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*Review*

# Drug Repurposing in Pediatric Brain Tumors: Posterior Fossa Ependymoma and Diffuse Midline Glioma under the Looking Glass

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**Abstract**

Tumors of the Central Nervous System (CNS) represent the leading cause of cancer-related deaths in children. Current treatment options are not curative for most malignant histologies, and intense preclinical and clinical research is needed to develop more effective therapeutic interventions against these tumors, most of which meet the FDA definition for orphan diseases. Increased attention is being paid to the repositioning of already-approved drugs for new anticancer indications as a fast-tracking strategy for identifying new and more effective therapies. Two pediatric CNS tumors, posterior fossa ependymoma (EPN-PF) type A and diffuse midline glioma (DMG) H3K27-altered, share loss of H3K27 trimethylation as a common epigenetic hallmark and display early onset and poor prognosis. These features suggest a potentially common druggable vulnerability. Successful treatment of these CNS tumors raises several challenges due to the location of tumors, chemoresistance, drug blood-brain barrier penetration, and the likelihood of adverse side effects. Recently, increasing evidence demonstrates intense interactions between tumor cell subpopulations and supportive tumor microenvironments (TMEs) including nerve, metabolic, and inflammatory TMEs. These findings suggest the use of drugs, and/or multi-drug combinations, that attack both tumor cells and the TME simultaneously. In this work, we present an overview of the existing evidence concerning the most preclinically validated noncancer drugs with antineoplastic activity. These drugs belong to four pharmacotherapeutic classes: antiparasitic, neuroactive, metabolic, and anti-inflammatory. Preclinical evidence and undergoing clinical trials in patients with brain tumors, with special emphasis on pediatric EPN-PF and DMG, are summarized and critically discussed.

**Keywords:** pediatric CNS tumors; ependymoma; diffuse midline glioma; drug repositioning; mebendazole; valproic acid; haloperidol; metformin; celecoxib

## 1. Introduction

Tumors of the central nervous system (CNS) represent the leading cause of cancer-related morbidity and mortality in children in high-income countries [1,2]. In recent years, multiomics approaches have fueled significant advancements in our understanding of the molecular basis of pediatric neuro-oncogenesis. These efforts have allowed for robust stratification of histological tumor entities in clinically relevant molecular groups, which differ in epidemiologic, clinical, and biological profiles, and support the need for distinct treatment interventions [3,4]. Notwithstanding these advancements, current treatments have remained largely static, and 5-year survival rate for children with malignant CNS tumors only achieves a modest 57.5% [5]. Different from CNS cancers arising in adults, pediatric CNS tumors are characterized by a lower mutational burden [6], reduced number of epigenetic alterations [7], and a characteristic spatiotemporal distribution suggestive of a causal link to dysregulation of developmental program(s) during CNS embryogenesis [8]. A central trend emerging from recent research is that pediatric CNS tumors are derived from regionally distinct and temporally restricted neural cells-of-origin. Such tumors are broadly stratified as tumors of glial origin and include gliomas and ependymomas (EPN), and tumors of neuronal origin such as medulloblastoma (MB) and atypical teratoid/rhabdoid tumors (AT/RTs) [9].

Among glial tumors arising in the posterior fossa (PF), EPN type A (PFA) and diffuse midline glioma (DMG) share some commonalities, such as hindbrain origin, midline location, early onset, poor prognosis, and a unique epigenetic profile. There is currently a high demand for new chemotherapeutic options for children with EPN and DMG [10,11]. Due to the location within brainstem and diffuse nature of DMGs, these tumors are typically unresectable, and standard of care currently relies on focal radiotherapy (RT) alone or in combination with antitumor agents. Such approaches typically show a marginal influence on the course of disease as median overall survival (OS) is 9–11 months, and only 2% of children survive 5 years [12]. EPN are chemoresistant tumors with a tendency to recur [13],

and mainstays of treatment are surgery and adjuvant RT [14,15]. Although gross total resection is still the strongest predictor of outcome [16], it is only achieved in approximately 60% of children with EPN [17]. Moreover, the use of RT in very young patients is limited because of the deleterious side-effects of radiation on a developing nervous system [18], and the use of chemotherapy in recurrent EPN has not translated into clear survival advantages. EPN arising in the PF accounts for almost 70% of tumors observed in children, and these are further characterized as PFA and EPN type B (PFB). PFA is the commonest form in children and has the worst outcome, with 56% of patients showing 10-year OS [19], whereas PFB commonly displays a more indolent behavior [19].

Tumorigenesis appears to be epigenetically driven in PFA tumors as these tumors generally lack commonly recurrent oncogenic events but show aberrant EZHIP expression in nearly all cases [20]. In contrast, a number of genetic alterations drive development of DMGs. H3K27M mutations are present in almost 90% of DMGs and define a specific tumor type identified as DMG H3K27-altered [3,21,22]. Ubiquitous alteration of H3K27M co-segregates, in different combinations, with other genetic aberrations including p53 loss-of-function and alterations of signaling genes such as PDGFR$\alpha$, activin receptor type-1 and PI3K/mTOR [23]. Although PFA and DMG have distinct DNA methylation and genetic backgrounds, both are characterized by common epigenetic hallmarks, namely, a CpG island methylator phenotype, overall DNA hypomethylation, and a global loss of H3K27 trimethylation with focal gains within CpG islands [10]. H3K27 hypomethylation is caused by inhibition of the PRC2 complex occurring via competitive binding of the PRC2 catalytic subunit EZH2 with H3K27M in DMG [24] and overexpressed EZHIP in PFA [25]. These aberrant epigenetic event prevents the spread of H3K27me3 marks beyond PRC2 high-affinity sites [26]. Interestingly, these mechanisms are 100% mutually exclusive, and rare DMGs with wild type H3K27 show aberrant expression of EZHIP [27] and H3K27M mutations are found in rare PFA tumors that display low EZHIP expression [28]. Although distinct transcriptomes characterize both PFA and DMG, a commonly shared signature is the loss of PRC2-mediated gene repression and spurious activation of earlier developmental programs in neural stem cells that are crucial for oncogenesis [29,30]. Given the H3K27 hypomethylation at the core of PFA and DMG with H3K27M mutation, it is tempting to speculate that these tumors may share new and more effective chemotherapeutic interventions that cross histological boundaries.

The development of a new drug, from de novo discovery to final registration, requires an estimated time frame of 13–15 years, and an average expenditure of 2–3 billion US dollars [31]. To expedite this process, drug repositioning is an attractive approach as it allows the use of approved or investigational drugs for indications other than their originally intended use [32,33]. Because pharmacokinetics, pharmacodynamics, and safety profiles have already been established in the initial preclinical and Phase I studies, these compounds may rapidly enter into Phase II and Phase III studies. This serves to shorten the overall developmental timelines to only 3–9 years [31]. As opposed to molecularly-targeted agents, repurposed medicines have the potential to hit, in a simultaneous fashion, multiple unrelated pathways involved in tumorigenesis. Such therapeutics may function through off-target mechanisms, and may prove to be strategic weapons against malignancies driven by otherwise undruggable targets such as EZHIP. Despite these advantages, the number of non-oncological drugs that has been repurposed for oncology use is currently quite low [34].

The first breakthroughs in drug repurposing occurred serendipitously, as was the case of mebendazole (MBZ), an antihelmintic drug that was found to prevent glioblastoma (GBM) engraftment in mouse models [35]. Owing to advancements in biology and bioinformatics, more systematic approaches for the identification of repurposed drug candidates have been developed. These approaches are broadly divided into computational approaches, experimental approaches, and multiparametric pharmacogenomics strategies that allow for mechanistic insights on the identified compounds [36–38]. To date, 268 noncancer therapeutics have shown promising off-label anticancer profiles, although the majority of the studies have focused on adulthood cancers [39]. While this cost-effective approach could be particularly beneficial for childhood cancers, most of which meet the FDA definition of an orphan disease, drug repurposing discourages pharmaceutical companies from developing novel drugs [40]. Moreover, the distinct biology and vulnerabilities of childhood cancers suggest that adult oncology drugs may have limited applicability in pediatric cohorts. In pediatric CNS tumors, candidates for drug repurposing should fulfill unique characteristics, including: (1) blood-brain barrier (BBB) penetrance; (2) favorable safety profiles in infants and children; (3) proven preclinical efficacy in brain cancer stem cell-driven models; (4) pharmacokinetic properties that allow the drug to reach therapeutically effective concentrations at the tumor site; (5) synergy with approved anticancer treatments [41].

In recent years, mutually supportive interactions between tumor cells and the surrounding tumor microenvironments (TMEs) have increasingly been acknowledged as a driving force for both tumor progression and response to therapy [42–44]. A number of FDA-approved conventional medicines that target the innervated niche, the metabolic TME, and/or the inflammatory TME have shown antitumor activity, making them valuable candidates for combination therapy [45]. Some of these already-marketed agents are currently under investigation for drug repurposing in pediatric CNS tumors.

In this manuscript, we summarize recent progress in our understanding of the brain cancer killing activity of the most paradigmatic, preclinically validated noncancer medicines from four pharmacotherapeutic drug classes: antiparasitic, neuroactive, metabolic, and anti-inflammatory. After touching on the most relevant literature in adult CNS tumors, we examine clinical trials evaluating the safety, toxicity and clinical benefits of MBZ, valproic acid, metformin (MET) and celecoxib against pediatric CNS tumors, with a principal focus on EPN and DMG.

## 2. Antiparasitic Drugs

Promising preclinical evidence, coupled with algorithm-based analyses, suggest repurposing of antiparasitic drugs as promising anticancer candidates. This view is supported because of pleiotropic disease-fighting properties, easy access, low cost, and a well-established safety profile of these drugs in humans [46]. Of all these agents, only mebendazole (MBZ) has advanced to clinical trials for treatment of CNS tumors.

