Pediatric Diffuse Midline Gliomas: An Unfinished Puzzle

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Abstract: Diffuse midline glioma (DMG) is a heterogeneous group of aggressive pediatric brain tumors with a fatal prognosis. The biological hallmark in the major part of the cases is H3K27 alteration. Prognosis remains poor, with median survival ranging from 9 to 12 months from diagnosis. Clinical and radiological prognostic factors only partially change the progression-free survival but they do not improve the overall survival. Despite efforts, there is currently no curative therapy for DMG. Radiotherapy remains the standard treatment with only transitory benefits. No chemotherapeutic regimens were found to significantly improve the prognosis. In the new era of a deeper integration between histological and molecular findings, potential new approaches are currently under investigation. The entire international scientific community is trying to target DMG on different aspects. The therapeutic strategies involve targeting epigenetic alterations, such as methylation and acetylation status, as well as identifying new molecular pathways that regulate oncogenic proliferation; immunotherapy approaches too are an interesting point of research in the oncology field, and the possibility of driving the immune system against tumor cells has currently been evaluated in several clinical trials, with promising preliminary results. Moreover, thanks to nanotechnology amelioration, the development of innovative delivery approaches to overcross a hostile tumor microenvironment and an almost intact blood-brain barrier could potentially change tumor responses to different treatments. In this review, we provide a comprehensive overview of available and potential new treatments that are worldwide under investigation, with the intent that patient- and tumor-specific treatment could change the biological inauspicious history of this disease.

Keywords: pediatric diffuse midline glioma (DMG); diffuse intrinsic pontine glioma (DIPG); immunoncology; target therapy; immunotherapy

1. Introduction

Diffuse midline gliomas (DMGs) are one of the most devastating pediatric cancers, representing about 20% of all pediatric central nervous system (CNS) tumors, with approximately 200 to 300 new cases diagnosed each year in the United States [1,2].

Most DMGs occur between the ages of 5 and 10 years, with a peak at 7 years and no gender predilection [3]. The intrinsic localization of this tumor into midline structures contributes to the poor outcome of those patients; the widespread infiltrative nature as well as the critical anatomical location precludes surgical resection, while the presence of an intact blood-brain barrier (BBB) [4] hinders drug penetration into the tumor.

The term “DMG” replaced the previous nomenclature “diffuse intrinsic pontine glioma (DIPG)”, usually used for the primitive pontine midline gliomas, with the aim of emphasizing that these lesions are not solely centered in the pons/brainstem, but may also originate...
in the other midline structures, such as the thalami, the ganglio-capsular region, the cerebellum, cerebellar peduncles, the third ventricle, the hypothalamus, the pineal region, as well as the spinal cord [5], as postulated by the latest World Health Organization (WHO) classification of CNS tumors (WHO CNS 5) [6].

The discovery of recurrent somatic mutations lead to lysine 27 to methionine (p.Lys27Met) substitution in histone 3 (H3) gene variants H3F3A and HIST1H3B, encoding histone H3 variants H3.3 and H3.1, respectively, collectively referred to as H3K27M- in approximately 70% of DMG samples [4,7], which has completely revolutionized the knowledge of this disease and highlighted the analyses of the tumor tissue, especially for research purposes. It represents the major oncogenic event initiating tumorigenesis, disrupting cell physiology by altering the epigenetic regulation of their genes expression [8,9].

Despite current therapies involving radiotherapy and multiple chemotherapies, the prognosis is still poor, with a 2-year survival rate of <10% [10].

In this review, we discuss the main clinical, biological, and radiological characteristics.

2. Diagnosis

2.1. Clinical Features

All structures on the midline could be involved, with different signs and symptoms, including headaches, cranial nerve palsies, as well as motor or sensitive focal deficits. In a lesion involving the thalamus, the most frequent symptoms are weakness on one or both sides of the body or focal motor deficits.

For typical DIPGs, clinical symptoms and signs have a frequently short latency (a median time of 3–6 months) with a triad of cerebellar signs (ataxia, dysmetria, and dysarthria), long tract signs (hypertonia, hyperreflexia, and motor deficits), and cranial nerve palsies (especially VI and VII cranial nerves, isolated or multiples).

Spinal localizations could be difficult to detect, until they manifest with focal or generalized motor deficits.

Metastatic disease (MD) is reported in about 13% of cases with a median time from diagnosis of 7.2 months (range 4.6 months–2.2 years); intraparenchymal metastasis usually involves supratentorial, infratentorial, or spinal regions, but it could also concern ventricular or leptomeningeal dissemination (LMM) [11,12]. Of note, patients with supratentorial metastasis experienced a better overall survival (OS) when compared with patients with intraventricular disease. However, MD did not reduce OS, probably because the local progression and rapid involvement of vital structures remain the main causes of death [13].

2.2. Biological Landscape

The fundamental step in understanding DMG biology came in 2012, when mutations in H3.3 histone were detected in almost 70% of DMG samples, and in 12–19% of cases with similar variants (namely H3.2 and H3.1 variants) [7]. H3K27M alteration lead to a global epigenetic dysregulation, due to the inactivation of the polycomb repressive complex 2 (PRC2), through an interaction between the enhancer of zest homologue-2 (EZH2) and the mutant histone [14]. This phenomenon resulted in a global DNA hypomethylation, with consequent transcriptional depression at these specific loci and the dysregulation of multiple cellular processes [15].

Castel and Coll in 2015 described ninety-seven DIPG, and all but one were found to harbor either a somatic H3K27M alteration or loss of H3K27 trimethylation. They reported firstly a new mutation in HIST2H3C, thus impacting on prognosis. Tumors harboring a mutation in H3.3 exhibit radioresistance, with an higher tendency to relapse and to metastatic progression than those reported in H3.1 variants. H3.3K27M-altered DIPG showed a pro-neural/oligodendrogial phenotype and a pro-metastatic phenotype, while H3.1-K27M-mutated tumors exhibited a mesenchymal/astrocytic phenotype and a pro-angiogenic/hypoxic signature [16].
These results have been confirmed in the pivotal study published in 2017, showing that more than one thousand pediatric high-grade gliomas (HGG) and DIPG. In this study, H3.3K27M was detected in almost 60–70% of DIPG, and it was associated with a worse OS (median 11 months), while H3.1 and H3.2 variants showed a relatively longer OS (median 15 months) and a lower risk of metastasis spread [17]. In addition to the K27M status, other changes, such as the overexpression of EZHIP and alterations in the epidermal growth factor receptor (EGFR), have been reported, which were recently included in the latest 2021 WHO classification of CNS tumors as “H3K27-altered tumors” [6].

Beyond H3K27M alteration, other concomitant changes in the expression of several genes that strongly regulate embryonic morphogenesis, the activity of transcription factors, and cellular growth have been detected, including MHC class I polypeptide-related sequence A (MICA), platelet-derived growth factor receptor-α (PDGFRA), and cyclin-dependent kinase inhibitor 2A (CDKN2A) [18], as well as mutations in ACVR1, TP53, or components of the PI3K/mTOR/MAPK pathways [18,19].

TP53 mutations occur in about 40% of DIPG and represent the second most frequent mutation, correlated with a worsening OS [20]. This mutation allows tumor cells to evade death signaling, leading to unrulled proliferation. However, even in TP53 wild-type tumors, about 80% of cases report a mutation in the protein phosphatase, Mg2+/Mn2+-dependent 1D (PPM1D), which seems to determine an overexpression of TP53 and of other proteins involved in DNA damage response.

