in clinical trials [115,116]. In recurrent glioblastoma, drug delivery of carboplatin with LIPU was found to be feasible and safe [116]. New preclinical and clinical trials are exploring enhanced drug delivery using ultrasound-based techniques to overcome BBB impenetrability, and thereby deliver effective treatment to DMG patients (Table 1) [115]. Current clinical trials for DMG are investigating the application of ultrasound-targeted therapy specifically using SONALA-001 and MR-Guided Focused Ultrasound (MRgFUS) energy as a new sonodynamic therapy (NCT05123534; Table 2). This technology utilizes the ability of cancer cells to preferentially uptake aminolevulinic acid (ALA) [117,118]. Initial experiments showed that various cancer cells were able to uptake large amounts of ALA, and, when exposed to blue light, glowed pink, thereby facilitating surgical resection [119]. Interestingly, accumulation of ALA's byproduct induced cell death when cells were exposed to high-energy light, a process called photodynamic therapy (PDT) [119]. Feasibility of this PDT for brain tumors was demonstrated in a clinical trial (NCT04559685) by using a combination of noninvasive MRgFUS to produce high-intensity light in the brain in combination with ALA [118,120]. This sonodynamic therapy (SDT) in combination with a proprietary ALA analog (SonALAsense) is now being tested for brain tumors including DMGs (NCT05123534; Table 2). Patients receive an intravenous dose of SonALAsense prior to MRI which is used to focus ultrasound waves over the entire tumor, generating light through a process called sonoluminescence. The light activates a byproduct of ALA (SONALA-001) that triggers programmed cell death only in cancer cells because of their efficient uptate of ALA [117–119].

Another method to improve drug delivery is convection-enhanced delivery (CED), which bypasses the BBB and avoids drug stability issues and systemic toxicities. This method allows repeated intratumoral administration and achieves high drug concentrations within the brain tumor [121]. To perform CED, a cannula is implanted directly into the brain or the tumor to create a conduit for delivery of the drug via a pressure gradient between the tumor itself/treatment site and the external environment. This permits adequate delivery of a high drug concentration while bypassing the BBB and minimizing drug toxicity by preventing systemic absorption [122]. CED is being explored for many therapeutic approaches in animal models of HGGs, including DMG. A trial studying CED of 124I-omburtamab, a radiolabeled monoclonal antibody targeting the immune checkpoint inhibitor CD276 (NCT01502917; Table 2), showed increased overall survival by 3-4 months in children with DMG who previously had been treated with radiotherapy [123].

Finally, new nanoparticle delivery technologies are drawing attention for the treatment of brain tumors. Nanoparticle delivery can be used in conjuction with other drug delivery methods, including CED. A current trial, now in phase 1 (NCT04264143; Table 2), is evaluating the maximum tolerated dose of MTX110, a water-soluble nanoparticle formulation of the HDAC inhibitor panobinostat, and gadolinium delivered via CED in pediatric patients newly diagnosed with DMGs.

To ensure effective drug delivery to DMG tumors, the effectiveness of combinations of drugs that are not BBB-penetrable but may target DMG cells with efflux inhibitors is being investigated. These preclinical models may advance to clinical trials. Among them, Asby et al. combined the CDK4/6 inhibitor palbociclib, which does not cross the BBB, with the rapamycin analog temsirolimus, an mTOR inhibitor. mTOR inhibitors have been shown to reduce efflux and increase drug concentrations in CNS and other malignancies (Table 1) [124–129]. Asby showed that this combination of inhibitors led to decreased viability in DMG cells [124]. Further evaluation of palbociclib in rat hippocampus also conferred absence of neurotoxicity, suggesting that this combination may be a viable option not only to increase tumor susceptibility to treatment, but also to counter both inherent resistance related to CDK4/6 and difficulties associated with BBB penetration by palbociclib [124].

In summary, while many promising therapeutics have been developed in the last decade, efforts to translate these into effective treatment options for patients have been hampered by the challenges of drug delivery. Improving drug delivery for DMG thus remains a critical area of research that must continue to expand alongside drug development efforts for effective treatment of DMG.

Conclusion

Diffuse midline glioma is a devastating diagnosis for patients. The aggressive nature of the tumor and its delicate location within the brain are significant obstacles to developing therapeutic regimens. Decades have been spent researching adequate therapies in an effort to mitigate this bleak prognosis. Yet despite multiple promising and ongoing preclinical studies and clinical trials, minimal improvements in long-term outcome have been achieved.

The most prominent barriers to effectively treating DMG are drug delivery (i.e., BBB penetrance), inherent resistance mechanisms, and drug toxicities. For example, BBB efflux transporters and inherent or acquired resistance to therapies have limited the efficacy of the most promising drugs for DMG. Nevertheless, efforts to overcome these barriers continue as drug delivery methods such as CED and combination therapies of drugs are developed as therapeutic paradigms to evade resistance. Ongoing studies are investigating factors that lead to resistance to radiotherapy. Studies of tumor hypoxia, for example, provide compelling evidence that medications inhibiting hypoxia may counteract this resistance mechanism and thus prove to be effective against DMG by enhancing its radiosensitivity.

The identification of unique genetic markers and mutations has also greatly impacted the trajectory of preclinical studies and clinical trials for DMG. Detection of the H3K27M mutation in DMG has increased our knowledge of this tumor and its pathobiology exponentially, particularly regarding the design of novel molecularly targeted treatments. Promising preclinical studies using epigenetic modifiers, or other molecularly targeted agents like ONC201 (imipridone), represent significant advances in the treatment of DMG and are advancing to clinical trials for H3K27M-positive DMGs. In addition, immunotherapies, namely CAR T-cell therapy, oncolytic viruses, and vaccine therapy, have emerged as promising approaches for effectively treating this disease. The studies and clinical trials of these and other emerging therapeutics and treatment delivery modalities that we have discussed here represent active efforts to make preclinical findings therapeutic realities. They offer promise for patients as they reveal new therapeutic targets and approaches for treating DMG.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Aleeha Noon: Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Stefanie Galban:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2023.100899.

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