### *2.1 Mebendazole*

#### 2.1.1 Preclinical Studies

Mebendazole (MBZ) is an antiparasitic drug with 40 year history of safe use in humans and meets many of the ideal features for a repurposed drug to treat CNS tumors [47,48]. Like all benzimidazoles used for treating helminth infestations, MBZ inhibits parasite microtubule polymerization via high-affinity binding to a colchicine-sensitive site on $\alpha$-tubulin. This property allows MBZ to dysregulate microtubule-mediated transport of secretory vesicles, glucose uptake, and ATP formation [49]. Beside its microtubule destabilizing properties, MBZ exerts pleiotropic effects on a number of cancer-related pathways at clinically achievable concentrations, such as angiogenesis, apoptosis, cell cycle progression, epithelial-to-mesenchymal transition, metastatic spread, antitumor immune response, and protein kinase inhibition [50–52]. Its role as a blocker of VEGFR2 activity was discovered by molecular fit computations on 3671 FDA approved drugs across 2335 human protein crystal structures. This work allowed investigators to map new drug-target interactions and predict novel uses for this drug [53]. Notably, experimental validation demonstrated that MBZ inhibits VEGFR2 kinase activity as well as angiogenesis at doses comparable to its well-established antihelmintic effects. Proteo-chemometric computational methods, coupled with kinase assays, have identified other previously unrecognized targets of MBZ including ABL1, MAPK1/ERK2, MAPK14/p38a, BCR-ABL, and BRAF [54,55]. A recent study that used computational cell cycle profiling to prioritize FDA-approved drugs with repurposing potential demonstrated that MBZ was one of 36 agents with the strongest cytotoxic effect of a panel of 884 drugs that also contained known anticancer agents [56]. Other unexpected anticancer activities of MBZ were discovered by *in silico* analyses followed by *in vitro* and/or *in vivo* validation, such as induction of DNA damage [56] and differentiation [57]. For example, MBZ is the second of the top 20 differentiation-promoting candidates in a library of 1235 drugs that were screened through a computational approach that leveraged drug-induced gene expression changes in the differentiation state of leukemia cells [58].

MBZ has been reported to efficiently suppress proliferation in cell lines from a wide range of cancers, including leukemia, breast, and colorectal cancer [59–61], while sparing noncancerous immortalized cells at the same dose. The growing interest in MBZ as a repurposed medicine against CNS tumors stems from its physicochemical properties of possessing no charge, lipophilicity, and relatively small size as these are features that suggest favorable neuropenetrance after systemic administration [62]. However, of the known three polymorphs of MBZ (*i.e.*, polymorphs A, B, and C) only polymorph C only reaches therapeutically effective concentrations in murine intracranial allograft models and brain tissues, achieving a brain to plasma ratio of 0.8 [63].

The IC50s for cell viability of MBZ in brain tumor models varies from 0.1–0.3 $\mu$M in GBM cell lines [35,64], up to 0.5 $\mu$M in non-neuronal cell lines and cultured DMG cells dosed with MBZ [65,66], and that these dose ranges fall in a clinically attainable range [67]. Of interest, multiple single-agent screens of 2706 drugs targeting 860 distinct cellular mechanisms uncovered that MBZ was among the 371-potency-selected agents in analysis of six DMG cell culture models [66]. Seminal preclinical studies convincingly provided evidence that MBZ prolongs survival in intracranial glioma [35] and MB [68]. Moreover, MBZ was associated with reduced vascularity in tumor, but not in normal tissues [69]. MBZ also suppresses the assembly of the primary cilium [65], a microtubule-based structure that plays a fundamental role in signal transduction of sonic hedgehog (Shh) and other cancer-related pathways, and is essential for initiation and maintenance of MB allografts in mice [70]. A recent study leveraging *in silico* analysis of the hub genes of GBM and a connectivity map platform for drug repurposing, found that multiple azole compounds had potential anti-GBM activity [71]. However, only the benzimidazoles flubendazole, fenbendazole, and MBZ were proven to efficiently suppress DNA synthesis, cell migration and invasion, G2/M cell cycle arrest in *in vitro* validation experiments [72]. An *in vitro* drug screening on a panel of 19 cell lines derived from childhood solid tumors, including CNS tumors, interrogated drug candidates in a collection of approximately 3800 approved and investigational compounds [40]. In this study, MBZ and other benzimidazoles were identified among the 736 compounds with robust bioactive profiling, and were found to be effective against the majority of the cell lines in this panel.

**Table 1. Ongoing studies registered at Clinicaltrials.Gov (current as of 18 September 2022) investigating MBZ as a repurposed drug against adult and pediatric CNS tumors.**

| NCT | Official title | Conditions | Phase | Status | Other therapeutic agents | Population | Dates | Outcome Measures | Results |
|---|---|---|---|---|---|---|---|---|---|
| NCT01729260 | Phase I Study of MBZ in Newly Diagnosed HGG Patients Receiving TMZ | HGG | I | Completed | Standard postoperative chemoradiation TMZ | 24 patients; age ≥18 yr. | Study Start: April 4, 2013; Last Update Posted: May 7, 2021 | MTD; OS | [82] |
| NCT02644291 | Phase I Study of MBZ Therapy for Recurrent/Progressive PBTs | BSG, HGG, other PBTs | I | Completed | | 16 patients; 1–21 yr. | Study Start: May 2016; Last Update Posted: June 23, 2022 | Adverse events; 2-year OS | |
| NCT01837862 | A Phase I Study of MBZ for the Treatment of Pediatric Gliomas | LGG, HGG, including DIPG | I, II | Recruiting | Standard chemotherapy drugs for the treatment of pediatric BTs | 36 patients; 1–21 yr. | Study Start: October 22, 2013; Last Update Posted: April 13, 2022 | MTD of MBZ in combination; 3-year EFS; 3-year OS | |

Trials against adult and pediatric CNS tumors are reported in the upper and lower part of the table (separated by a bold line), respectively. Tumors of the main topic of this review are in bold. BSG, brain stem glioma; BTs, brain tumors; DIPG, diffuse intrinsic pontine glioma; EFS, event-free survival; HGG, high-grade glioma; LGG, low-grade glioma; MBZ, mebendazole; MTD, maximum tolerated dose; OS, overall survival; PBTs, pediatric brain tumors; TMZ, temozolomide; yr., years.

MBZ acts synergistically with ionizing radiation (IR) and several chemotherapeutic agents in different preclinical models of CNS tumors. In human (GBM14) and murine (GL261) glioma cells, MBZ enhances DNA-damaging effects of IR by targeting interphase microtubules at doses significantly lower than that needed for inducing mitotic arrest [73]. Mechanistically, this radiosensitizing effect is linked with inhibition of DNA damage repair (DDR) signaling through interference with the cytoplasmic-nuclear trafficking of DDR proteins. MBZ has also been used *in vitro* and *in vivo* as an adjuvant to frontline chemotherapeutics for the clinical management of CNS tumors showing enhanced growth-inhibitory effects. Of interest, improved DDR response is a mechanism of radioresistance shared by glioma stem cells and many types of glioma cells, including pediatric glioma, and that this highlights the importance of further preclinical and clinical evaluation of MBZ in combination with radiotherapy (RT) [74].

The combination of temozolomide (TMZ) with MBZ exerted a greater anti-proliferative effect than TMZ alone in patient-derived cultures and GBM cell lines, although this combination failed to significantly prolong survival in orthotopic glioma mouse models when compared to MBZ monotherapy [35]. In MB lines, MBZ overcomes resistance to the Shh inhibitor vismodegib mediated by mutant smoothened (SMO) receptor, and exerts an additive inhibition of canonical Shh signaling in combination with vismodegib [65]. Although these studies suggest potential combinatorial efficacy effects from MBZ, caution must be taken when including MBZ in treatment protocols since antagonistic effects of MBZ with different therapeutics have been reported by collateral activation of other pathways such as NF-kB [75] and the MEK-ERK [76] pathways.

#### 2.1.2 Clinical Studies

The first studies investigating anti-tumor activity and safety of MBZ in the clinic were two case reports on individual patients with metastatic adrenocortical carcinoma [77] and metastatic colon cancer [78]. After failure of all treatment approaches, MBZ monotherapy was administered at the standard antihelmintic dose of 100 mg twice daily. This dosing showed a favorable safety profile accompanied by long-term disease control in both cases. Data on larger numbers of cancer patients confirmed an acceptable toxicity profile, and overall long-term safety of MBZ, even at much higher doses (up to 4 g/day), although antitumor efficacy was not consistently reported [79,80]. All of the ten patients with refractory advanced gastrointestinal cancer studied developed rapid progressive disease while on single-agent MBZ at individualized doses targeted to a serum concentration of 300 ng/mL [79]. Another study used MBZ as an adjuvant therapy in association with FOLFOX4 and bevacizumab in 20 patients with metastatic colorectal carcinoma. Here the investigators reported significant improvement of overall response rate (ORR) and elevation of progression free survival (PFS) with respect to the control group of 20 patients treated with FOLFOX4 and bevacizumab [80]. However, the study failed to show significant variation in one year OS between the two arms.

Two safety trials were designed to determine the highest tolerable dose of MBZ in an adjuvant setting using frontline drugs for recurrent [81] or newly diagnosed adult high-grade glioma (HGG) patients (NCT01729260 [82]). Both trials reported good drug tolerability, although the small number of patients enrolled in these trials preclude drawing conclusions concerning MBZ efficacy in the treatment of glioma (Table 1, Ref. [82]). Pharmacokinetic analysis documented dose-related plasma levels of MBZ with large inter-patient variability, suggesting that administration of 75–100 mg/kg/day for future trials would provide safety, acceptable toxicity, and adequate plasma concentrations to reach those that result in antineoplastic activity *in vitro* [82]. However, a Phase II trial of MBZ (1600 mg thrice daily) with TMZ or lomustine in 88 patients with recurrent GBM failed to show clinical benefit with OS being no better than historical controls [83].

