


REVIEW

Chemotherapy in pediatric brain tumor and the challenge of the blood–brain barrier

Johid Reza Malik¹  | Anthony T. Podany^{1,2}  | Parvez Khan³  |
Christopher L. Shaffer²  | Jawed A. Siddiqui³  | Janina Baranowska-Kortylewicz⁴  |
Jennifer Le⁵  | Courtney V. Fletcher¹  | Sadia Afruz Ether¹  | Sean N. Avedissian^{1,2} 

¹Antiviral Pharmacology Laboratory, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA

²Pediatric Clinical Pharmacology Program, Child Health Research Institute, University of Nebraska Medical Center, Omaha, Nebraska, USA

³Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska, USA

⁴Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA

⁵University of California San Diego Skaggs School of Pharmacy and Pharmaceutical Sciences, San Diego, California, USA

Correspondence

Sean N. Avedissian and Johid Reza Malik, Antiviral Pharmacology Laboratory, University of Nebraska Medical Center, 986145 Nebraska Medical Center, Omaha, NE 68198-6145, USA.
Email: sean.avedissian@unmc.edu and jomalik@unmc.edu

Funding information

National Institute of Allergy and Infectious Diseases, Grant/Award Number: K23 AI134307; National Institute of Mental Health, Grant/Award Number: K23 MH125734

Abstract

Background: Pediatric brain tumors (PBT) stand as the leading cause of cancer-related deaths in children. Chemoradiation protocols have improved survival rates, even for non-resectable tumors. Nonetheless, radiation therapy carries the risk of numerous adverse effects that can have long-lasting, detrimental effects on the quality of life for survivors. The pursuit of chemotherapeutics that could obviate the need for radiotherapy remains ongoing. Several anti-tumor agents, including sunitinib, valproic acid, carboplatin, and panobinostat, have shown effectiveness in various malignancies but have not proven effective in treating PBT. The presence of the blood–brain barrier (BBB) plays a pivotal role in maintaining suboptimal concentrations of anti-cancer drugs in the central nervous system (CNS). Ongoing research aims to modulate the integrity of the BBB to attain clinically effective drug concentrations in the CNS. However, current findings on the interaction of exogenous chemical agents with the BBB remain limited and do not provide a comprehensive explanation for the ineffectiveness of established anti-cancer drugs in PBT.

Methods: We conducted our search for chemotherapeutic agents associated with the blood–brain barrier (BBB) using the following keywords: Chemotherapy in Cancer, Chemotherapy in Brain Cancer, Chemotherapy in PBT, BBB Inhibition of Drugs into CNS, Suboptimal Concentration of CNS Drugs, PBT Drugs and BBB, and Potential PBT Drugs. We reviewed each relevant article before compiling the information in our manuscript. For the generation of figures, we utilized BioRender software.

Focus: We focused our article search on chemical agents for PBT and subsequently investigated the role of the BBB in this context. Our search criteria included clinical trials, both randomized and non-randomized studies, preclinical research, review articles, and research papers.

Finding: Our research suggests that, despite the availability of potent chemotherapeutic agents for several types of cancer, the effectiveness of these chemical agents in treating PBT has not been comprehensively explored. Additionally, there is a scarcity of studies examining the role of the BBB in the suboptimal outcomes of PBT treatment, despite the effectiveness of these drugs for other types of tumors.

KEYWORDS

blood–brain barrier, central nervous system, chemotherapy, oncology, pediatric brain tumor