TP53 mutation usually occurs with the amplification of PDGFRA, which is the most common one (about 30% of cases), and it is strictly implicated in the RTK-RAS-PI3K-AKT signaling pathway. PDGFRA determines the activation of PI3K and MAPK pathways, and it is usually coupled with H3.3 mutations [21], thus explaining its association with major clinical aggressiveness [22].

Poly ADP-ribose polymerase (PARP1), a protein essential for the repair of single strand DNA breaks induced by alkylating agents and radiation, is overexpressed in about 54% of DIPG [23].

Activin A receptor type 1 (ACVR1), a member of the bone morphogenetic protein signaling pathway, has been detected exclusively in approximately 30% of DIPG [24] and it was significantly associated with younger age, longer survival, and the presence of H3.1 variant (about 80% of cases) or PIK3CA/PIK3R1 mutations [25]. Its role in tumorigenesis still remains unclear. This mutation has been previously reported only as a germline mutation in a congenital autosomal dominant disease of the connective tissue called fibrodisplasia ossificans progressive (FOP), but the typical ACVR1 alteration found in DIPG (p.Gly328Val) has not been reported in FOP patients. Therefore, the real connection between DIPG and FOP patients is still under investigation.

Deletions of cell cycle regulatory genes CDKN2A/CDKN2B are not frequent in DIPG, but the dysregulation of the cell cycle checkpoint has been reported in about 25–30% of DIPG, with the amplification of CCND2 and deletions of CDKN2C predominating ones [21].

Mutations of chromatin remodeling genes in telomeric regions (ATRX) are less common in DIPG than supratentorial HGG, showing ATRX mutations, commonly mutated in almost all H3.3 G34-mutant gliomas, and only in a slow percentage of H3.1 mutated DIPG (about 9%).

The MAPK pathway is a well-known pathway transducing growth and differentiation signals, mostly found altered in pediatric low-grade gliomas [26]. Recent molecular discoveries reported BRAFV600E mutation in about 30% of DMG or hemispheric HGG, but rarely in DIPG, correlating with a moderately improved prognosis [17,23].
Different studies investigated molecular subgrouping among DIPG, taking into consideration histological, epigenetic, and genomic features, with the intent to stratify patients and identify higher-risk subgroups.

In 2011, Paugh et al. subclassified DIPG into three subgroups, based on the most recurrent genetic alteration, a PDGFRA alteration found in 47% of DIPGs, a RB amplification in another 31% of samples, and the third part with both pathways involved [21].

Puget defined two DIPG subgroups, the mesenchymal and the proliferative one, according to the predominant histological features [19].

Other subsequent classification proposals concern microRNA investigations, methylation, and protein profiling, identifying two subgroups with N-Myc or PTCH1 upregulation [27].

Buczkowicz et al. stressed the importance of the tumor mutation rate by identifying three different classes, namely Myc-N-amplified, H3K27-altered, and the silent group, with few copy number alterations and low mutation rates, but there was no evidence of the survival impact of tumor mutational rate [24].

However, the most significant subclassification with a real impact on prognosis remains the one postulated in 2012 by Koxang, distinguishing two subgroups, harboring or not harboring H3K27 mutation, with worse or better prognosis, respectively [7].

Therefore, even if H3.3 alteration confirms its negative prognostic role, there remains much to be discovered about a profoundly heterogeneous pathology, with the intent to fulfill the current knowledge gap of the past 50 years, when biopsy approach was not routinely performed, resulting in few tissue samples available for molecular and epigenetic investigations.

2.3. Radiological Findings

MRI remains the gold standard for the diagnosis of DMG. In particular, for DIPG, typical findings include a T1-hypointense and T2-hyperintense lesion involving >50% of the pons [28] with high perfusion and restricted diffusion sequences [29,30]. A retrospective analysis with a radiological and pathological central review of 22 cases enrolled in institutional trials, with associated immune histochemical analyses, demonstrating the high-frequency detection of H3K27M alterations when MRI features are carefully assessed, confirming the consistency of integration imaging features with biological markers [31]. Moreover, it seems that specific MRI features could be used to discriminate the H3K27M mutational status of lesions not involving the pons, demonstrating a greater contrast enhancement with thicker enhancing margins and a lower degree of edema is more frequent in DMG and H3K27M-altered, compared to the wild-type (WT) group [5].

More informations are provided by an interesting recent report on the preliminary examination of HERBY trial patients. They detected that the larger part of midline tumors was radiologically well-defined with absent or minor perilesional edema. Thalamo-pulvinar tumors showed the greatest proportion of moderate or strong enhancement, with the greater part of intratumoral necrosis being reported. Different patterns of diffusion were highlighted, as well as LMM, which resulted in an expected worse outcome. There were no differences in survival according to location, tumor enhancement, or diffusion restriction. The results from the HERBY trial have recently been incorporated into the Response Assessment of Pediatric Neuro-Oncology (RAPNO) guidelines for pediatric HGG [32].

Similar studies encourage the need for a deeper integration of radiological and histological findings in order to correctly stratify all patients.
Positron emission tomography (PET) imaging with aminoacid tracers, such as 18-F-dihydroxy-phenylalanine (F-DOPA), is a new diagnostic method that has been largely used in the oncology field in the last few decades. Preliminary studies seem to correlate with a higher uptake of tracer with more aggressiveness and, as we recently learned, with H3K27M mutational status. Prior results were first reported by Morana and colleagues, demonstrating that a higher uptake of F-DOPA is associated with a worse prognosis [33]. These data are still under debate, and further investigations are needed for the routine use of this methodic [34].

3. Current Treatments

The dismal prognosis makes DMG treatment one of the major challenges in pediatric neuro-oncology. Most established prognostic factors are summarized in Table 1.

| Favorable Prognostic Factors | Unfavorable Prognostic Factors |
|-----------------------------|--------------------------------|
| Age < 3 years [35–37]        | Age > 10 years                 |
| Duration of symptoms ≥ 3 months [38,39] | Duration of symptoms ≤ 3 months [32] |
| Absence of cranial nerve palsies or long tract involvement at diagnosis [40] | Improved perfusion [41,42] |
| Significant reduction in steroids needing | Presence of a ring enhancement [22] |
| Rapid amelioration of neurological signs [44] | Higher choline: N-acetylaspartate ratio than the median of 2.1 [45] |
| H3.1 alteration             | H3.3 alteration p53 mutation |
| Tumor volume reduction after therapy [46] | LMM [47] or metastatic disease [48] |

| Detection of lactate and N-acetyl aspartate in MRI spectroscopy (MRS) [49,50] |

There have been several studies worldwide, but none of them significantly changed the median OS, which is invariably stable at 9–12 months from initial diagnosis. Time to progression (TTP) ranged from 5 to 9 months, and the outcome remains poor for more than 90% of children, who died within 2 years from initial diagnosis.