In the pediatric setting, a pilot trial (NCT02274987) has examined the feasibility of personalized therapeutic interventions in 15 children with newly diagnosed DMG using molecularly targeted therapy with up to four FDA approved drugs. These drugs were selected by an integrated approach leveraging genetic profiling with whole exome sequencing (WES), RNA sequencing, and *in silico* drug-gene matching [84]. Although the study was not designed to assess therapeutic efficacy, no survival benefit was reported in patients (n = 8) who followed treatment recommendation *vs* patients (n = 7) who underwent standard treatment. In this study, MBZ was selected to target PDGFRA copy number gain or PDGFRA overexpression and was administered in combination with other agents to 6 children.

A dose escalation clinical testing of oral MBZ as a monotherapy has recently been completed in pediatric patients with no longer responsive HGG or MB (NCT02644291). The study was aimed at analyzing adverse events and 2-year OS as a secondary endpoint. A phase I, II trial (NCT01837862) studying MBZ in combination with frontline treatments for gliomas is currently enrolling pediatric patients with low-grade glioma (LGG) and HGG, including diffuse intrinsic pontine glioma (DIPG), to find out the maximum tolerated dose (MTD) and 3-year clinical benefits. However, no records of completed clinical trials in children are available at this time.

Further clinical experimentation on MBZ should be formulated to address critical questions such as poor aqueous solubility and systemic availability of the agent, poor gastrointestinal absorption, and extensive first pass metabolism. Each of these variables result in large inter-patient pharmacokinetic variability and hamper achieving adequate therapeutic concentrations *in vivo*. These shortcomings may be overcome by nanocarrier-based formula-

tion, prodrug design, and solid dispersion approach to increase bioavailability and reduce pill burden [46].

## 3. Neuroactive Drugs

The TME has become increasingly appreciated as a major driver of cancer growth and the nervous system has emerged to be a crucial component as well [85,86]. A number of neurochemicals such as glutamate, norepinephrine, an acetylcholine, and secreted factors such as NGF, BDNF, and GDNF have been described as promoting tumor-supportive signaling. This suggests existing neuroregulatory therapeutics may be repurposed in an off-label setting with conventional cancer therapies, a notion that has ignited the emerging field of cancer neuroscience [87,88]. These new avenues are especially attractive for CNS tumors which establish an intense, reciprocal cross-talk with the surrounding nervous system. Neuroactive drugs convey several advantages, such as high neuro-penetrance and well-known safety profiles in clinical settings. Moreover, epidemiological studies have shown that cancer risk is inversely correlated with antipsychotic drug treatment in adult patients with schizophrenia; however, data particular to the pediatric population is scant in nature [89].

Primary tumors of the CNS are principally driven by glutamatergic signaling through a network of excitatory synapses [90]. Functional neuroglioma synapses that promote tumor invasion and growth have been characterized between pre-synaptic glutamatergic neurons and post-synaptic GBM [91] and DMG cells [92]. The excitatory postsynaptic current appears to be mediated by a transient rise of calcium through calcium-permeable glutamatergic $\alpha$-amino-3-hydroxy-5- methyl-4-isoxazole propionic acid receptors (AMPAR) [88]. In support of this finding, pharmacological blockade of AMPAR by the antiepileptic drug perampanel inhibits proliferation of tumor cells in orthotopic animal models of patient-derived GBMs [91] and DIPGs [85]. Glioma cells, in turn, release growth stimuli, such as glutamate, activating a bidirectional positive feedback loop that augments neuronal excitability and tumor proliferation [85]. Although often considered a byproduct of mechanical pressure, seizures that accompany CNS tumors can be linked to increased peritumoral hyperexcitability by glutamate-secreting neoplastic cells [85], and this has been observed in adult and pediatric glioma xenografts [88,93]. Therefore, disrupting electrical hyperactivity with anti-epileptics may result in control of both tumor growth and seizures. Although retrospective studies and small prospective clinical trials have investigated the anti-cancer benefit of anti-epileptics in patients with CNS tumors, a definite conclusion has not yet been reached.

### *3.1 Valproic Acid*

#### 3.1.1 Preclinical Studies

Valproic acid (VPA) is a short branched-chain fatty acid with a long-established use in humans as a mainstream antiepileptic medicine. Significant effort on understanding the embryotoxic effects of in-uterus exposure to VPA [94] led to the discovery of its anti-proliferative and differentiation-inducing capabilities in transformed cells of neural origin both in culture [95] and *in vivo* [96] in the 1990's, fueling investigation of VPA as an alternate cancer-fighting therapeutic. Since these pioneering studies, evidence has gradually accumulated regarding the antineoplastic activity of VPA in several model systems, and this topic has been the focus of numerous reviews [97–99]. The mechanisms that underpin the antiepileptic and antitumoral activity of VPA are not clearly defined, but are very likely distinct in nature [98,100]. VPA anticonvulsant action is clearly related to the blocking of voltage-dependent sodium channels and to increased brain concentrations of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) through VPA-induced modulation of enzymes involved in GABA metabolism. Effective VPA concentrations for these actions are 0.5–1 mM in *in vitro* competitive assays and in cultured hippocampal neurons [101].

VPA is also a relatively weak inhibitor of class I histone deacetylases (HDAC). IC50s for VPA in *in vitro* HDAC assays are within, or close to, its therapeutic range (0.35–0.7 mM in serum) and result in dose-dependent histone hyperacetylation [100,102]. As an epigenetic modeler of chromatin structure, VPA is involved in oncogenic silencing and recruitment of tumor suppressive transcription factors [103] that ultimately trigger apoptosis, autophagy, or differentiation, with concurrent inhibition of cell-cycle progression, invasion, and tumor angiogenesis [97–99]. Mechanistically, VPA modulates several distinct cancer-related pathways, including MAPK, $\beta$-catenin, and GSK-3 signaling pathways.

Several seminal reviews in the field have thoroughly examined the cancer-related effects of VPA in various glioma models [99,103,104]. Generally, IC50s of VPA at 72 h are far above the upper limit of clinically therapeutic concentration (1 mM) when tested in the majority of glioma cell lines [105–108]. However, 1 mM VPA is able to induce a time-dependent, oxidative stress-related, autophagy as well as activation of MAPK signaling, H3 and H4 histone acetylation, upregulation of the cell-cycle inhibitor p21, differentiation, and inhibition of VEGF secretion in glioma cells and HUVEC tube formation [105,109,110]. Higher doses of VPA (5–20 mM) are needed to reduce stress-related molecules such as paraoxonase 2 (PON2), an endogenous free-radical scavenging enzyme, that increases the apoptosis inducing reactive oxygen species (ROS) [107]. This *in vitro* VPA activity translates to *in vivo* glioma models where VPA inhibits tumor growth and angiogenesis while inducing differentiation and apoptosis [107,110,111].

Few studies are available concerning the activity of VPA in preclinical models of pediatric CNS tumors. In medulloblastoma cell lines, VPA at clinically safe concen-

trations (0.6 and 1 mM) necessitate prolonged exposure (14 days and longer) to induce potent growth inhibition, apoptosis, and differentiation, although detectable H3 and H4 hyperacetylation are noted as earlier as 3 days after drug addition [112,113]. In both heterotopic and orthotopic MB xenografts, VPA treatment significantly suppresses tumor growth and angiogenesis, while enhancing histone hyperacetylation, apoptosis, and differentiation.

In search for more effective treatment strategies against DMG, VPA was included in a chemical screen of 83 of the most promising therapeutic agents identified in preclinical models [114]. However, it was not effective when tested in a panel of seven patient-derived DMG lines, possibly because the maximum dose used was 10 $\mu$M and the readout used was cell viability at 72 h. Indeed, in a subsequent study that focused on dose-dependent effects of VPA in H3K27M mutant DMG, IC50s ranged between 2.96 mM and 5.1 mM [115]. In this study, VPA antiproliferative effects were associated with dose-dependent augmentation of histone acetylation and apoptosis in tumor cells, whereas at the highest VPA dose tested (5 mM) minimal toxicity to rat hippocampal neuronal and glial cells was observed.

#### 3.1.2 Clinical Studies

The HDAC-inhibitory activity of VPA, together with its radio and chemo-sensitizing potential [115–118] and the limited side-effects in humans, implicate VPA as a potential neo-adjuvant therapeutic useful in multimodal treatment approaches. Currently, more than 80 trials are evaluating the safety and clinical benefits of VPA in combination with standard of care, or investigational therapies, in a vast array of cancers, including adult and pediatric CNS tumors (Table 2, Ref. [119–122]).

Results obtained, to date, concerning the efficacy of VPA treatment show conflicting significance in glioma patients. A number of retrospective analyses have indicated VPA has moderate activity in newly diagnosed GBM patients treated with standard of care TMZ-based chemotherapy and radiotherapy, linking improved outcomes to the radio and chemo-sensitizing properties of VPA [123–125]. Common drawbacks for the majority of the studies reported are the small sample sizes, the historical bias, and the paucity of information on VPA administration protocols that did not assure these studies achieved anticancer concentrations *in vivo* with the dosing regimens used. Indeed, a pooled analysis of prospective clinical trials in a much larger cohort of patients (n = 1869) with newly diagnosed GBM did not uncover any association between use of the antiepileptic levetiracetam, or VPA, as add-on drugs to standard RT + TMZ and improvement of either PFS or OS [126]. However, it is very likely that patients received typical anti-seizure prophylaxis at doses of 5–10 mg/kg/day, although no information is specifically provided on VPA doses used. In another retrospective analysis of 359 patients with gliomas, VPA use positively correlated with a survival benefit in the GMB (WHO IV) group, whereas in Grade II/III gliomas VPA it was associated with a decrease in PFS and more rapid malignant progression. Conversely, higher doses of VPA (up to 25 mg/kg/day) added to concomitant RT + TMZ therapy (NCT00302159) resulted in an improvement of OS of patients with HGG over historical controls (Median OS of 29.6 months *vs* OS of 8.6–19.3 months previously reported in other studies) [119], and was associated with little late toxicity in a follow-up study [120]. A Phase II trial is currently under way to assess the efficacy of VPA plus sorafenib and sildenafil for the treatment of recurrent HGG (NCT01817751).