1 | INTRODUCTION

Pediatric brain tumors (PBTs) comprise 25% of all childhood cancers,^{1,2} and are one of the leading causes of cancer-diagnosed death in children.³ From surgical removal of PBT followed by radiation therapy and adjuvant chemotherapy to immunotherapy, PBT treatment has substantially improved throughout the years.^{1,4,5} Improvement in gross total resection (GTR) or subtotal resection (STR) of PBT has resulted in the targeted removal of the tumor and enhanced radiological treatment.⁶ For example, proton beam treatment is one promising radiation therapy as it allows targeted dosing at high levels with reduced surrounding tissue damage.⁷ Unfortunately, despite advancements, radiation therapy can cause hearing loss, impaired neurocognition, and alteration in neuroendocrine function, among other adverse events.^{8–10} The detrimental effect of radiotherapy in the modulation of BBB integrity was examined in immunocompetent and immunocompromised mice, and it was found that 12-h irradiation in immunocompetent mice caused alteration of efflux transporter activity compared to immunocompromised mice indicating a role of proinflammatory molecules in BBB structural changes.¹¹ Similarly, human clinical trials, in vitro, and in vivo studies have shown leaky BBB caused by irradiation of central nervous system (CNS).^{12–14} Cell death augmented by radiotherapy is one of the underlying mechanisms for leakage in the BBB, and one report showed a 15% decline in endothelial cell population post 24-h irradiation with a 25 Gy dose.¹⁵ The reactive oxygen species (ROS) production from irradiation can indirectly damage the BBB by inducing apoptosis which starts as early as 4-h after irradiation, and the effect was observed maximum post 12-h of irradiation.^{16–18} Though several PBTs respond well to radiation, this comes at the cost of potential long-term neurological consequences, particularly a problem in young children with developing brains.¹⁹ Immunotherapy of PBTs is emerging as a novel adjuvant monotherapy in the post-radiotherapy setting, and early clinical trials show overall safety, feasibility, and

survival benefit in patients.²⁰ While it is still progressing, the adverse effects of immunotherapy are a serious concern, with immune-related adverse effects showing up as early as 3 months post-therapy.²¹ Unfortunately, there is limited success with combinations of radiotherapy, neurosurgery, and chemotherapy.^{22–25} Considering the overall side effects of the current therapy to treat PBTs,²⁶ chemotherapy becomes the treatment choice for controlling the residual and micrometastatic tumors that cannot be removed by surgery.²⁷ However, BBB, being important for regulating which molecules can pass from the blood into the brain, can hinder drug penetration leading to suboptimal drug concentrations in the CNS.^{28,29}

In 1979, chemotherapy became part of standard-of-care therapy as an adjuvant to surgery and radiation in PBTs. In patients with medulloblastoma (MB) with or without metastases, the addition of chemotherapy as an adjuvant significantly improved the event-free survival rate up to $86\% \pm 9\%$.¹ Several combinations of anti-tumor drugs have been optimized to a range of 4–9 treatment cycles, depending on the risk assessment of the disease.³⁰ Interestingly, in many cancers, including MB, it has been observed that children tolerate chemotherapy better than adults.³¹ While the exact mechanism of this tolerance is unknown, it is believed to be a combination of altered hepatic metabolism, diminished resistance to treatment regimens, and fewer concurrent disease states as compared to adult patients.³² Nonetheless, chemotherapy causes significant adverse effects, such as post-treatment pancytopenia, encephalopathy, ataxia, and motor weakness, among many other undesirable effects.³³ A systemic approach for chemotherapy involving the evaluation of molecular basis/checkpoints, the related epigenetics, and the genomic level study has increased efficacy and lowered the risk for toxicity in patients.^{34–36} Through the HIT-2000 trial, it was established that a systemic drug regimen in combination with intraventricular methotrexate is better than craniospinal irradiation in children >4 years of age for the treatment of nonmetastatic MB.³⁷ Even for treating high-risk PBTs in children >3 years of age, chemotherapy

can be applied to the radiologically inaccessible residual tumor. In a randomized clinical trial of 261 patients with group 3 MB subtype, carboplatin use during radiotherapy increased survival rate from 54% to 73%.³⁸

Despite the remarkable success of chemotherapy in PBTs, improvements in patient survival remain a significant concern and challenge, and tumor subtype-associated differential success under chemotherapeutic treatment needs to be comprehensively explored.²⁷ This premise requires understanding and characterizing anti-tumor drug pharmacology in all histologically and clinically diverse PBT subtypes. Within the scope of this review, we discuss the most common PBTs and their chemotherapies with an emphasis on the BBB's role in drug delivery and the efficacy of treatment.

2 | PBT AND THE BLOOD-BRAIN BARRIER

Among the different pediatric brain cancers, MB is most prevalent, representing approximately 20% of all brain-related cancers in children.^{39,40} Cerebellum-originated MBs have been found among all ages, but children with a median age of 5 years show the highest incidence of MBs.⁴¹ MBs are the first PBT to have its own Medulloblastoma Advanced Genomics International Consortium (MAGIC), which has provided insight into the molecular basis of MBs, leading to better clinical results.⁴² In 2006, the WHO classified the subgroup of cancers within MBs into four subtypes: Wnt/Integrated (WNT)-activated, Sonic Hedgehog (SHH)-activated, group 3 and group 4 with distinct genetic makeup which is essential for clinical differentiation.^{43,44}