Currently, radiotherapy (RT) remains the mainstay of treatment at diagnosis, and even at first or second relapse. The standard of care for newly diagnosed patients is focal intensity-modulated radiation therapy (IMRT) to the primitive tumor (54–60 Gy, divided in 1.8–2 Gy fractions, given once daily for 5 days per week over 6 weeks) [40]. This treatment results in temporary symptom relief, as well as moderate delaying tumor progression, in about 70–80% of patients. Unfortunately, this effect shrinks after a few months with the restart of tumor growth and potentially distant dissemination, with a median TTIP after RT often shorter than 6 months [51]. A large review of aggregate data from more than 2000 patients in about 70 studies has revealed a median OS of approximately 11 months for patients treated with RT, not excluding the use of a hypofractionated regimen, considering the possibility of multiple courses of radiation [52]. The results of a matched cohort analysis demonstrate a similar OS rate with a hypofractionated regimen (13 or 16 fractions in 3 to 4 weeks) compared with a conventional radiation therapy regimen (30 fractions in 6 weeks) for patients with newly diagnosed DIPG [53], but without any survival benefits. The transient response to RT enforced researchers to attempt to increase radiation dose using higher doses of radiation (up to 7000 cGy), resulting in increased toxicity without any improvement in OS [54], as well as a hypofractionated regimen, which was demonstrated to be feasible but with no advantages on survival [55].
Re-irradiation represents the only salvage treatment for recurrent disease and a palliative therapeutic option. The largest series of re-irradiated cases were published by Janssens et al. on behalf of the SIOPE HGG/DIPG working group [56]. Thirty-one patients who underwent re-irradiation were compared with 39 patients who were not selected for re-irradiation, with a moderate OS benefit with re-irradiation (13.7 versus 10.3 months). Patients with a greater time interval from the initial diagnosis to first radiation therapy benefited more with re-irradiation, probably for a more indolent disease.

Several other studies confirmed a statistically significant median survival benefit after re-irradiation for recurrent DIPG, ranging from 3 to 4 months. The maximum doses reported in the literature ranged from 30 to 36 Gy (1.8 Gy/day), according to the time passed since their first radiation therapy to permit some recovery of brainstem tolerance [57].

Different types of adjuvant and neoadjuvant therapies have been tested, and many other trials are still ongoing, with the intent to change the natural history of the disease. Radiation sensitization with different agents such as topotecan, cisplatin, carboplatin, temozolomide, or motexafin gadolinium is already described [58], but none of these drugs demonstrated to be effective [59–61].

Additional chemotherapy before, during, or after radiotherapy, including temozolomide, carboplatin, cisplatin, tamoxifen, and high-dose myeloablative chemotherapy (such as those used in high-risk medulloblastoma) demonstrated moderate responsiveness to treatment, but it unquestionably failed to determine the advantages of OS or PFS, resulting in increased toxicities and hospitalizations [51].

Doz et al. obtained a median OS of almost 11 months using carboplatin during RT [62] and multiple chemotherapeutic agents (tamoxifen, cisplatin, or high-dose methotrexate), with the intent to delay radiotherapy to clinical or radiological progression. Therefore, their approach requires a long recovery, a high risk of infections, and the development of severe toxicities [63].

The German HIT–GBM protocols assessed a variety of chemotherapeutic strategies in the HIT–GBM protocols, but none of them showed a superior OS [64].

In a single national institution study, Massimino et al. evaluated four different regimens in DIPG treatment, including high-dose chemotherapy followed by myeloablative treatment; cisplatin/etoposide followed by isotretinoin before, during, and after local RT, or a combination of chemo-radiotherapy and single vinorelbine before, during, and after radiotherapy. The results have not been encouraging [65].

Temozolomide has long been investigated in DMG, but it failed to obtain the expected benefit on survival rates [60,66]. Therapeutic failures could be related to the presence of an unmethylated methyl-guanine methyltransferase (MGMT) promoter, which rapidly removes methyl and alkyl groups from the O6 position of guanine, directly contrasting the mechanism of action of temozolomide. The MGMT promoter has been found hypermethylated mostly in the H3.3/G34 group and less in tumors with K27 mutation [67], thus probably leading to DMG resistance to alkylating agents reported in several trials [60,68–70].

Wagner et al. reported a moderately better median PFS in patients with DIPG treated with adjuvant chemotherapy after RT compared with patients treated with RT alone (11.3 months versus 9.5 months) [71], but no significant improvements in OS. Similar results were reported in other randomized trials [72,73].
The role of tumor resection for midline pediatric DMG remains uncertain, while, impressively, the HERBY trial showed no evidence of a different event-free survival (EFS) rate according to the surgical approach; meanwhile, patients who experience a (rare) near total resection (NTR) or debulking survived longer [74], as stated in other investigations [75].

For the future, we can hypothesize that only combinations of traditional therapy with epigenetic therapy, immunotherapy, or nanotechnologies for drug delivery may lead to the development of curative approaches [76].

4. Target Therapies for DMG

Due to the advanced understanding of DMG molecular pathology, several studies have tried to investigate new potential therapeutic approaches with molecular drugs targeted against a specific pathway. These findings support and motivate the need for a biopsy assessment of the tumor to correctly define the potential therapeutic targets, as recently stated in two global multi-institutional trials (NCT01182350 and NCT02233049).

A linear comparison of the many different studies available is quite difficult due to the high variability of eligibility criteria, primary and secondary outcomes, the assessment of response and progression, statistical design, and endpoints, which are still far from an international standardization [70].

Critical research was conducted in order to capture all available clinical trials with registration in the ClinicalTrials.gov portal, investigating DMG and DIPG.

We included all clinical trials based on the investigation: (1) DMG/DIPG and (2) DMG/DIPG and other CNS tumors.

Currently, 115 trials followed the appropriate inclusion criteria. Ninety-nine percent of them were interventional, and 3.6% were observational. A phase category was reported for 109 (94%) of the registered trials. Phase I is the most common phase design (n = 68, 60%). Thirty-eight trials (33%) were phase II, and a total of four (3.4%) were phase III.

As of June 2022, only 14 (12%) trials have published their results: 3 are specific to DIPG/DMG, while the others, including DMG, are amongst other pediatric CNS tumors. None of them demonstrated a significant change in progression-free survival (PFS) and OS. Study characteristics are reported in Table 2.

To date, 57 interventional studies are recruiting for newly diagnosed and/or recurrent DMG/DIPG, with the larger part being coordinated by a medical institute in the USA. The major part of them is reported in Table 3.
### Table 2. DIPG/DMG trials completed.