In the pediatric setting, the first report concerning the clinical efficacy of VPA is a single-case study on a 10-year-old boy with a radio and chemoresistant GBM, treated with a VPA monotherapy to reach a plasma trough level greater than 1 mM [127]. This is the threshold concentration associated with an anti-cancer effect and is 2 to 3-fold above concentrations commonly obtained in children with epilepsy. Although a complete remission was documented after 10 months on VPA, drug-related drowsiness led to discontinuation of VPA and tumor recurrence after 16 months. In two subsequent underpowered retrospective analyses in heavily pretreated pediatric patients with CNS tumors of different histologies, including EPN [128] and DIPG [129], prophylactic valproate treatment, although it was well tolerated, resulted in no, or barely, statistically significant survival benefits. A phase I study (NCT00107458) found dose-limiting somnolence at drug exposures required to maintain biologically relevant threshold levels, whereas VPA administered to reach trough concentrations up to 0.7 mM showed acceptable toxicity [121]. H3 and H4 hyper-acetylation in peripheral blood mononuclear cells (PBMCs), as surrogate markers of HDAC inhibition, were observed independently of VPA dose in half of the patients. Based on these findings, a multi-institution, phase 2 clinical trial of radiation and VPA, followed by maintenance VPA and bevacizumab (NCT00879437) was conducted in 38 children with glioma, specifically 20 with DIPG and 18 with HGG [122]. The treatment strategy was generally well tolerated, but no improvement in EFS or OS when compared with historical values was observed, although anecdotally encouraging tumor responses were observed.

More recently, VPA has been used in combinations with other drugs in pilot studies focused on evaluating personalized treatments for DIPG patients to specifically target FOSB-overexpressing tumors (4/15 cases) [84] or as a therapeutic backbone at a plasma trough levels of 0.5–0.7 mM (6/9 cases) [130]. Although no significant improvement of OS was observed in either study in the personalized treatment cohort compared to controls, 2 long-term survivors (>2 years) out of 9 patients were reported in one study, one of whom received a multidrug regimen that also included VPA [130].

**Table 2. Ongoing studies registered at Clinicaltrials.Gov (current as of 18 September 2022) investigating VPA as a repurposed drug against adult and pediatric CNS tumors.**

| NCT | Official title | Conditions | Phase | Status | Other therapeutic agents | Population | Dates | Outcome measures | Results |
|---|---|---|---|---|---|---|---|---|---|
| NCT00302159 | A Phase II Clinical Trial of the HDACi VPA in Combination With TMZ and RT in Patients With HGG Multi-Institutional Trial | HGG | II | Completed | RT, TMZ | 43 patients; 18–90 yr. | Study Start: March 2006; Last Update Posted: August 18, 2016 | PFS and OS at 6, 12 and 24 months | [119,120] |
| NCT01817751 | Phase 2 Study of Sorafenib, VPA, and Sildenafil in the Treatment of Recurrent HGG | HGG | II | Active, non-recruiting | sorafenib, sildenafil | 47 patients; age ≥18 yr. | Study Start: April 11, 2013; Last Update Posted: March 23, 2022 | PFS at 6 and 12 months | |
| NCT00107458 | A Phase I Study of VPA in Children with Recurrent/Progressive Solid Tumors Including CNS Tumors | CNS tumors, including DIPG and EPN | I | Completed | | 26 patients; 2–21 yr. | Study Start: May 2005; Last Update Posted: August 7, 2014 | Toxicity | [121] |
| NCT00879437 | A Phase 2 Study of VPA and Radiation, Followed by Maintenance VPA and Bevacizumab in Children With Newly Diagnosed HHG or BSG | HGG, BSG | II | Completed | bevacizumab, RT | 38 patients; 3–21 yr. | Study Start: September 1, 2009; Last Update Posted: July 21, 2021 | 1-yr EFS | [122] |
| NCT03243461 | International Cooperative Phase III Trial of the HIT-HGG Study Group for the Treatment of HGG, DIPG and Gliomatosis Cerebri in Children and Adolescents <18 Years (HIT-HGG-2013) | HGG WHO III/IV, DIPG | III | Completed | TMZ, RT | 167 patients; 3–17 yr. | Study Start: July 17, 2018; Last Update Posted: June 6, 2022 | Effects of VPA with respect to historical controls | |

Trials against adult and pediatric CNS tumors are reported in the upper and lower part of the table (separated by a bold line), respectively. Tumors of the main topic of this review are in bold. BSG, brain stem glioma; CNS, central nervous system; DIPG, diffuse intrinsic pontine glioma; EFS, event-free survival; EPN, ependymoma; HDACi, histone deacetylase inhibitor; HGG, high-grade glioma; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TMZ, temozolomide; RT, Radiation Therapy; VPA, valproic acid; yr., years.

**Table 3. Ongoing studies registered at Clinicaltrials.Gov (current as of 18 September 2022) investigating MET as a repurposed drug against adult and pediatric CNS tumors.**

| NCT | Official title | Conditions | Phase | Status | Other therapeutic agents | Population | Dates | Outcome measures | Results |
|---|---|---|---|---|---|---|---|---|---|
| NCT01430351 | A Phase I Lead-In to a 2 × 2 × 2 Factorial Trial of TMZ, Memantine, Mefloquine, and MET as Post-Radiation Adjuvant Therapy of GBM | GBM | I | Active, non-recruiting | TMZ, Memantine, Mefloquine | 144 patients; age ≥18 yr. | Study Start: September 14, 2011; Last Update Posted: June 18, 2021 | Toxicity; PFS and OS | [185] |
| NCT02780024 | MET and Neo-adjuvant TMZ and Hypofractionated Accelerated Limited-margin RT Followed by Adjuvant TMZ in Patients With GBM (M-HARTT STUDY) | Newly diagnosed GBM | II | Active, non-recruiting | TMZ, HART | 50 patients; age ≥18 yr. | Study Start: March 2015; Last Update Posted: March 2, 2022 | OS; Toxicity | [186] |

**Table 3. Continued.**

| NCT | Official title | Conditions | Phase | Status | Other therapeutic agents | Population | Dates | Outcome measures | Results |
|---|---|---|---|---|---|---|---|---|---|
| NCT02149459 | Improving the Response of Recurrent Glioma to RT Through Metabolic Intervention | BTs | I | Unknown | RT KD | 18 patients; age ≥18 yr. | Study Start: June 2014; Last Update Posted: October 27, 2017 | Adverse events; PFS, OS, ORR | [187] |
| NCT04691960 | A Pilot Study of KD and MET in GBM: Feasibility and Metabolic Imaging | GBM | II | Recruiting | KD | 36 patients; age ≥18 yr. | Study Start: August 2016; Last Update Posted: December 22, 2021 | Tolerability | |
| NCT03243851 | Efficacy and Safety of Low Dose TMZ Plus MET as Combination Chemotherapy Compared With Low Dose TMZ Plus Placebo in Patient With Recurrent or Refractory GBM | GBM | II | Completed | TMZ, | 81 patients; age ≥19 yr. | Study Start: November 2016; Last Update Posted: September 9, 2021 | 6 months PFS; 6 months OS | |
| NCT05183204 | A Phase 2 Trial of Paxalisib Combined With a KD and MET for Newly Diagnosed and Recurrent GBM | GBM | II | Not yet recruiting | Paxalisib, KD | 33 patients; age ≥18 yr. | Study Start: January 2022; Last Update Posted: January 10, 2022 | PFS at 6 months; OS | |
| NCT04945148 | Oxidative Phosphorylation Targeting In Malignant Glioma Using MET Plus RT TMZ | GBM, IDH-wildtype | II | Not yet recruiting | RT, TMZ | 640 patients; age ≥18 yr. | Study Start: October 2022; Last Update Posted: August 3, 2022 | Safety; tolerability; PFS, OS, ORR | |
| NCT01528046 | A Phase I Trial of Dose Escalation of MET in Combination With Vincristine, Irinotecan, and TMZ in Children With Relapsed or Refractory Solid Tumors | Solid tumors, primary BTs | I | Active, non-recruiting | vincristine, irinotecan, TMZ | 26 patients; 1–18 yr. | Study Start: September 2012; Last Update Posted: April 6, 2022 | MTD; Antitumor activity | |
| NCT02040376 | Placebo Controlled Double Blind Crossover Trial of MET for Brain Repair in Children With Cranial-Spinal Radiation for MB | BTs | III | Completed | previous cranial, radiation | 24 patients; 5–21 yr. | Study Start: March 2014; Last Update Posted: September 2022 | Efficacy of MET in fostering brain repair | [188] |
| NCT05230758 | Phase III Randomized Double-blind Placebo-controlled Trial of MET for Cognitive Recovery and White Matter Growth in Paediatric MB Patients | MB, cognitive impairment | III | Not yet recruiting | | 140 patients; 7–17 yr. | Study Start: February 15, 2022; Last Update Posted: February 9, 2022 | Effect of MET on behavior and the brain in children treated for BTs | |

Trials against adult and pediatric CNS tumors are reported in the upper and lower part of the table (separated by a bold line), respectively. Tumors of the main topic of this review are in bold. BTs, brain tumors; GBM, glioblastoma; KD, ketogenic diet; MB, medulloblastoma; MET, metformin; MTD, maximum tolerated dose; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TMZ, temozolomide; RT, Radiation Therapy; yr., years.

The only trial so far to advance to a phase III study is under way in 49 centers (International cooperative Trial, NCT03243461) and is enrolling children and adolescents with HGG to evaluate the efficacy of VPA as a neo-adjuvant to TMZ and RT with respect to historical controls.