Diffuse intrinsic pontine glioma (DIPG), a high-grade glioma (HGG), is an aggressive PBT with poor survival that accounts for ~75% of brain stem tumors in children.³⁹ The WHO classification for pediatric high-grade gliomas (pHGGs) indicates diffused astrocytoma as grade II, grade III for anaplastic astrocytomas, and grade IV for glioblastomas.⁴⁵ The histone mutations HIST1H3B, H3F3A, and G34 are considered a predominant subgroup in pHGGs. Detailed molecular characterization and epigenetics of pHGGs have been carried out by various researchers.^{46–49} Although there has been progress in understanding the subgroups of HGG, distinguishing it from the adult form of HGG, improvement for pediatric glioma is needed as mortality remains high at 43% for children up to 14 years old with PBT.⁵⁰

Ependymoma is a relatively less common PBT (<https://tumourclassification.iarc.who.int/login?redirecturl=%2Fchapters%2F45>), and constitutes around 10% of the total childhood brain tumors reported.⁵¹ Interestingly,

there seems to be a male predominance among ependymoma cases in patients <5 years of age.^{51,52} A literature review by Sun and colleagues evaluated sex discrepancy in brain tumor biology.⁵³ They found that brain tumors occur more frequently in males compared to females regardless of age, tumor histology, or region of the world. They hypothesized that sexually dimorphic mechanisms might control tumor cell biology, as well as immune and brain microenvironmental responses.⁵³ According to the WHO classification, the subgroup of ependymoma includes subependymoma and maxillary ependymoma (grade I), classic (grade II), and anaplastic (grade III).⁵⁴ Given the intrinsic association of the CNS and the BBB in drug pharmacology, designing successful chemotherapy regimens and understanding the PBT-BBB-drug axis is crucial for maximizing therapeutic effects.

2.1 | Blood-brain barrier

The BBB is a protective vascular barrier keeping the brain safe from the detrimental effect of toxins and pathogens.^{55–57} The structural component of the BBB primarily includes microvascular brain endothelial cells (MBECs) lining the cerebral blood vessels,⁵⁸ pericytes that share the basement membrane with endothelial cells,⁵⁹ and astrocytes with their tendrils for communication with neighboring cells^{60,61} (Figure 1). Expression of tight junction proteins, namely occludins, claudins, junctional adhesion molecules, and cytoplasmic accessory proteins by MBECs, astrocytes, and pericytes play a pivotal role in barrier formation.^{62–64} Despite tight junction formation by peripheral capillary endothelial cells, the TEER (Trans epithelial electrical resistance) value observed is 2-fold less when compared to the BBB, pointing to a bidirectional paracellular transport of molecules across the capillary endothelial cells.^{65,66} The BBB-associated brain endothelial cells are distinct from capillary endothelial cells and exhibit extensive fenestration and enhanced tightness of intercellular junctions with lower pinocytotic function.^{65,67,68} The unique features of the BBB enable ionic homeostasis and optimal nutrition maintenance in the CNS.⁶⁹ There is passive permeability for essential water-soluble nutrients across the BBB, while other nutrients engage with specific transporters for nervous tissue requirements.^{70,71} It is important to understand the specific role played by the BBB in PBTs as the BBB can present physiological obstacles for pharmacologic agents used in the treatment of PBTs.

The constituent cells of the BBB express efflux transporters, including ATP-binding cassette (ABC) proteins P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP). These efflux transporters, as crucial as they are to BBB regulation, can pump out pharmacologically