| Number of Trial | Study Name                                                                 | Phase | Countries | Start-End Date | Enrollment Size | Primary Outcome | Secondary Outcome | Results |
|-----------------|-----------------------------------------------------------------------------|-------|-----------|----------------|----------------|------------------|-------------------|---------|
| NCT03566199     | MTX110 by CED in Treating Participants with Newly Diagnosed Diffuse Intrinsic Pontine Glioma (PNOC015) | I     | USA       | 2019–2021      | 7 patients     | Safety and tolerability of repeated MTX110 infusions | Clinical efficacy | 1 AE; 7/7 patients died for PD phase II expansion cohort was not activated at behest of pharmaceutical supplier |
| NCT01182350     | Molecularly Determined Treatment of Diffuse Intrinsic Pontine Gliomas (DIPG-BATS) | II    | USA       | 2011–2016      | 53 patients    | OS after biopsy | AE biopsy-related | No AE biopsy-related |
| NCT02607124     | A Phase I/II Study of Ribociclib, a CDK4/6 Inhibitor, Following Radiation Therapy | II    | USA       | 2015–2020      | 11 patients    | AE; 1-year OS | /                 | 4/11 patients developed SAE; 11/11 patients died for PD |
| NCT01189266     | Vorinostat and Radiation Therapy Followed by Maintenance Therapy with Vorinostat in Treating Younger Patients With Newly Diagnosed Diffuse Intrinsic Pontine Glioma | I/II  | USA       | 2010–2021      | 79 patients    | MTD, 2-year OS | /                 | 2 patients completed the trial; 50 patients left for lack of efficacy |
| NCT00036569     | A Phase II Study of Pegylated Interferon Alfa 2b (PEG-Intron(Trademark)) in Children With Diffuse Pontine Gliomas | II    | USA       | 2002–2012      | 32 patients    | 2-year OS | Median TTP | No improvement in OS, probably delaying TTP |
| NCT00879437     | Valproic Acid, Radiation, and Bevacizumab in Children with High-Grade Gliomas or Diffuse Intrinsic Pontine Gliomas | II    | USA       | 2009–2021      | 38 patients    | 1-year EFS, percentage of SAE grade ≥ 2 | Median EFS, median OS | No benefit on EFS and OS |
| NCT01514201     | Veliparib, Radiation Therapy, and Temozolomide in Treating Younger Patients with Newly Diagnosed Diffuse Pontine Gliomas | I/II  | USA       | 2012–2019      | 66 patients    | MTD; OS; DLTs | /                 | No SAE, but no benefits on EFS and OS |
| NCT01836549     | Imetelstat Sodium in Treating Younger Patients with Recurrent or Refractory Brain Tumors | I/II  | USA       | 2013–2018      | 43 patients    | Objective response (at least 50%) | PFS | Terminated (due to several intracranial hemorrhages and recommendation by the PRTC DSMB) |
| NCT01774253     | Erivedge (Vismodegib) in the Treatment of Pediatric Patients with Refractory Pontine Glioma | II    | USA       | 2013–2015      | 9 patients     | PFS | SAE; OS, QoL | Terminated (lack of enrollment and commercial availability of drug) |
| NCT03387020     | Ribociclib and Everolimus in Treating Children with Recurrent or Refractory Malignant Brain Tumors | I     | USA       | 2018–2020      | 22 patients    | MTD | Objective responses | MTD identified |
### Table 2. Cont.

| Number of Trial | Study Name                                                                 | Phase | Countries          | Start-End Date | Enrollment Size | Primary Outcome       | Secondary Outcome | Results                                                                 |
|-----------------|----------------------------------------------------------------------------|-------|--------------------|----------------|------------------|----------------------|--------------------|-------------------------------------------------------------------------|
| NCT03257631     | A Study of Pomalidomide Monotherapy for Children and Young Adults with Recurrent or Progressive Primary Brain Tumors | II    | USA/Europe         | 2017–2022      | 52 patients     | Objective responses | Long-term SD, PFS, OS | No patient in DIPG group achieved objective responses or SD              |
| NCT01502917     | Convection-Enhanced Delivery of 124I-Omburtamab for Patients with Non-Progressive Diffuse Pontine Gliomas Previously Treated with External Beam Radiation Therapy | I     | USA                | 2012–February 2022 | 50 patients (expected) | MTD; toxicity | OS | Terminated (stopping rule was met per protocol as a result of the last two subjects experiencing dose limiting toxicities) |
| NCT00880061     | An Open-Label Dose Escalation Safety Study of CED of IL13-PE38QQR in Patients with Progressive Pediatric Diffuse Infiltrating Brainstem Glioma and Supratentorial High-Grade Glioma | I     | USA                | 2009–2015      | 7 patients      | Feasibility and safety | Objective response on MRI, clinical and patient-specific | Terminated; preliminary results on 4 patients |
| NCT03178032     | Brain Infusion of the DNX-2401 Virus Through the Cerebellar Peduncle       | I     | Spain              | 2017–2021      | 12 patients     | Safety, tolerability, and toxicity | OS at 12 months; complete/partial response in MRI | Terminated; results published |

AE: adverse event; CED: convection-enhanced delivery; PD: progression disease; MTD: maximum tolerated dose; TTP: time to progress.