Overall, although VPA treatment has not been reported to yield satisfactory results in a number of studies, some patients have responded well to VPA when used in combination with other treatments. Therefore, further prospective studies are warranted to better understand potential clinical benefits of VPA in the treatment of CNS tumors.

### *3.2 Dopamine Pathway and Cancer*

A close connection between neurotransmitters and cancer progression has come into understanding over the course of the last decades. A recent bibliometric analysis of papers published in the last 20 years that linked these two distinct fields, identified dopamine receptors (DRs) as a newly emerging research hotspot [131]. Dopamine (DA) has consistently been reported to affect a variety of cancer-related processes, such as cell signaling, survival, proliferation and invasion in both *in vitro* and *in vivo* models of various tumor types [132]. In addition, DA also acts on the TME particularly through inhibition of angiogenesis and modulation of the immune response. Although the mechanisms underlying DA anticancer properties remain poorly defined, a number of target-agnostic screens have identified dopamine receptor D2 (DRD2) and other DRs as actionable therapeutic oncology targets [133,134]. ONC201 is a first-in-class member of a novel class of anti-cancer compounds termed imipridones. Imipridones exert potent antitumor activity by selective antagonism of DRD2 [135]. Mechanisms of action of ONC201 encompass stimulation of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) expression, and activation of FOX3a-mediated apoptosis through inhibition of AKT/ERK [136]. ONC201 also activates the mitochondrial serine protease caseinolytic protease P (ClpP) [137]. ClpP is involved in protein quality control by degrading misfolded or damaged proteins, and while the role of ClpP in cancer remains elusive, it is upregulated in many different tumor types including breast, lung, and CNS cancers. ONC201 is currently under evaluation in almost 30 clinical trials in advanced cancers and is in late-stage clinical development for recurrent gliomas that harbor the H3 K27M mutation. As an investigational agent without clinical indications other than oncological diseases, a detailed discussion of ONC201 is not included in this review.

#### Haloperidol: Preclinical Studies

Given the potential relevance of DRD2 in cancer, other DRD2 modulators can be potentially be repurposed for cancer therapy. The highly brain-penetrant antipsychotic haloperidol is FDA-approved for treating schizophrenia, Tourette syndrome, severe behavioral disorders, and hyperactivity in children [138]. Haloperidol has also shown promise in preclinical models of various cancer types, making it a good candidate for repurposing in the treatment of pediatric CNS tumors. Beside antagonizing DRD2 with an IC50 of approximately 30 nM, haloperidol also exerts anti-adrenergic (a1), anti-histaminic (H1), and anti-cholinergic action. However, haloperidol is associated with various side-effects including extrapyramidal syndromes [139]. In addition, it has an antagonist effect on Sigma-1 receptors that have been implicated in VEGF synthesis and cell proliferation [140,141].

As an antineoplastic agent, haloperidol is an inducer of autophagy, apoptosis, and cell cycle arrest as well as being an inhibitor of MAPK activity [142]. In different GBM cell lines, haloperidol suppresses cell proliferation with IC50s ranging between 20 and 40 $\mu$M, and, moreover, induces a significant increase in apoptosis [141]. However, the effective concentrations *in vitro* are approximately 3 orders of magnitude greater than therapeutic plasma concentrations (2–50 nM) in both adult [141] and pediatric [143] populations. These values are significantly higher than that required for haloperidol inhibition of DRD2, suggesting that mechanisms other than DRD2 antagonism underpin the antiproliferative effects of haloperidol. Other studies have reported that DRD2 agonists increase formation, proliferation and invasiveness of GBM tumorspheres, whereas molecular knockdown or pharmacological inhibition by DRD2 antagonists, including haloperidol, decreased spheroid formation at concentrations close to those clinically attainable [144,145]. Cotreatment of cells with TMZ and haloperidol results in a synergistic suppression of cell growth, an effect linked to TMZ-mediated upregulation of DRD2 expression [141,146].

An inverse relationship between DRD2 and EGFR has been observed in a portion of clinical GBM samples in publicly available datasets [147]. Moreover, high EGFR expression is associated with chemoresistance to two different DRD2 antagonists, haloperidol and ONC201, in a panel of GBM patient-derived xenografts (PDXs) tested both *in vitro* and *in vivo*. These findings indicate that constitutive activation of EGFR signaling, through ectopic overexpression of a EGFRvIII mutant, confers resistance to DRD2 antagonists. Notably, a statistically significant inverse correlation between EGFR expression and ONC201 efficacy was reported in a phase II GBM trial (n = 15), suggesting that EGFR can serve as a chemo-predictive biomarker of sensitivity to DRD2 inhibition.

In contrast, others have reported haloperidol possesses pro-angiogeneticas well as neuroprotective effects. For example, chronic exposure of up to 72 h to relatively low concentrations of haloperidol (5 $\mu$M) induces upregulation of expression and secretion of BDNF and VEGF from the human GBM cell line T98-G [148,149].

Many of the anticancer effects caused by haloperidol *in vitro* can only be observed at high concentrations, which are also likely to promote off-target receptor engagement. Therefore, further preclinical investigation on the potential repurposing of haloperidol is warranted.

## 4. Metabolic Drugs

Metabolic reprogramming is a hallmark of cancer by promoting uncontrolled cell proliferation and increased survival [150–152]. Spurred on by the discovery of the interplay between the metabolic and epigenetic landscapes, metabolic alterations are now widely recognized as onco-requisite factors in pediatric hindbrain tumors [153]. Therefore, non-oncology drugs that target cellular metabolism are attractive therapeutic candidates for infants with PFA and DMG [154].

PFA tumors and patient-derived cells display enhanced glycolysis and tricarboxylic acid (TCA) cycle activity as well as global loss of H3K27 trimethylation [155, 156]. Glucose dependency and upregulation of glycolytic and hypoxia-related programs is a hallmark of PFA with respect to normal brain and other EPN groups. This specific metabolic landscape reshapes the epigenome of PFA tumors through diminished methylation as well as increased demethylation and acetylation at H3K27. Mechanistically, EZHIP over-expression blocks PRC2-mediated H3K27 trimethylation, whereas H3K27 hyperacetylation and hypomethylation are maintained by an hypoxia-induced epigenetic mechanism that involves high levels of acetyl-CoA and $\alpha$-ketoglutarate ($\alpha$-KG)-mediated activation of H3K27 histone demethylases KDM6A and KDM6B [156]. A similar link between metabolism and epigenome occurs in DMG H3-altered tumors through the H3K27M oncohistone. Specifically, with respect to histone H3 wild-type DMG, DMG H3-altered tumors display upregulation of key genes involved in glycolysis and TCA cycle metabolism and this parallels an altered metabolite profile, exhibiting increased $\alpha$-KG concentration that enables maintenance of H3K27me3 hypomethylation [157]. Interestingly, *in vivo* magnetic resonance spectroscopy reveals higher concentrations of glutamate and citrate in both PFA and DMG H3-altered patients when compared to PFB and H3 wild-type DMG patients, respectively. Consistently, blocking $\alpha$-KG production by glutaminolysis inhibitors hampers growth of PFA and DMG H3-altered cells, and this effect can be rescued by exogenous $\alpha$-KG.

Tumor metabolism is a principal driver of EPN heterogeneity [158]. Single cell-transcriptomics has provided evidence that each EPN subgroup has unique metabolic programs. PFA subtype shows higher metabolic activity compared to other subgroups, allowing for increased metabolic adaptation to different environmental contexts and this correlates with PFA's clinically aggressive behavior. Among the metabolic pathways, mitochondrial oxidative phosphorylation (OXPHOS) is a major contributor to EPN heterogeneity within tumor subgroups and non-neoplastic cells of the same cell type. Mitochondrial dysfunction has also been implicated in pediatric HGG, as these tumors commonly display a significantly reduced mitochondrial (mt)DNA copy number with respect to normal brain and glycolytic phenotype. In pediatric patient-derived HGG models, depletion of mitochondrial DNA (mtDNA) leads to enhanced cell migration and invasion, therapeutic resistance, and *in vivo* tumorigenicity. Shifting glucose metabolism from glycolysis to mitochondrial oxidation via AMPK activation, or PDK inhibition, restores mtDNA abundance and induces growth inhibition and apoptosis, thus paving the way for translation into clinical studies [159].

Although a number of studies suggest preclinical efficacy of targeting cancer metabolism, and a few trials have been completed or initiated to explore the clinical benefit of metabolic drugs or metabolic treatment in adult CNS tumors [160], only metformin (MET) is currently under evaluation in children with CNS tumors.

### *4.1 Metformin*

#### 4.1.1 Preclinical Studies

MET is a veteran, first-line therapy for patients with Type 2 Diabetes (T2D), shows a very favorable safety profile, simplicity of administration [161,162], and high blood brain barrier penetrability [163]. The first hints to an anticancer activity associated with MET stemmed from epidemiological studies that indicated an inverse relationship between treatment of diabetic patients with MET at standard clinical doses and the incidence of cancer [164,165] or overall cancer mortality rates [166]. A recent systematic review and meta-analysis of cancer outcomes in MET users demonstrated that MET could be a useful adjuvant agent in patients with colorectal and prostate cancer [166]. By contrast, several studies failed to show an improved survival in diabetic women with breast cancer as a result of MET treatment [161]. Conclusive evidence in support of the use of MET as an add-on drug in anticancer therapy is still lacking, perhaps because most epidemiological studies are retrospective and present methodological limitations. This view strongly advocates for randomized controlled trials (RCTs) to investigate dose, duration, and efficacy of MET treatment in cancer patients [166].