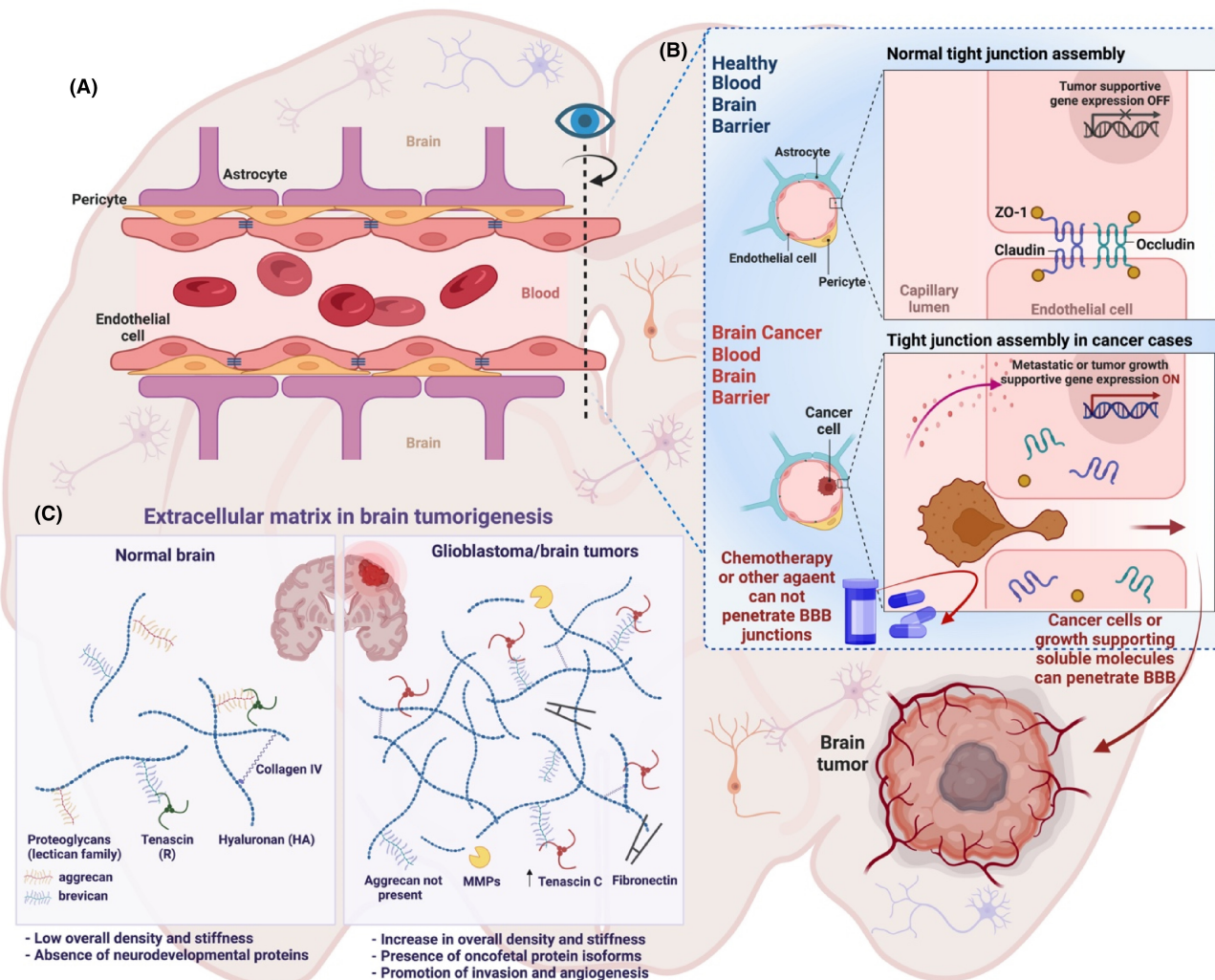


FIGURE 1 Presentation of BBB interplay with PBT, (A) Constituent cells of BBB and the in vivo environment, (B) enlarged part of CNS depicting BBB in healthy brain and brain tumor with the associated BBB inhibition of drug entry, and (C) molecular-level comparison of the normal brain with a brain tumor. BBB, blood–brain-barrier; PBT, pediatric brain tumor; CNS, central nervous system; MMPs, matrix metalloproteases. *Figure was generated utilizing [Biorender.com](https://www.biorender.com).

important molecules from the brain.^{72,73} The critical role of these protein pumps has been shown in knock-out animals, confirming that many small molecules used as drugs are a substrate for these protein pumps and are excluded by the BBB resulting in lower efficacy of the drugs.^{74–76} The integrity of the BBB in the tumor region, also known as blood tumor barrier (BTB), varies based on the type and subtypes of tumor.⁷⁷ Limited studies have shown BBB integrity modulation for different PBTs (Table 1). Midline glioma (DMG) is a subtype of HGGs where the BTB is seen intact, whereas adult glioblastoma has been shown to express a leaky BTB.^{77,78} Likewise, it was demonstrated that there is a substantial difference in the BBB of WNT-activated and SHH-activated MBs subtypes.⁷⁹ The establishment of aberrant vascular networks in the WNT-activated MB impacts paracrine signaling activity, which creates a non-functioning BBB and allows enhanced

chemotherapeutic concentrations as compared to SHH-activated MB. The heterogeneous alteration in the BBB in adult brain tumor and PBTs and their subtypes affect the permeability, bioavailability, and chemotherapeutic response of potential therapeutic chemoagents.^{79,80} Even though molecular identification and targeted therapy for PBTs have come a long way, better strategies are needed to improve drug penetration and thereby the efficacy of current and future therapeutic agents.