### Table 3. DMG/DIPG trials still recruiting.

| Number of Trial | Study Name                                                                 | Phase | Countries | Start Date | Enrollment Size | Primary Outcome   | Secondary Outcome          |
|-----------------|----------------------------------------------------------------------------|-------|-----------|-------------|------------------|---------------------|---------------------------|
| DIPG/DMG        | A Study of Low-Dose Bevacizumab with Conventional Radiotherapy Alone in Diffuse Intrinsic Pontine Glioma (LoBu/LarDIPG) | II    | India     | February 2020 | 40 patients     | OS                  | PFS, AE, steroid use, pattern of relapse, QoL |
| NCT04532229     | Nimotuzumab in Combined with Concurrent Radiochemotherapy in the Treatment of Newly Diagnosed Diffuse Intrinsic Pontine Glioma (DIPG) in Children | III   | China     | April 2021   | 48 patients     | OR                  | 1-year OS, PFS           |
| NCT04771897     | A Study of BXQ-350 in Children With Newly Diagnosed Diffuse Intrinsic Pontine Glioma (DIPG) or Diffuse Midline Glioma (DMG) (KONQUER) | I     | USA       | May 2021     | 22 patients     | AE, MTD             | OS, QoL                   |
| Number of Trial | Study Name                                                                 | Phase | Countries | Start Date   | Enrollment Size | Primary Outcome | Secondary Outcome          |
|----------------|---------------------------------------------------------------------------|-------|-----------|--------------|----------------|-----------------|---------------------------|
| NCT04943848    | rHSC-DIPGVax Plus Checkpoint Blockade for the Treatment of Newly Diagnosed DIPG and DMG | I     | USA       | January 2022 | 36 patients    | MTD, DLT       | 1-year OS, TTP            |
| NCT03992015    | Gemcitabine in Newly Diagnosed DIPG                                       | Early I | USA       | September 2016 | 10 patients    | PK testing level | -                         |
| NCT0577735     | Stereotactic Biopsy Split-Course Radiation Therapy in DMG (SPORT-DMG Study) | II    | USA       | October 2021  | 18 patients    | TTP            | QoL, PFS, OS; SAE         |
| NCT0749641     | Neoantigen Vaccine Therapy Against H3.3-K27M Diffuse Intrinsic Pontine Glioma (ENACTING) | I     | China     | March 2021   | 30 patients    | Safety, 1-year OS | MTD, median PFS and OS    |
| NCT0471897     | A Study of BXQ-350 in Children with Newly Diagnosed Diffuse Intrinsic Pontine Glioma (DIPG) or Diffuse Midline Glioma (DMG) (KONQUER) | I/II  | USA       | February 2021 | 22 patients    | MTD; SAE, PK    | OR, OS, QoL                |
| NCT03126266    | Re-Irradiation of Progressive or Recurrent DIPG                           | NA    | Canada    | April 2017   | 27 patients    | Second PFS     | OS                        |
| NCT0396657     | Brain Stem Gliomas Treated with Adoptive Cellular Therapy During Focal Radiotherapy Recovery Alone or With Dose-Intensified Temozolomide (Phase I-BRAVO) | I     | USA       | May 2018     | 21 patients    | Safety and feasibility, DLT | PFS, OS                   |
| NCT03620032    | Study of Re-irradiation at Relapse Versus RT and Multiple Elective rt Courses (DIPG) | II    | Italy     | November 2015 | 54 patients    | PFS            | PFS, OS, RT toxicity, QoL |
| NCT05009992    | Combination Therapy for DMG                                              | II    | USA       | October 2021  | 216 patients   | PFS, OS        |                           |
| NCT04264143    | CED of MTX110 Newly Diagnosed Diffuse Midline Gliomas                    | I     | USA       | March 2020    | 9 patients     | AE, MTD        | PFS, OS                   |
| NCT0563357     | 131I-omburtamab Delivered by CED in DIPG Patients                         | I     | USA       | October 2021  | 36 patients    | Safety         | PFS                       |
| NCT04804709    | Non-Invasive FUS With Oral Panobinostat in Children with Progressive DMG | I     | USA       | March 2021    | 3 patients     | SAE            | 6-month PFS; 6-month OS, OS; PFS |
| NCT04196413    | GD2 CAR T Cells in Diffuse Intrinsic Pontine Gliomas (DIPG) and Spinal Diffuse Midline Glioma (DMG) | I     | USA       | September 2020 | 54 patients    | Safety, feasibility, DLT | OS; PFS                  |
| NCT05478837    | Genetically Modified Cells (KIND T Cells) for the Treatment of HLA-A*0201-Positive Patients With H3.3K27M-Mutated Glioma (PNCC018) | I     | USA       | July 2022     | 12 patients    | MTD; safety    | Manufacturing feasibility |
| NCT05479939    | Biological Medicine for DIPG Eradication 2.0 (BIOMEDE 2)                | I     | France, USA | July 2022    | 368 patients   | MTD, safety of infusions | Manufacturing feasibility |
| Number of Trial | Study Name | Phase | Countries | Start Date | Enrollment Size | Primary Outcome | Secondary Outcome |
|----------------|------------|-------|-----------|------------|-----------------|-----------------|-------------------|
| NCT02960230    | H3.3K27M Peptide Vaccine with Nivolumab for Children With Newly Diagnosed DIPG and Other Gliomas | I/II | USA, Switzerland | November 2016 | 50 patients | AE; OS | - |
| NCT03696355    | Study of CDC-0084 in Pediatric Patients with Newly Diagnosed Diffuse Intrinsic Pontine Glioma or Diffuse Midline Gliomas | I | USA | October 2018 | 27 patients | MTD; SAE; RR; DoR; OS; PFS | |
| NCT01922076    | Adasosertib and Local Radiation Therapy in Treating Children with Newly Diagnosed DIPG | I | USA | April 2013 | 46 patients | MTD; SAE | PK; RR; PFS; OS; |
| NCT04758533    | Clinical Trial to Assess the Safety and Efficacy of AloCELYVIR with Newly DIPG in Combination With Radiotherapy or Medulloblastoma in Monotherapy (AloCELYVIR) | I/II | Spain | April 2021 | 12 patients | DLT | OS, AE |
| NCT03605550    | A Phase 1B Study of PTC596 in Children with Newly Diagnosed Diffuse Intrinsic Pontine Glioma and High-Grade Glioma | I | USA | August 2018 | 54 patients | MTD, AE, PK | PFS, OS |
| NCT03652545    | Multi-antigen T Cell Infusion Against Neuro-Oncologic Disease (REMINDS) | I | USA | December 2018 | 32 patients | Read | OR |
| NCT04049669    | Pediatric Trial of Indoximod with Chemotherapy and Radiation for Relapsed Brain Tumors or Newly Diagnosed DIPG | II | USA | October 2019 | 140 patients | 8 months PFS, 12 months OS | OS, PFS TTP |
| NCT04911621    | Adjuvant Dendritic Cell Immunotherapy for Pediatric Patients with HGG or DIPG (ADDCIT-pedGLIO) | I/II | Belgium | September 2021 | 10 patients | Safety and feasibility | OS, PFS, TTP |
| NCT04837547    | PEACH TRIAL Precision Medicine and Adoptive Cellular Therapy (PEACH) for Neuroblastoma and DIPG | I | USA | September 2021 | 24 patients | DLT | AE, safety, feasibility; OS, PFS, ORR |
| NCT02644460    | Abemaciclib in Children with DIPG or Recurrent/Refractory Solid Tumors (AflacST1501) | I | USA | February 2016 | 60 patients | DLT, MTD, PK | AE, hematological toxicities |
| NCT03416530    | ONC201 in Pediatric H3 K27M Gliomas | I | USA | January 2018 | 130 patients | RP2D | - |
| NCT02525692    | Oral ONC201 in Recurrent GBM, H3 K27M Glioma, and Medulloblastoma | II | USA | August 2015 | 89 patients | PFS | - |
| NCT04541082    | Phase I Study of Oral ONC206 in Recurrent and Rare Primary Central Nervous System Neoplasms | I | USA | September 2020 | 102 patients | MTD | - |
| NCT04732065    | ONC206 for the Treatment of Newly Diagnosed or Recurrent DMG and Other Recurrent Malignant CNS Tumors (PNOC-02) | I | USA, Switzerland | August 2021 | 250 patients | DLT, MTD | PK parameters |
| NCT04185038    | Study of B7-H3-Specific CAR T Cell Locoregional Immunotherapy for DIPG/DMG and Recurrent or Refractory Pediatric Central Nervous System Tumors | I | USA | December 2019 | 90 patients | Safety and feasibility | Distribution of CNS-CART cells, RR |
| NCT02359565    | Pembrolizumab in Treating Younger Patients with Recurrent, Progressive, Refractory HGG, DIPG, Hyper-Mutated tumors, Ependymoma, or Medulloblastoma | I | USA | May 2015 | 110 patients | AE, OR | PFS, EFS, OS, radiological response |
| Number of Trial | Study Name                                                                 | Phase | Countries | Start Date | Enrollment Size | Primary Outcome | Secondary Outcome |
|----------------|-----------------------------------------------------------------------------|-------|-----------|------------|----------------|-----------------|-------------------|
| NCT05009992    | Combination Therapy for the Treatment of DMG II                             | II    | USA       | August 2021 | 216 patients    | 6-months PFS; 7-months OS |                   |
| NCT03893487    | Fimepinostat in Treating Brain Tumors in Children and Young Adults (PN0C2016) | I     | USA       | August 2019 | 30 patients     | BBB penetration   |                   |
| NCT03243461    | International Cooperative Phase III Trial of the HIT-HGG Study Group (HIT-HGG-2013) | III   | Germany   | July 2018   | 167 patients    | EFS             |                   |
| NCT03598244    | Voltinib in Treating Patients with Recurrent or Refractory Primary CNS Tumors | I     | USA       | October 2018 | 50 patients     | MTD, RP2D       | CR, PR, PK        |
| 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 | I/II  | USA       | October 2018 | 130 patients    | AE, SAE, DLT, PK, OR, OR |                   |
| NCT04099797    | C7R-GD2.CAR T Cells for Patients with GD2-Expressing Brain Tumors (GAIL-B) | I     | USA       | February 2020 | 34 patients     | DLT             | RR                |
| NCT01837862    | A Phase I Study of Mebendazole for the Treatment of Pediatric Gliomas       | I     | USA       | October 2013 | 36 patients     | MTD             | EFS, OS, PR o CRR |
| NCT04239092    | 9-ING-41 in Pediatric Patients with Refractory Malignancies                | I     | USA       | June 2020    | 68 patients     | AE              |                   |
| 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, Ewing Sarcoma, and Osteosarcoma (CLOVER-2) | I     | USA       | April 2019   | 30 patients     | DLT             | EFS, OS, dosimetry |
| NCT03389802    | Phase I Study of APX005M in Pediatric CNS Tumors                           | I     | USA       | February 2018 | 45 patients     | AE, MTD, DLT, PK | ORR, PFS, OS      |
| NCT04295759    | INCB8309 in Treating Children with Recurrent/Progressive HGG                | I     | USA       | May 2020     | 28 patients     | AE, MTD, CMAX   | PFS, OS, TTP      |
| NCT01884740    | Intravenous Infusion of Eribulin and Bevacizumab for Relapsed/Refractory Intracranial Glioma In Patients Under 22 | I/II  | USA       | June 2013    | 30 patients     | ORR             | AE, PFS, OS       |
| NCT0709680     | Study Of Palbociclib Combined With Chemotherapy in Recurrent/Refractory Solid Tumors | I/II  | USA       | May 2019     | 167 patients    | EFS, DLT, AE    | AE, CR or PR, DoR, PFS, OS, PK, Tmax |
| NCT04870844    | CBL0137 for the Treatment of Relapsed or Refractory Solid Tumors, Including CNS Tumors and Lymphoma | I/II  | USA       | January 2022 | 38 patients     | DLT, anti-tumor effect | AE, min-max SC, clearance, IR, OS, PFS |
### Table 3. Cont.