The antineoplastic properties of MET are mediated by both a direct action on cancer cells and indirect effects on the TME [167,168]. Primarily, MET affects tumor cell metabolism by the downregulation of the mTOR pathway [169], and the blockade of mitochondrial complex 1 that results in decreased ATP production and a consequent energetic stress [170]. It has been shown that MET exerts dual and opposing roles on mitochondrial respiration, stimulating mitochondrial biogenesis at pharmacological doses, or reducing it through decreasing adenine nucleotide levels at supra-pharmacological levels [171]. Indirect effects of MET on host metabolism are regulated

by AMPK-dependent inhibition of hepatic gluconeogenesis and subsequent reductions in blood glucose and insulin. In addition, MET affects the TME by virtue of its anti-inflammatory [166] and anti-angiogenetic properties [167].

Preclinical studies conducted *in vitro* and *in vivo* have shown antineoplastic MET activity in models of different types of cancer [151,168]. In glioma models, MET inhibits cell proliferation and invasiveness, induces apoptosis, autophagy, and GBM stem cell differentiation [167,168]. However, the doses used in these preclinical investigations of up to more than 10,000 mg/L, are generally far above the plasma levels achievable in the clinic (0.465–2.5 mg/L) [172]. Recently, exosome-mediated co-delivery of MET and cPLA2 siRNA to target mitochondrial and phospholipid metabolism has shown promise in treatment of GBM PDXs and this combination exerted a significant increase in survival [173]. Therefore, potential use of MET as an add-on drug in glioma therapy, also in virtue of its radiosensitizing [174,175] and chemotherapy-sensitizing effects [176–178] warrants further preclinical evaluation.

In PFA tumors and cell lines with EZHIP overexpression, an enhanced glycolytic phenotype is associated with H3K27 acetylation enrichment at hexokinase-2, pyruvate dehydrogenase, and AMPKa-2 [156]. MET treatment reduces EZHIP expression while increasing H3K27me3, suppressing TCA cycle metabolism, and limiting tumor growth *in vitro* and in patient-derived animal models as well as sensitizing cells to the antitumoral effects of the HDAC inhibitor panobinostat [156]. Interestingly, sensitivity to MET appears to be dependent on EZHIP expression and shows reduced sensitivity in neural stem cells expressing catalytically inactive EZHIP. Together these data suggest that EZHIP can epigenetically rewire metabolic pathways in therapeutically-targetable PFA tumors. A possible limitation of the study is that MET exerts growth impairment of EPN cells in cultures and mouse models at supra-physiological concentrations that are generally thought to be unachievable in patients.

In DIPG models, dual targeting of mitochondrial function by a combination of MET and dichloroacetate synergistically suppresses cell proliferation through ROS-mediated DNA damage and apoptosis, and promotes radiosensitization both *in vitro* and *in vivo* [159], similarly to what has been reported in GBM models [179].

#### 4.1.2 Clinical Studies

MET is currently under investigation in clinical trials of a large array of human malignancies, with more than 60 studies currently active. Importantly, the maximal daily dose of oral MET administered in the majority of these trials is between 1000 and 2000 mg/day (median 1700 mg) and that this is within the established dosing range used to treat patients with T2D [180].

A number of studies have addressed how MET influences progression of CNS tumors, however, discrepancies in these data warrant further observation. In a matched case-control analysis, a negative relationship was observed between long-term and poorly controlled diabetes and the risk of glioma, that this effect was stronger in men and older individuals, and that antidiabetic treatment was unrelated to risk [181]. One of the first studies to examine the anti-tumor activity of MET in primary GBM reported a statistically significant longer PFS in diabetic patients with MET therapy when compared to other diabetic patients (10.13 *vs* 4.67 months; $p = 0.043$) and other patients without diabetes (10.13 *vs* 6.7 months; $p = 0.018$) [182]. However, a seminal retrospective analysis on 1093 glioma patients found a favorable relationship between MET intake and OS and PFS in patients with grade III gliomas, but not grade IV gliomas [183]. Conversely, MET as monotherapy, or in combination with other drugs, yielded no improvement in life expectancy [184] in a pooled analysis of MET use and outcomes in 1731 individuals with newly diagnosed GBM from three randomized trials.

A dozen clinical trials focused on the use of MET in patients with CNS tumors are in the recruiting phase (Table 3, Ref. [185–188]). A phase I factorial study (NCT01430351) examined the tolerability of TMZ in various combinations with three repurposed agents, specifically, MET, mefloquine, and memantine after completion of standard of care for GBM. Although an encouraging 2-year survival rate of 43% was observed, the trial was not sufficiently powered to evaluate the efficacy of interventions [185]. The clinical benefits of MET as an add-on agent to radiotherapy and chemotherapy has been assessed in a limited number of Phase II clinical trials in patients with newly diagnosed or progressive GBM. Based on encouraging preclinical results demonstrating that the combination of MET and TMZ yields superior antitumoral activity in GBM cells and tumors over monotherapy using any single agent [177], the efficacy and safety of low dose TMZ with or without MET were compared in patients with non-responsive GBM (NCT03243851). However, no results are currently available for this study. The addition of MET to TMZ and hypofractionated, accelerated radiotherapy (NCT02780024; M-HARTT STUDY) for the treatment of GBM patients was well tolerated and safe, and favorable outcomes were observed in those patients with low methylation levels of MGMT [186].

An ongoing multicentric Phase II study (NCT04945148; OPTIMUM) is aimed at evaluating the effect of MET to target OXPHOS in association with the standard first-line treatment with RT and TMZ in participants with GBM with wild-type IDH. This tailored approach is based on the observation that GBM with wild-type IDH present a unique and homogenous energetic metabolism that is specifically dependent on OXPHOS, and likely suppressed by MET, an oral inhibitor of mitochondrial complex 1. Newly diagnosed wild-type IDH GBM patients with the OXPHOS+ signature will

be enrolled in the trial, with the major outcomes to be measured being PFS, OS and ORR.

Two ongoing pilot Phase II clinical studies (NCT04691960; NCT05183204) intend to evaluate the feasibility and activity of MET and a ketogenic diet (KD), a high fat, low carbohydrate diet, in the treatment of HGG. Because glucose is thought to be a driver of tumor growth, lowering blood glucose levels by KD and MET are hypothesized to have therapeutic potential in GBM. A previous safety study (NCT02149459) [187] found that combined metabolic therapy and RT in adult patients with WHO III and WHO IV HGG was well tolerated.

To our knowledge, no work on the cancer-related properties of MET in pediatric patients with CNS tumors has been published. In a pilot precision medicine study by Mueller and colleagues [84], MET was used in a 25 month old boy with H3 wild-type anaplastic astrocytoma to target IGF1R overexpression, in combination with MBZ, etoposide and carboplatin. This child progressed at 27.4 months from initiation of on-study therapy.

There is only one active trial on MET in children with primary CNS tumors and other solid tumors (NCT01528046). This study is aimed at analyzing the safety of MET as an adjuvant agent in a backbone regimen of vincristine, irinotecan, and TMZ, and as a secondary endpoint, MET antitumor activity.

Two other trials are focused on examining the potential of MET to promote brain repair in children treated with cranial radiation for MB. This study is based on preclinical observations in rodents where MET promotes neurogenesis and prevents brain injury by fostering recovery of sensorymotor and cognitive function [188]. In mice with transient middle cerebral artery occlusion, MET rescue of BBB disruption is mechanistically associated with a decrease in neutrophil infiltration, cytokine expression, endothelial injury, and an enhancement of neurobehavioral outcomes [189]. Beside stroke therapy, these MET effects may also counteract the pro-inflammatory and pro-angiogenetic microenvironment that promotes neuro-oncogenesis. Indeed, the use of MET is associated with better performance than placebo in tests of declarative and working memory in 24 long-term survivors of pediatric MB (NCT02040376) [188]. Based on these encouraging results, a multicenter phase III trial (NCT05230758) is currently investigating the effects of MET on cognitive function and the brain in an estimated population of 140 children treated for CNS tumors.

## 5. Anti-inflammatory Drugs

Inflammation plays a crucial role in the process of tumorigenesis, and either supports or suppresses all stages of tumor development through modulation of critical crosstalk between tumor cells and the inflammatory TME [190].

Pediatric CNS tumors appear to have neither high immunogenicity nor highly inflammatory immune microenvironment unlike their adult counterparts [191,192] and, therefore, are commonly viewed as representing immunologically "cold" tumors. Pilocytic astrocytoma (PA) and EPN show significantly higher levels of infiltrating myeloid and lymphoid cells when compared to higher grade lesions such as GBM and MB [193]. In addition, PA and EPN convey a more activated myeloid phenotype [193]. Inflammatory response has been identified as a predominant molecular signature of PFA [194]. With respect to PFB, PFA tumors promote an immunosuppressive TME through secretion of various cytokines such as IL-6 [194], IL-16, and IL-1$\beta$ [195]. These molecules activate tumor infiltrating myeloid cells and microglia through a paracrine mechanism. Primary DMGs display a largely non-inflammatory TME, defined by minimal T cell infiltrate, low to absent expression of chemokines and cytokines, and non-inflammatory phenotype of tumor-associated microglia/macrophages [196–198]. However, recent evidence shows that DMGs typically display higher leukocyte chemo-attractant expression than other pediatric HGGs [199]. In silico-analysis of immune cell infiltration across pediatric CNS tumors identifies three broad clusters associated with tumor types and subtypes and are correlated with prognosis [200], potentially informing future immunotherapeutic options.

Regulation of inflammation is increasingly emerging as an actionable strategy for the prevention and treatment of cancer. A number of clinical trials exploiting immunotherapies with targeted mechanisms are currently under way in pediatric CNS tumors [199]. However, in the present review we will only focus on the paradigmatic nonsteroidal anti-inflammatory drug celecoxib, a non-specific agent that targets chronic inflammation.