To effectively treat MB and the other PBTs, a chemotherapeutic agent must be capable of crossing the BBB to obtain optimal CNS concentrations.^{79,81,82} A drug's molecular size plays a critical role in traversing the BBB. Currently, only 5% of the available drug can pass through the BBB, emphasizing the need to explore and modulate both BBB and therapeutic agents.^{83–85} To achieve desired CNS concentrations of potential anti-tumor agents, it is

TABLE 1 Level of damage to the blood–brain–barrier integrity in different types of PBT*.

PBT type	PBT subtypes with Intact BBB	PBT subtypes with Mild BBB alteration	PBT subtypes with Leaky BBB	References
Medulloblastoma	1. SHH ⁶⁷ 2. Group 4 subtype ²¹⁶	3. Group 3 Subtype ²¹⁶	4. WNT-activated medulloblastoma ⁶⁷	Phoenix 2016 ⁷⁹ , Mo 2002 ²⁴⁰
Glioma	1. DIPG (DMG) ²¹⁸ 2. Periphal glioma ²¹⁷	3. pHGG ²¹⁸	4. Late Glioma or core of Glioma ²¹⁷	Gao 2018 ²⁴¹ , Wei 2002 ²⁴²
Ependymoma*	1. Intracranial ependymoma ²¹⁹ 2. Spinal ependymoma ²¹⁹ (based on ABC transporter expression)	3. Myxopapillary ependymomas ¹⁹⁹ (based on ABC transporter expression)	No data available	Ginguené 2010 ²¹⁴ , Duda-Szymańska ²⁴³

Note: Intact BBB: Healthy and functional BBB with no signs of damage. Mild BBB alteration: Minimum damage to the BBB with little increased porosity. Leaky BBB: BBB with minimum inhibition of molecules across it, a non-functional BBB.

Abbreviations: ABC transporter, ATP-binding cassette transporters; BBB, blood–brain–barrier; DMG, diffuse midline glioma; PBT, pediatric brain tumor; pHGG, pediatric high-grade glioma; SHH, sonic hedgehog; WNT, wingless/integrated.

*There are inconclusive reports of BBB integrity in pediatric ependymomas. Thus, this is hypothesized based on the expression of ABC transporters.

essential to understand the role of the BBB and its modulation for enhanced drug penetration.

3 | CHEMOTHERAPEUTIC TREATMENT OF PEDIATRIC BRAIN TUMORS (PBTs)

3.1 | Medulloblastoma (MB)

Chemotherapeutic agents like cisplatin, carboplatin, lomustine, cyclophosphamide, and vincristine are commonly used in MB treatment^{1,86–90} (Table 2). In a pediatric study, the progression-free survival (PFS) of children with high-risk MB improved from 65% to 86% and 79% for 3 and 5 years, respectively, in those treated with craniospinal irradiation and vincristine.⁸⁸ In other pediatric clinical trials of maintenance chemotherapy, lomustine, cisplatin, and vincristine were used to inhibit the resurrection of the disease.^{87,88,90,91} Patients 3–10 years of age receiving adjuvant therapy with chemotherapy experienced a 96% 2-year survival rate compared to a 59% 2-year survival rate with radiotherapy alone.^{90,91} Furthermore, patients with advanced stages of MB demonstrate greater benefit of adjuvant chemotherapy compared to early stage MB.⁸⁸ Several studies have shown better response to chemotherapy alone for most MB subtypes like desmoplastic, extensive nodular, or classic MB, since it alleviates the use of radiation therapy.^{92–98} Rutkowski et al demonstrated that histopathology analysis was a strong independent prognostic indicator for 8-year event-free survival and overall survival, where de-escalation of chemotherapy may be appropriate in young children with desmoplastic/nodular and extensive nodularity type of MB histopathology.⁹² In a different study, the standard risk of MB showed an expected overall survival of about 85% in patients with craniospinal irradiation followed by adjuvant chemotherapy.^{1,99,100} However, in high-risk MB, this regimen has only a 50% cure rate, where intensive treatment with high-dose chemotherapeutic agents increases the survival from 20% to 40% and 60% to 70%.^{101,102} In this pursuit of chemotherapy in MB, the phase I study of sonidegib (LDE225) on PBT and phase II for relapsed MB exhibited anti-tumor activity for patients with relapsed Hh MB, but it was not active against non-Hh MB.¹⁰³