| Number of Trial | Study Name                                                                                                                                   | Phase | Countries                          | Start Date  | Enrollment Size | Primary Outcome | Secondary Outcome |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------|-------|------------------------------------|-------------|-----------------|-----------------|--------------------|
| NCT05135975     | A Study of Cabozantinib as a Maintenance Agent to Prevent Progression or Recurrence in High-Risk Pediatric Solid Tumors                   | II    | USA                               | October 2021| 100 patients    | 1-year PFS      | 1–2–5 year OS, 2–5 year PFS, DoR, AE |
| NCT04730349     | A Study of Bempegaldesleukin (BEMPEG: NKTR-214) in Combination with Nivolumab in Children, Adolescents, and Young Adults with Recurrent or Treatment-Resistant Cancer (PIVOT IO 020) | I/II  | USA                               | June 2021   | 234 patients    | DLT, AE, SAE, PK, ORR | PFS, OS |
| NCT04238819     | A Study of Abemaciclib (LY2835219) in Combination with Temozolomide, Irinotecan, and Abemaciclib in Combination with Temozolomide in Children and Young Adult Participants with Solid Tumors | I     | USA, Europe, Asia                  | November 2020| 60 patients     | DLT, PK         | ORR, DoR, CBR, DCR |
| NCT05298995     | GD2-CAR T Cells for Pediatric Brain Tumors                                                                                                 | I     | Italy                             | May 2022    | 54 patients     | Safety and MTD  | Expansion infiltration, TTP, EFS, OS |
| NCT05090003     | A Study of the Drug Selinexor with Radiation Therapy in Patients with Newly Diagnosed DIPG H3K27M-Mutant HGG                               | I/II  | USA                               | October 2021| 36 patients     | MTD, EFS, OR    | -                  |
| NCT05123534     | A Phase 1/2 Study of Sonodynamic Therapy Using SONALA-001 and Exablate 4000 Type 2 in DIPG Patients                                           | II    | USA                               | November 2021| 18 patients     | Safety; MTD    | OR, TTP, OS |
| NCT05169944     | Magrolimab in Children and Adults with Recurrent or Progressive Malignant Brain Tumors (PNO0025)                                         | I     | USA                               | December 2021| 24 patients     | Definition of phase II-MTD, SAE | - |
| NCT05096481     | PEP-CMV Vaccine Targeting CMV Antigen to Treat Newly Diagnosed Pediatric HGG and DIPG and Recurrent Medulloblastoma                        | II    | USA                               | October 2021| 120 patients    | 4-months PFS, 1-year PFS, 1 year-OS | 1-year PFS in rMB, 1-year OS in rHHG |
| NCT05278208     | Lutathera for Treatment of Recurrent or Progressive High-Grade CNS Tumors or Medulloblastomas Expressing SST2A                        | I/II  | USA                               | March 2022  | 65 patients     | MTD, SAE PFS    | OR                  |

SAE: severe adverse event; OR: objective response; SC: serum concentration; CED: convection-enhanced delivery; FUS: focus ultrasound; EFS: event-free survival; PK: pharmacokinetic; RR: response rate; DoR: duration of response; CBR: clinical benefit rate; DCR: disease control rate; RP2D: recommended phase II dose.
Mechanisms of Targeting DMG: Where We Are

Molecularly-guided therapies that have been investigated and continue to be developed are summarized in Table 4.

Table 4. Molecularly targeted agents in clinical development for the treatment of DMG. Details are provided in the main text.

| Target Therapeutic Agents Study (Reference or Clinical Trial) |
|---------------------------------------------------------------|
| HDAC panobinostat [77] (NCT02717455)                           |
| HDAC/LSD1 corin [78]                                           |
| H3K27M demethylase GSKJ4 [79]                                 |
| FACT complex curaxin (CBL0137) NCT04870944                   |
| EZH2 tazemetostat [80]                                        |
| HDAC vorinostat [81]                                          |
| PRC1 PTC028 NCT03605550                                     |
| EGFR nimotuzumab [82,83], NCT03620032                         |
| EGFR erlotinib [84,85]                                        |
| EGFR gefitinib [86]                                           |
| PDGFRA dasatinib NCT00996723                                 |
| PDGFRA crenolanib NCT01393912                                |
| VEGFR-2, EGFR vandetanib [87]                                 |
| PBRK/AKT/mTOR everolimus NCT03696355, NCT05009992, NCT02420613 |
| ACVR1 LDN-193189 or LDN-214117 [88,89]                        |
| BCL2 venetoclax [90]                                          |
| proteasome marizomib NCT03345905                             |
| CDK 4/6 palbociclib, ribociclib [91,92], NCT03434262         |
| PARP1 niraparib [93]                                          |
| XPO1 selinexor [94] (NCT05099003)                            |
| blood–brain barrier BXQ-350 [95]; NCT04264143; NCT03086616  |
| blood–brain barrier CED [95]; NCT00880061; NCT04264143; NCT03086616 |
| blood–brain barrier Focused ultrasound NCT05123534           |
| B7-H3 omburtamab NCT05063357; NCT01502917                    |
| DRD2/3 ONC201 [96–99], NCT03416530                           |
| STAT3 AG490 [100]                                            |
| AURKA phthalazinone pyrazole [101]                           |
| PLK1 volasertib [101,102]                                     |
| Cancer vaccines H3.3-K27M targeted neoantigen peptide [103]   |
| Cancer vaccines rHSC-DIPGVax NCT0494384                       |
| Oncolytic adenovirus AloCELYVIR [103], NCT04758533           |
| Oncolytic adenovirus DNX-2401 [104], NCT03178032             |
| GD2 CAR T cells NCT04196413; NCT04099797; NCT0418503; NCT 05298995 [105] |
| HER2 and EGFRvIII CAR T cells [106]                          |

Below, we discuss in detail the therapies on which clinical data have been published. Several studies aim to target one of the epigenetic mechanisms found in DMG, alone or in different combinations, as DMG single therapies have been documented as less effective [51].

It has been postulated that acetylation can inhibit an interaction between H3K27M tumors and the PCR2 complex, resulting in a normalized epigenetic status. Mainly driven by this hypothesis, previous studies have investigated the use of inhibitors of histone deacetylases (HDAC), which were strongly demonstrated to be effective in several DMG models in preclinical trials [107–109]. Among them, prior data on panobinostat were confirmed in a phase I trial with encouraging results, and others are in progress to overcome the main challenges of developing drug resistance and the limited BBB penetration of panobinostat [77] (NCT02717455). However, when pre-treated DMG cells are re-challenged with panobinostat, they developed resistance, thus indicating that probably combinational therapies are needed. Among HDAC inhibitors, vorinostat failed to improve outcome [81] in a phase I/II study conducted by the children oncology group (COG).
A novel DNA intercalating anticancer drug that has been demonstrated to significantly inhibit DNA methylation and subsequent cancer initiation targeting the FACT complex in DMG cells is uraxin \[110\]. Recently, a phase I/II trial has been opened concerning the FACT complex-targeting Curaxin (CBL0137), and phase I has opened for several types of neoplasms, including DIPG and DMG, with OS and MTD determination as primary aims (NCT04870944).