### *5.1 Celecoxib*

#### 5.1.1 Preclinical Studies

Celecoxib is a potent selective cyclooxygenase-2 (COX-2) inhibitor that functions by blocking the COX-2/prostaglandin E2 (PGE2) signal axis [201]. COX-2 catalyzes the rate-limiting step in the conversion of arachidonic acid to PGE2, that has been found to promote tumor development by exerting pro-survival, pro-angiogenetic, and proliferative effects as well as maintaining an inflammatory state that helps to escape immunity [202]. COX-2 is overexpressed in many tumors, is found in greater abundance in HGG than either LGG or normal brain tissue, and possesses a reverse relationship with patient prognosis [203].

In addition to its action on the immune/inflammatory and metabolism TMEs [45], celecoxib has emerged as a potent antineoplastic in different malignancies, including glioma [190,204]. Its anti-proliferative and anti-metastatic properties are linked to its ability to affect signaling pathways crucial in oncogenesis such as AKT, NF-$\kappa$B, STAT3, and MMP. In addition, it also acts in a COX-2 independent manner as a "mitocan" and "pro-oxidant agent" in environ-

**Table 4. Most relevant ongoing studies registered at Clinicaltrials.Gov (current as of 18 September 2022) investigating celecoxib as a repurposed drug against adult and pediatric CNS tumors.**

| NCT | Official title | Conditions | Phase | Status | Other therapeutic agents | Population | Dates | Outcome measures | Results |
|---|---|---|---|---|---|---|---|---|---|
| NCT00047294 | Phase II Study Of TMZ, Thalidomide And Celecoxib In Patients With Newly Diagnosed GBM In The Post-Radiation Setting | GBM | II | Completed | TMZ, Thalidomide | 55 patients; 18–120 yr. | Study Start: April 2001; Last Update Posted: July 11, 2017 | PFS; OS | [220] |
| NCT00112502 | A Randomized, Factorial-Design, Phase II Trial of TMZ Alone and in Combination With Possible Permutations of Thalidomide, Isotretinoin and/or Celecoxib as Post-Radiation Adjuvant Therapy of GBM | Recurrent/progressive GBM | II | Completed | isotretinoin, TMZ, thalidomide | 178 patients; age $\geq$18 yr. | Study Start: September 2005; Last Update Posted: October 18, 2021 | Median PFS; Median OS | [221,222] |
| NCT00504660 | Combination of 6-Thioguanine, Capecitabine, Celecoxib and TMZ or CCNU for Recurrent Anaplastic Glioma and GBM | HGG | II | Completed | 6-TG, Capecitabine, TMZ or CCNU, Thalidomide | 75 patients; age $\geq$18 yr. | Study Start: September 2003; Last Update Posted: January 11, 2012 | 12 months PFS for anaplastic glioma; 6 months PFS for GBM | [223] |
| NCT02770378 | A Proof-of-concept Clinical Trial Assessing the Safety of the Coordinated Undermining of Survival Paths by 9 Repurposed Drugs Combined With Metronomic TMZ (CUSP9v3 Treatment Protocol) for Recurrent GBM | GBM | I, II | Completed | TMZ, Aprepitant, Minocycline, Disulfiram, Sertraline, Captopril, Itraconazole, Ritonavir, Auranofin | 10 patients; age $\geq$18 yr. | Study Start: November 2016; Last Update Posted: October 5, 2021 | DLT; OS; PFS | [224,225] |
| NCT00165451 | Anti-Angiogenic Chemotherapy: A Phase II Trial of Thalidomide, Celecoxib, Etoposide and Cyclophosphamide in Patients With Relapsed or Progressive Cancer | Neoplasms (including EPN, DIPG) | II | Completed | Thalidomide, Etoposide, Cyclophosphamide | 20 patients; 1–21 yr. | Study Start: June 2001; Last Update Posted: July 7, 2011 | Feasibility; Adverse effects; Biological activity | [226] |
| NCT01756989 | ANGIOCOMB Antiangiogenic Therapy for Pediatric Patients With Diffuse Brain Stem and Thalamic Tumors | BSG | II | Completed | Thalidomide, Etoposide | 50 patients; 1–16 yr. | Study Start: January 2005; Last Update Posted: May 8, 2014 | Survival | [227,228] |

**Table 4. Continued.**

| NCT | Official title | Conditions | Phase | Status | Other therapeutic agents | Population | Dates | Outcome measures | Results |
|---|---|---|---|---|---|---|---|---|---|
| NCT01285817 | A Phase II Clinical Trial Using Metronomic Oral Low-dose Cyclophosphamide Alternating With Low-dose Oral Methotrexate With Continuous Celecoxib and Weekly Vinblastine in Children and Adolescents With Relapsed or Progressing Solid Tumors | Solid tumors, CNS tumors (EPN, HGG, LGG) | II | Unknown | Vinblastine, Methotrexate, Cyclophosphamide | 90 patients; 4–21 yr. | Study Start: November 2010; Last Update Posted: March 2016 | PFS; RR | [229,230] |
| NCT01331135 | Sirolimus in Combination With Metronomic Therapy in Children With Recurrent and Refractory Solid Tumors: A Phase I Study | Solid tumors, CNS tumors | I | Completed | Sirolimus, Etoposide, Cyclophosphamide | 18 patients; 1–30 yr. | Study Start: April 2011; Last Update Posted: August 9, 2017 | RR (CR, PR, SD); Adverse events | [231] |
| NCT01356290 | A Phase II Study of Metronomic and Targeted Anti-angiogenesis Therapy for Children With Recurrent/Progressive MB, EPN and ATR | MB, EPN, ATRT | II | Recruiting | Bevacizumab, Thalidomide, Fenofibric acid, Etoposide, Cyclophosphamide, Cytarabine | 100 patients; up to 19 yr. | Study Start: April 2014; Last Update Posted: April 8, 2022 | RR; OS; PFS; Toxicity feasibility; QoL | |
| NCT02574728 | AflacST1502: A Phase II Study of Sirolimus in Combination With Metronomic Chemotherapy in Children With Recurrent and/or Refractory Solid and CNS Tumors | Solid tumors, CNS tumors | II | Recruiting | Sirolimus, Etoposide, Cyclophosphamide | 60 patients; 1–30 yr. | Study Start: June 2015; Last Update Posted: November 1, 2021 | RR (CR, PR, SD); Adverse events | |

Trials against adult and pediatric CNS tumors are reported in the upper and lower part of the table (separated by a bold line), respectively. Tumors of the main topic of this review are in bold. ATRT, atypical teratoid rhabdoid tumor; CCNU, lomustine; CNS, central nervous system; CR, complete remission; DLT, dose limiting toxicity; EPN, ependymoma; HGG, high grade glioma; GBM, glioblastoma; LGG, low grade glioma; MB, medulloblastoma; OS, overall survival; PR, partial response; PFS, progression-free survival; QoL, quality of control; RR, response rate; SD, stable disease; 6-TG, 6-thioguanine; TMZ, temozolomide; yr., years.

mental contexts of low oxygen or glucose [201,205]. As a mitocan, celecoxib impairs mitochondrial biogenesis in tumor cells. As a "pro-oxidant agent", it inhibits the OXPHOS by disturbing electron transfer within the respiratory cycle, which leads to reactive oxygen species (ROS) overload and triggering mitochondrial ROS-induced apoptosis [201,206]. Celecoxib also targets cancer stem cells by down-regulating WNT signaling, inhibiting EMT, and decreasing angiogenetic and growth promoting factors such as VEGF, FGF, and MMPs that restrain angiogenesis, metastasis, and stemness [201,205,207]. Computational proteochemometric analysis found that celecoxib ranked among top hits for binding to CDH11 [53], an adhesion protein that, in glioma, regulates cell migration and survival *in vivo* [208].

The therapeutic dose of celecoxib used to suppress inflammation is 300 mg, with plasma levels of approximately 2–7 $\mu$M, whereas that for cancer this does is 400 mg twice a day [209]. Half-maximal inhibitory concentrations for COX-2 are in the submicromolar range (0.29–0.87 $\mu$M) [201], whereas the IC50 values of celecoxib as an anticancer agent range between 20 and 70 $\mu$M across all tumor cell lines tested.

In preclinical models of glioma, celecoxib has proved to be the most potent selective COX-2 inhibitor to impair cell proliferation *in vitro*, although the high effective doses of 25–100 $\mu$M suggest COX-2-independent pathways [210–212]. A variety of mechanisms underlie the anti-proliferative effects of celecoxib, including proteosomal degradation of survivin [211], p53-dependent G1 cell cycle arrest [213], potent reduction of WNT/$\beta$-catenin/Tcf [212,214] and STAT3 signaling pathways [215], and down-regulation of the CCL2/CCR2 and CXCL10/CXCR3 signaling axes [216]. In addition, celecoxib reduces self-renewal capacity and increases apoptosis in GBM [217] and medulloblastoma-derived stem cells [215]. Recent evidence implicates COX-2 as a target of miRNA-128 through direct binding to the 3′UTR of the COX-2 transcript [218]. Additionally, a miR-128 inhibitor significantly increased COX-2 mRNA expression and proliferation of glioma cells.

There is scarce literature on celecoxib as single agent in pediatric CNS tumor models, and the available evidence is mostly restricted to MB, where celecoxib hampers tumor growth *in vitro* and *in vivo* [215,219]. Of note, in the work of Lin and coworkers conducted in DIPG, celecoxib was found to be among the 371-potency-selected agents uncovered in a panel of 2706 approved and investigational drugs [66].

#### 5.1.2 Clinical Studies

Celecoxib has been included in a large number of clinical trials in CNS tumors (Table 4, Ref. [220–231]) due to a favorable side effect profile, antiangiogenic properties, tumor sensitization to chemotherapy, targeted therapy, and radiation therapy [215,232,233].

In the adult setting, a phase II clinical trial (NCT00047294) enrolling patients with newly diagnosed GBM examined the addition of metronomic celecoxib and thalidomide as antiangiogenic agents to adjuvant, post-irradiation TMZ. Although the three-drug combination was well tolerated, no significant survival advantage was reported [220]. The outcome in terms of PFS and OS was examined in the adjuvant setting following chemoradiation in GBM patients (NCT00112502). In this factorial study, dose-dense (dd)-TMZ was used as the backbone for combinations with one or more 3 chemotherapy agents that included celecoxib. Although the multiple cytostatic agent regimen was safe and generally well tolerated in combination with dd-TMZ [221], a follow-up study failed to demonstrate superiority of any of the combinations *vs* dd-TMZ alone [222].