3.1.1 | Sunitinib

The U.S. Food and Drug Administration (FDA) approved sunitinib (SU11248, Sutent), a tyrosine kinase inhibitor used as a multi-target agent in cancer angiogenesis.¹⁰⁴

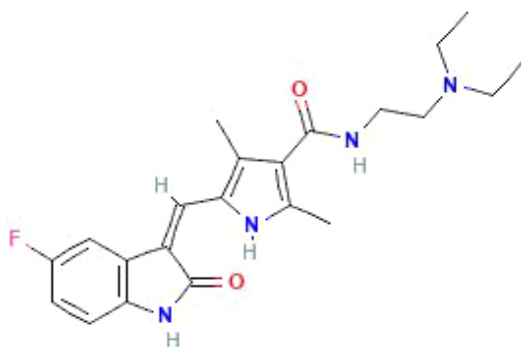
TABLE 2 A summary of important anti-tumor drugs under trial/investigation for PBT treatment*.

Drug/therapy	Condition/ disease	Mechanism of action	Clinical/preclinical trials	Observed side effects	References
Cisplatin	MB	DNA crosslinks/adducts formation	Phase III	Anemia, nephrotoxicity, and excitotoxic neuronal death	Packer 2006 ^{1,87} , Packer 1988 ⁹⁰ , Packer 1994 ⁸⁹ , Sirachainan 2018 ²³⁶
Carboplatin*	MB	DNA alkylating agent	Phase III	Peripheral neuropathy, Low blood cell count, and abnormal magnesium level	
Lomustine	MB	DNA crosslinks and alkylating agent	Phase III	Myelosuppression, ulcers, nephrotoxicity, and poor appetite	
Cyclophosphamide	MB	DNA crosslinks formation	Phase I	Apoptotic and excitotoxic neuronal death, a decline in neurogenesis, cytokine dysregulation, reduced glutathione/glutathione peroxidase	
Vincristine	MB	Inhibitor of mitosis at metaphase by interaction with tubulin	Early phase I	Neuronal apoptosis, blurred or double vision, and drooping eyelids	
Sunitinib*	MB	Tyrosine kinase inhibitor	Phase I clinical trials	Increased liver enzymes and low levels of thyroid hormones	Rock 2007 ¹⁰⁴
Valproic acid*	MB	Enhanced neurotransmission of GABA	Phase II clinical trials	Congenital anomalies, drowsiness, tremors, dysphoria, and thrombocytopenia	Li ¹¹¹
Combination of gemcitabine and ribociclib	MB	CDK4/6 inhibitor	Phase I	Hematologic toxicity, CNS exposure	Pribnow 2022 ²³⁷
TB-403	MB	Neutralizing Ab, Neuropilin1-PIGF signaling inhibitor	Phase I	Vomiting, diarrhea, nausea, fatigue, decreased appetite, and decrease in immune cell count	Saulnier-Sholler 2022 ¹²⁸
Panobinostat*	DIPG	Histone deacetylase inhibitor	Preclinical studies on humans and mouse	Thrombocytopenia, Lymphopenia, neutropenia, low calcium, low albumin, low phosphorus, low hemoglobin, and fatigue	Bradner 2010 ¹⁴⁵ , Grasso 2015 ²³⁸
Difluoromethylornithine (DFMO)*	DIPG	Inhibition of the polyamine synthesis pathway	Preclinical trials	Ototoxicity, gastrointestinal upset, proteinuria, and anemia	Wallace 2003 ¹⁵⁵ , Rea 2004 ¹⁵⁴
Temozolomide*	DIPG	Causes reduction in O ⁶ -methylguanine methyltransferase (MGMT), required for DNA repair, and this reduction in the enzyme results in an increased level of O ⁶ -methylguanine in DNA, leading to a higher cytotoxic effect	Phase III clinical trials	Stomatitis, dyspepsia, hemorrhoids, convulsions, and hemiparesis	Brada 200 ¹⁶⁸ , Yung 2000 ¹⁶⁹ , Yung 1999 ¹⁷⁰
Bevacizumab*	DIPG	Antagonist of VEGF	Phase III clinical trials	Belching, nephrotic syndrome, hypertensive crisis, leukopenia, and proteinuria	Ferrara 2004 ¹⁸⁹ , Friedmann 2009 ¹⁸⁸
5-azacytidine	Ependymoma	DNA methylation inhibitor	Pilot clinical trial	Febrile neutropenia, bruising, and leukopenia	Sandberg 2019 ²²⁰
Pembrolizumab	Ependymoma	Immune checkpoint inhibitor	Phase II	Anemia and hyperglycemia	Blumenthal 2016 ²³⁹