EGFR overexpression is found in approximately 80–85% of HGG biopsies, and it opened up the possibility of immunotherapy among these incurable tumors, and its potential curative effect has been demonstrated \[111\].

Anti-EGFR drug trials, such as those concerning nimotuzumab \[82\], gefitinib \[86\], or erlotinib \[84\], demonstrated limited benefits in a small subset of patients. However, nimotuzumab, a humanized IgG1 monoclonal anti-ERBB1/EGFR antibody, with specific activity against EGFRvIII, has shown similar outcomes to more intensive chemotherapy regimens, with fewer side effects, low toxicities, and no need for prolonged hospitalization, thus leading to the continued investigation of nimotuzumab as an adjuvant therapy in pediatric glioma \[112\]. This administration, in combination with vinorelbine, is the standard of care in the new national phase III open-label randomized study, coordinated by Foundation IRCCS National Institute of Tumors of Milan (NCT03620032) \[83\].

El-Khoululi and Coll reported the results of a phase I/II open-label single-arm study of multi-targeted therapy, with bi-weekly anti-vascular-endothelial growth factor (VEGF) bevacizumab and standard chemotherapeutic agent irinotecan combined with daily erlotinib. They demonstrated that this approach is safe and mostly well tolerated, but unfortunately has little impact on prognosis (13.8 months versus 10 months) \[85\].

A phase II study on valproic acid associated with radiation, followed by maintenance of valproic acid and bevacizumab in children with DIPG, showed no significant impact on PFS and OS, respectively, after 7.8 and 10.3 months, with a one-year EFS of 12% \[113\]. A phase II study of gefitinib, in combination with RT, showed a 2-year OS of 19.6% and PFS longer than 36 months in three patients \[86\].

A phase I trial of vandetanib, a selective VEGFR-2 and EGFR inhibitor conducted by Broniscer et al., reported a 2-year OS of 21.4% \[87\].

A DIPG-BATS study, a phase I clinical trial coordinated by Saint Jude, stressed the new paradigmatic approach, evaluating the rational combination therapies of novel therapeutic agents, based on the tumor type and molecular characteristics of recurrent brain tumors, including DIPG.

The PI3K/AKT/mTOR pathway has been identified as a promising target for therapeutics for DMGs due to its frequent dysregulation in more than 50% of DMGs harboring a dysregulation on this downstream. The rapamycin analog everolimus, largely used for different types of CNS tumors, has been investigated in DMG as well, especially in combination therapy. Among the several combinations, ribociclib and everolimus, investigated in a phase I clinical trial, were demonstrated to be well-tolerated, with pharmacokinetic properties similar to those in adults. Potential therapeutic ribociclib concentrations could be achieved in CSF and tumor tissue, although interpatient variability was observed (NCT02813135). Recently, we published a single-center report, confronting two DIPG cohorts: one treated with radiotherapy and nimotuzumab/vinorelbine, and the other one receiving a patient-specific second-line treatment at progression. We reported a significant increased median OS in the personalized treatment and control cohort (20.26 and 14.18 months, respectively), with everolimus, in particular, achieving the best OS \[114\].

The CDK4/6 pathway directly regulates the cell cycle and, in human cancers, it is usually overexpressed, leading to its constitutional activation and oncogenic aberrant proliferation \[115\]. CDK alterations are described in about 30–40% of DMG. Three CDK inhibitors, namely palbociclib, ribociclib, and abemaciclib, have been tested in DMG patients. Palbociclib and ribociclib showed good results in preclinical settings, but failed to improve survival in preliminary phase I trials \[91,92\], probably due to the fording of synergic therapies. Different combinations are under investigation, including temo-
zolomide (with or without irinotecan) (NCT04238819), everolimus, and erlotinib (with concomitant radiotherapy).

Probably all of these treatment failures may be caused by multifactorial causes, such as the presence of drug efflux transporters, the immunosuppressive tumoral microenvironment, and the low ability of the tested drugs to cross an almost intact BBB, and other resistance mechanisms are still under investigation [116].

These speculations have paved the way for further reflections and investigations, including testing new potential therapeutical molecules, such as selinexor, a selective inhibitor of karyopherin exportin-1 (XPO1)-mediated nuclear export (SINE) [94] (NCT05099003), or BXQ-350, a drug with two main components (saposin c (SapC), expressed as human lysosomal protein, and the phospholipid dioleoyl phosphatidylserine (DOPS), a cell membrane phospholipid (clinical formulation BXQ-350) [117] (NCT04771897)).

To overcome the tumor microenvironment and reach an adequate concentration of therapeutic agent inside the tumor mass, four studies using a convection-enhanced delivery (CED) are still ongoing (NCT04264143; NCT03086616; NCT05063357; NCT01502917). In short, CED is a neurosurgical approach involving the stereotactic insertion of a catheter through the brain to directly deliver therapeutic agents to the region of interest. This approach involves the generation of a pressure gradient through slow infusion via intraparenchymal microcatheters to create fluid convection within the brain, increasing the penetration and distribution of the therapeutic agent. Interstitial infusion to the brainstem via CED has been proven to be safe and feasible in multiple animal models, and a recent phase I clinical trial in children with DIPG validated this as safe in human patients [118]. In vivo studies have demonstrated that CED can achieve excellent biodistribution, affected by the physical properties of the drugs, such as its inverse relationship with molecular weight, which allows a direct infusion of drugs under controlled pressure into the tumor mass (specifically with an irinotecan liposoluble particle or a water-soluble panobinostat nanoparticle formulation named MTX110, whose investigations are ongoing (NCT04264143)).

Two other studies aim to investigate the role of omburtamab, a murine IgG1 monoclonal antibody, in recognizing CD276 (also known as B7-H3) and actively introducing it into the tumor by CED. This antibody is selectively marked with a radioactive substance, 124 or 131-Iodine omburtamab, which can determine tumor death, binding the target antigen and enhancing radio-induced tumor death [119]. Unfortunately, the study testing with 124-iodine has recently been interrupted for toxicities (NCT01502917), while the study with 131-iodine radionuclide is currently recruiting (NCT05063357).

Another extremely innovative CED application combined an experimental agent, named IL-13 pseudomonas exotoxin (IL13-PE), with a usual MRI contrast agent (gadolinium DTPA) to monitor drug delivery. The initial results published from the first four enrolled patients demonstrate that this approach is safe and guarantees an adequate drug distribution into tumor cells [95] (NCT00880061).

Another emerging drug delivery technique is the use of focused ultrasound (FUS) to destroy the integrity of the BBB during therapy administration and to improve drug delivery of chemotherapeutic agents or novel nanoparticle therapies. FUS, previously tested only on animal models, uses low-frequency ultrasound waves in combination with intravenously administered microbubbles to transiently open the BBB, without tissue injury by rearranging the endothelial tight junctions. Further investigations are needed in tumor models before the application to pediatric patients becomes feasible [120]. A trial exploring an MR-guided focused ultrasound energy in combination with SONATA-001 administrations is in progress (NCT05123534).