The combination of celecoxib with 6-thioguanine and capecitabine plus lomustine or TMZ (NCT00504660) [223] failed to achieve any marked improvement in PFS or OS over earlier studies on recurrent GBM. Conversely, this multidrug regimen proved to be promising and well tolerated for patients with high-grade anaplastic glioma. These patients fared better than those treated only with an alkylating agent, although the number of patients did not reach the preset statistical certainty. In this group of patients, PFS-12 was 44% (95% CI 29–67%) *vs* 21–33% of the most effective therapies in combined analysis of 16 previous studies.

Celecoxib has also been included in the polypharmacological regimen CUSP9v3, that uses nine repurposed non-oncological BBB-penetrating drugs in combination with metronomic TMZ in recurrent/progressive GBM (NCT02770378) [224,225]. Preclinical investigation showed that the 9-drug combination exhibited marked cytocidal, anti-proliferative, and antimigratory actions in GBM stem cells and cell lines [209,224]. It should be noted that seven of the drugs in this multi-agent strategy, including celecoxib, exerted limited anti-tumor effects within the dose range covering clinically achievable concentrations [225]. In a previous pilot study on 9 GBM patients, the CUSP9v3 approach was shown to be generally safe and well-tolerated, showing potential positive effects that require replication in larger trials [225].

In pediatric CNS tumors, different trials have reported a low toxicity profile of various multi-agent regimens that combine chemotherapy with various ancillary agents, including celecoxib, to both target tumor cells and modulate the TME [234,235]. A pilot study, NCT00165451, tested the feasibility of a four-agent antiangiogenic chemotherapy regimen with continuous oral celecoxib in heavily pretreated children with recurrent or progressive cancers, including EPN and DIPG [226]. This drug combination was well tolerated and associated with a prolonged disease-free status, mostly in patients with EPN, although the study was not powered for disease-specific efficacy evaluation. A similar five-drug antiangiogenic chemotherapy regimen

that also included metronomic fenofibrate, was trialed on 97 children with hematologic and solid tumors, including HGG, LGG, EPN and MB [236]. This trial confirmed acceptable toxicity in the majority of patients and noted clinical benefit only in EPN and LGG. In a phase II pilot study, NCT01756989, the combination of an antiangiogenic triple medication consisting of thalidomide, celecoxib, and etoposide was used in a metronomic fashion after local RT with topotecan in 8 children with inoperable DIPG (ANGIOCOMB protocol) [237]. This protocol was easily administered and well tolerated and associated with improved quality of life (QOL) for the patients over historical controls (eight patients with DIPG, who received RT only). In a larger cohort of patients (n = 41) the ANGIOCOMB protocol achieved a 12 and 24 month OS of 61% and 17%, with a prolonged QOL [227]. However, these results were not confirmed in another group of 17 children with refractory or high-risk malignancies where Grade III-V adverse events occurred in 76% of the patients [228].

Phase II multicentric trials focusing on the clinical efficacy of combinatorial regimens that also include celecoxib have been completed, or in the recruiting phase, in pediatric patients with recurrent/refractory solid and CNS tumors. The trial NCT01285817 examined the safety profile and anti-neoplastic efficacy of the SFCE METRO-01 regimen, based on a combination of cyclophosphamide, methotrexate, vinblastine, and continuous celecoxib. The cohort included 44 children with extra-cerebral tumors [229] and 29 children with CNS tumors, including EPN, HGG, and LGG [230]. Confirming a previous toxicity profile indicated in a pilot study in 16 children [234], the regimen was well tolerated, although it proved to be active only in patients with LGG with a potential clinical benefit in patients with neuroblastomas. The trial NCT01356290 (Metronomic and Targeted Anti-angiogenesis Therapy for Children With Recurrent/Progressive Medulloblastoma, Ependymoma and ATRT; MEMMAT), focused on testing the survival benefits of the antiangiogenic multidrug combination bevacizumab, thalidomide, celecoxib, fenofibrate, etoposide, and cyclophosphamide that was augmented with alternating courses of intraventricular etoposide and cytarabine in patients with recurrent EPN, MB or atypical teratoid/rhabdoid tumor (AT/RT). The results of this study are currently being evaluated. This trial was based on preliminary encouraging results reported in a cohort of 16 children with recurrent embryonal tumors [235], where treatment with this drug combination showed to be particularly beneficial in patients with MB, with both OS and EFS after 12 months of 85.7 $\pm$ 13%, *vs* the historical data of only 23–30%. By contrast, all patients with CNS PNET had died by 12 months. Very recently, a long-term follow-up of 29 patients with recurrent medulloblastoma prospectively treated according to the MEMMAT strategy between 2006 and 2016 has been published [238]. This study documented improved EFS and OS with respect to previously published series, with 9/29 patients still alive as of 07/2022. An ongoing phase II trial, NCT02574728, is assessing the response to treatment of daily celecoxib and sirolimus, a potent immunosuppressive agent, in combination with the common chemotherapy drugs etoposide, cyclophosphamide in patients with solid tumors, including EPN. A preliminary Phase I study, NCT01331135, aimed at determining the MTD, toxicities, and response to this treatment in a small cohort of children and young adults found that the combination was well tolerated [231]. Moreover, half of the patients experienced stable disease, some for extended durations, and one patient showed a partial response.

## 6. Conclusions

Off-label drug use in pediatric neuro-oncology must meet several challenges. As is the case for drug development in general, many non-cancer medicines have been repositioned in pediatric oncology based on epidemiological observations, case reports, case series, or single arm phase 2 studies compared to historical controls performed in adults [239]. Because these drugs show limited single-agent activity in cancer, their use in the clinic carries a substantial risk of failure. Moreover, assessment is ethically controversial in RCTs [240] or, alternatively, should require large and costly sample sizes to detect marginal benefits [241]. Given the issues with poor clinical trial accrual and rarity of many CNS cancers, RCTs may be impractical, even if enrolling different cancer types without molecular classification and risk stratification.

Anticancer efficacy in preclinical models is often achieved at supra-physiological doses as monotherapy, although clinically significant doses in rational combinatorial approaches have been shown to overcome cancer cell refractoriness. In clinics, non-chemotherapy drugs are often used in synergy with chemo/RT with the dual scopes of hitting multiple targets in tumor cells, and modulating tumor-microenvironmental contexts with cross-over effects. On the other hand, multidrug regimens may expose patients to undesirable toxicities in spite of limited clinical improvement, and, from a heuristic approach, factorial studies with agents that display limited individual efficaciousness may prove to be considerably complex [242–245]. However, incidental cases outlining encouraging results, even when these medicines have been administered as monotherapy, have been reported [127].

Recently, precision medicine has leveraged computational multiomics-based approaches for biomarker-guided drug selection, and interestingly some of the agents of choice for personalized treatments were used in an off-label fashion. In the NCT02274987 trial, five out of 17 FDA-approved drugs for children with DIPG were non-oncological medicines, namely MBZ, MET, propranolol, sertraline, and VPA, each of them known to target specific oncoproteins [84]. Similarly, VPA and MBZ were included in backbone therapy to molecularly-targeted agents for per-

sonalized treatment in another cohort of 9 children with DIPG [130].

Pluralistic evidence based on range of sources, including *in silico* bioinformatics, preclinical and clinical studies, QOL data, and real world evidence [246] can help drug repurposing efforts that corroborate, or even substitute for, traditional RCTs whenever impossible or even unethical [240,247]. The limited therapeutic landscape and dismal prognosis for some pediatric CNS tumors, including PF-EPN and H3K27M-DMG, call for development of new treatment options and the pursuing of all practicable strategies to urgently meet the unmet needs of such dreadful pediatric diseases.

## Abbreviations

$\alpha$-KG, $\alpha$-ketoglutarate; AMPA, $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AMPK, 5' AMP-activated protein kinase; BDNF, brain-derived neurotrophic factor; BSG, brain stem glioma; CDH11, cadherin 11; ClpP, caseinolytic protease P; CNS, central nervous system; COX-2, cyclooxygenase 2; CR, complete remission; DA, dopamine; DIPG, diffuse intrinsic pontine glioma; DMG, diffuse midline glioma; DRD2, dopamine receptor; D2DR, dopamine receptor; EFS, event free survival; EMT, epithelial-to-mesenchymal transition; EPN, ependymoma; FGF, fibroblast growth factor; GABA, gamma-aminobutyric acid; GSC, glioblastoma stem cell; HDAC, histone deacetylase; HGG, high-grade glioma; KD, ketogenic diet; MAPK, mitogen-activated protein kinase; MB, medulloblastoma; MBZ, mebendazole; MET, metformin; MMP, Matrix metalloproteinases; MTD, maximum tolerated dose; mtDNA, mitochondrial DNA; ORR; overall response rate; OXPHOS, oxidative phosphorylation; PBMC, peripheral blood mononuclear cell; PDK, pyruvate dehydrogenase kinase; PFS, progression free survival; PGE2, prostaglandin E2; PR, partial response; QOF, quality of life; RR, response rate; RT, radiation therapy; SD, stable disease; T2D, type 2 diabetes; TCA, tricarboxylic acid; TME, tumor microenvironment; TMZ, temozolomide; TRAIL, tumor necrosis factor-related apoptosis inducing ligand; VCR, vincristine; VEGF, vascular endothelial growth factor; VPA, valproic acid.

## Author Contributions

TS, and AR designed the study. TS, DL, PN and AS collected and analyzed the literatures. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

## Ethics Approval and Consent to Participate

Not applicable.


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## Conflict of Interest

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