Abbreviations: CDK4/6, cyclin-dependent kinase 4 or 6; CNS, central nervous system; DIPG, diffuse intrinsic pontine glioma; DNA, deoxyribonucleic acid; GABA, gamma amino-butyric acid; MB, medulloblastoma; PIGF, placental growth factor; VEGF, vascular endothelial growth factor.

Drugs denoted with () were discussed in this review. This table is not all-inclusive and only briefly summarizes some potential chemotherapeutic agents.

Chemical structure of Sunitinib. <https://pubchem.ncbi.nlm.nih.gov/compound/Sunitinib>



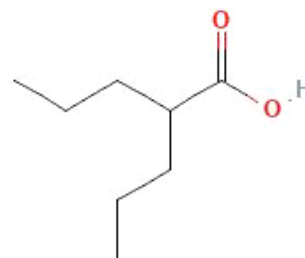
A preclinical report indicated inhibition of MB cancer by sunitinib involving the STAT3-AKT signaling pathway.¹⁰⁵ Pharmacologically, the plasma maximum concentration (C_{\max}) is observed between 6 and 12 h post-administration, and the bioavailability of oral sunitinib is estimated to be ~50%.^{106,107} Despite proven efficacy in treating renal cell carcinoma,¹⁰⁴ the success of sunitinib in treating pediatric MB is poor. One potential reason for this could be due to its limited penetration through an intact BBB.¹⁰⁸ A study by Sobanska and colleagues used a rabbit model to show that the exposure of sunitinib in plasma, aqueous humor, and CSF was different depending on the time of day of drug administration (8 am dose area under the curve [$AUC_{0-\text{time of last measurable concentration}}$], CSF: 55.5 ng*h/mL vs. 9 pm dose $AUC_{0-\text{time of last measurable concentration}}$, CSF: 66.3 ng*h/mL, respectively).¹⁰⁸ However, sunitinib penetration through the BBB was reported to be very low (<5%) and comparable in both dosing groups.

To overcome the limitations of the BBB, a study by Szalek and colleagues evaluated the antibiotic ciprofloxacin to modulate the BBB and enhance penetration of sunitinib.¹⁰⁹ They found that rabbits treated with sunitinib + ciprofloxacin had higher 24-h CSF exposures (AUC_{0-24} and C_{\max}) compared to those that only received sunitinib (50.4 vs. 155 ng*h/mL and 4.2 vs. 18 ng/mL).¹⁰⁹ As clinical outcome data in pediatrics for sunitinib are limited, a phase II clinical multicenter trial conducted by the Children's Oncology Group in 29 children found that sunitinib (as monotherapy) was reasonably well tolerated in children with recurrent ependymoma or high-grade glioma.¹¹⁰ However, the trial was closed at the time of interim analysis as there was no efficacy associated with sunitinib for recurrent PBT. The study concluded that sunitinib lacked anti-tumor activity as monotherapy.¹¹⁰

3.1.2 | Valproic acid

Valproic acid (VPA) is a histone deacetylase inhibitor (HDACi) that has shown promise in cancer therapeutics given that histone deacetylase is a key component of epigenetic machinery, and it regulates gene expression through increased histone acetylation, while behaving as oncogenes in some cancers like MB.

Chemical structure of VPA. <https://pubchem.ncbi.nlm.nih.gov/compound/ValproicAcid>



A report by Li and colleagues showed VPA inhibited cancerous growth in the MB cell line.¹¹¹ In a phase I clinical trial of children with brain cancer malignancies, the Children's Oncology Group showed that limiting VPA trough plasma concentrations to 75–100 µg/mL minimized toxicities.¹¹² In 2011, the FDA issued warnings for life-threatening side effects when VPA concentrations exceed 75 µg/mL.¹¹³ Consequently, balancing the benefit vs. toxicity of VPA is a clinical challenge in the treatment of cancer.