Furthermore, the identification of several intrinsic mechanisms underlying tumorigenesis has led to promising innovations, certainly including the discovery of the role of dopamine receptor D2 (DRD2) G protein-coupled receptor, which stimulates tumor growth and differentiation in tumor lines overexpressing this receptor [121], particularly expressed in the midline structures [122]. ONC201 is a selective oral antagonist of dopamine receptor D2/3 (DRD2/3) and also a potent agonist of the mitochondrial caseinolytic protease P
(ClpP). Once activated by ONC201, ClpP drives the degradation of mitochondrial respiratory chain enzymes and triggers apoptosis and cancer-selective cell death [123]. Preclinical models exhibit brilliant anti-cancer activity, inducing tumor necrosis factor-related apoptosis, with selective tumor cell death [124]. The first responses to single-agent ONC201 were reported in an adult patient with recurrent H3 K27M-altered thalamic glioma, who obtained a near-complete objective response (96%), with the complete regression of the primary thalamic lesion for more than 3 years during ONC201 treatment [96]. In the wake of these promising finding, early results of phase II clinical trial of 18 patients (7 adults and 11 children) demonstrated a median progression-free duration of 53.14 (range 41–81.9) weeks. Thirteen patients discontinued ONC201 due to clinical and/or radiographic disease progression and died due to their disease. The median time from ONC201 discontinuation to death was 3.9 (range 0.4–25) weeks. Among the 14 patients with recurrent disease, the median PFS is 14 weeks: 15 weeks for the 7 adults and 13 weeks for the 7 pediatric patients [97]. The first DIPG patient treated with adjuvant ONC201 obtained a radiological response and clinical improvement, with a reduction in facial palsy. He continued ONC201 monotherapy for 12 months before the progressive disease developed. A second patient achieved an 18-month PFS, and she is still on treatment. Moreover, the synergy of ONC201 in combination with epigenetic modulators targeting H3K27M (such as vorinostat), or ONC206, a more recent analog, was demonstrated to be effective in several preclinical data [98,99]. Nowadays, four-phase I/II clinical trials with ONC201 are recruiting for patients with H3K27-altered gliomas, one of them specific to the pediatric population (NCT03416530).

The role of cancer vaccines is well known in oncologic immunotherapy settings, but they have never been tested on DMG patients. Several clinical trials are ongoing to investigate the possible role of a vaccine containing an H3.3-K27M-targeted neoantigen peptide, presented by antigen-presenting cells (APCs), activating specific T-cells and triggering corresponding cytotoxic T-cell immune responses; thence, the final objective is to eliminate H3.3-K27M-expressing DIPG cells. The results of a phase I trial demonstrated a good profile of feasibility and tolerability, with a valid DIPG immune response detected in peripheral blood and cerebrospinal fluid, and phase II is ongoing [125].

A recent phase I trial aims to investigate the potential therapeutic role of a vaccine monotherapy (rHSC-DIPGVax), starting with an in-human study, combined with an anti-PD1 therapy (balstilimab), with the intent to induce both a more profound intra-tumoral response with the inhibition of negative co-regulatory pathways and the overcoming of the immunosuppressive microenvironment [126]. A subsequent part of this study will provide a combination of anti-CLTA4 therapy (zalifrelimab), taking advantage of its ability to induce T-cell proliferation, and memory formation (NCT0494384).

Moreover, another strategy promotes the use of oncolytic adenovirus to exert an anti-tumor ability. As shown in a phase I-II trial with AloCELYVIR, bone-marrow-derived allogeneic mesenchymal stem cells infected with an oncolytic adenovirus (ICOVIR-5) are currently under investigation (NCT04758533) [103]. Recently, a single-center trial (NCT03178032) was conducted by Gállego Pérez-Larraya and coll. DNA-2401, an oncolytic adenovirus that selectively replicates in tumor cells, was utilized in treating newly diagnosed DIPG. The patients received a single virus infusion through a catheter placed in the cerebellar peduncle, followed by radiotherapy. Over a median follow-up of 17.8 months (range 5.9 to 33.5), the median survival was 17.8 months, with one patient free of tumor progression at 38 months; however, its tumor was H3K27M wild-type, further confirming the worse prognosis of this mutation [104].

Adoptive T cell therapies have emerged as a promising approach for hematological diseases, but also solid tumors, such as neuroblastoma and other tumors expressing a target antigen on their surfaces.

Concerning CNS tumors, published data are available for 10 adult patients treated with CAR-T cells manipulated and redirected against antigens HER2 and EGFR variant III [106], with encouraging results concerning safety and feasibility, but dismal regarding survival benefits. In pre-clinical experiences, anti-GD2 CART cells strongly eradicated brainstem
tumors in orthotopic xenograft mouse models, but, at the same time, a significant number of mice died after CAR-T cell infusion, probably for local inflammatory infiltration and acute edema in the pontine region [127], demonstrating that further preclinical investigations are needed before its use in a clinical setting. Several obstacles need to be overcome to obtain therapeutic success: the heterogeneous distribution of target antigen, the antigen loss after CAR-T cells infusion, the possible development of neuro-inflammatory toxicity, and the inhibitory tumor microenvironment, which can reduce the infiltration of CART cells.

Different approaches are under investigation to improve CAR T-cell-based efficacy in solid neoplasms, including intrinsic costimulatory domains, genetic implementations, secreted cytokines, monoclonal antibodies, or chemical molecules [128].

Three clinical trials are currently recruiting applicants for DIPG, which typically express GD2 on their surface due to their neuroectodermal origin (NCT04196413; NCT04099797; NCT0418503). The results of the first four patients treated with CAR T cells were recently published by Majzner and colleagues [105]. The cells were administered intravenously, and the three patients who exhibited clinical benefit were given subsequent anti-GD2 CAR T cell infusions administered intracerebro-ventricularrly via the Ommaya reservoir. All these exhibited clinical and radiographic improvement. Of note, all four patients experienced tumor inflammation-associated neurotoxicity, reversible with intensive supportive care [105].

Moreover, the first national phase I clinical trial, involving GD2 CART cells in pediatric brain tumors, is going to be coordinated by an Italian institution (Bambino Gesù Children’s Hospital) and it will include three different cohorts of CNS tumors (NCT 05298995).

5. Conclusions and Future Directions

DMG is one of the major critical challenges in pediatric oncology, due to intrinsic molecular and epigenetic dysregulation, an intact BBB that hinders drug delivery, and a limited immune response to tumor antigens. Presently, no curative therapy has been found.

The scientific efforts accomplished during the last 20 years have led to a deeper knowledge of DMG and DIPG biology, and therefore to a better understanding of the different vulnerabilities and how to attack them.

The availability of target therapies, immunotherapy, and new advanced delivery systems with nanotechnologies has completely changed the paradigmatic approach of oncologic treatment and opened worldwide scientific researches in several clinical trials, with promising preliminary results.

However, especially concerning DIPG, it is quite difficult to immediately research a curative therapy, considering the almost lethality of this disease and the little progress made on OS in recent decades. In the same way, even clinical trial failures provide key insights for the continuation of care, driving further investigations and scientific interests.

With this critical reinterpretation of the obtained results, releasing negative results have an impact on learning and examining new potential therapies. Retrospective studies can therefore provide important information that may help incoming trials to point out what to investigate, and they strongly need to be encouraged, such as in the retrospective and prospective SIOPE DIPG Registry [129,130].

The goal would be to create an international research network to share clinical, radiological, and biological information worldwide, and thus identify the best therapeutic approach for every single patient and potentially change the inauspicious fate of this disease.

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