Ionized forms of VPA at a plasma pH of 7.4 render it less permeable through plasma membranes for passive diffusion,^{114,115} and likewise, VPA also has difficulty crossing the BBB. VPA's difficulty in penetrating the CNS is believed to be because VPA acts as a substrate for the ATP-binding efflux transporter on the BBB.¹¹⁶ Given the potential of VPA as an anti-tumor agent, current studies are exploring the acceleration of VPA influx through BBB. One such study determined that pre-treatment with *Gastrodia elata* extract substantially improved BBB penetration of VPA due to upregulation of influx transporters, specifically the OATP transporter.¹¹⁷ The study found that rats treated with *Gastrodia elata* at oral doses of 1 and 3 g/kg for 5 days increased the BBB AUC penetration ratio from 0.36 to 1.47 and 1.02, respectively.

3.1.3 | Carboplatin

Carboplatin is a platinum alkylating agent that covalently binds to DNA. Carboplatin is most commonly

used for ovarian cancers. However, carboplatin has shown potential for treatment of other cancers, with studies underway.

Chemical structure of Carboplatin. <https://pubchem.ncbi.nlm.nih.gov/compound/Carboplatin>



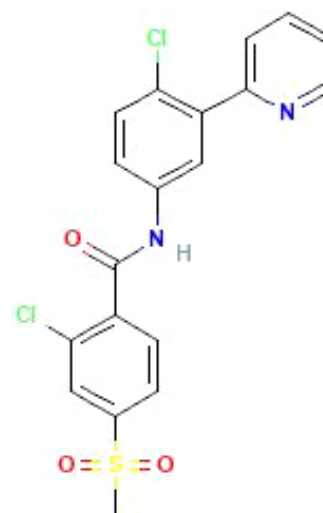
The molecular mechanism of carboplatin is similar to cisplatin but with lower side effects.¹¹⁸ In 2021, a randomized controlled trial in 261 children with MB found that carboplatin inclusion during radiotherapy enhanced survival by 19% compared to no carboplatin.³⁸ However, improved survival was only observed in the high-risk group 3 children with MB.³⁸

Preclinical in vivo studies have shown enhanced carboplatin penetration through the BBB when co-administered with RMP-7, a bradykinin analog.^{119,120} Specifically, Elliott and colleagues showed that intracarotid doses of RMP-7 from 0.01 to 9 µg/kg significantly increased the permeability of carboplatin into tumor tissue ($F [6, 144] = 10.92, p < 0.001$) and surrounding brain tissue ($F [6, 144] = 9.17, p < 0.001$) in a dose-dependent manner.¹¹⁹ A study by Matsukado et al¹²⁰ also showed that intracarotid infusions of RMP-7 increased the transport of carboplatin to tumors by 2.7 fold ($p < 0.001$). This could have clinical implications as they found that the RG2 glioma rats treated with carboplatin and RMP-7 had increased survival compared to those who only received carboplatin alone (37% vs. 74%).

3.1.4 | Vismodegib

Vismodegib is a small molecular inhibitor shown to efficiently inhibit relapse in SHH-activated MB, where the probability of drug resistance development is high.¹²¹

Chemical structure of Vismodegib. <https://pubchem.ncbi.nlm.nih.gov/compound/Vismodegib>



A phase I and phase II clinical trial concluded that vismodegib is an efficient and well-tolerated drug against pediatric and adult MB, and vismodegib achieves anti-MB activity by inhibiting the SHH signaling pathway.¹²² This trial reported an objective response rate of 37% for vismodegib but the drug showed no response (0% response rate) in a non-SHH type MB. Currently, there are limited data on vismodegib's ability to cross the BBB. A recent study published in early 2023 by Tylawsky and colleagues utilized a fucoidan-encapsulated vismodegib strategy to improve drug delivery across the BBB, and decrease the adverse effect of growth plate fusion observed at clinically effective doses.¹²³ They found that in their animal model, fucoidan-based nanoparticles encapsulating delivery of vismodegib exhibited good efficacy, reduced bone toxicity, and increased drug exposure to healthy brain tissue. This is especially significant to pediatric patients as growth plate fusion can stunt a child's growth potential. Overall, these findings demonstrate a potent strategy for targeted delivery that overcomes the BBB to achieve increased selective tumor penetration and has therapeutic implications for drug delivery to other diseases in the CNS.

3.1.5 | TB403

TB-403, a humanized recombinant IgG1 monoclonal antibody with high affinity to the receptor (Neuropilin-1) of the placental growth factor (PIGF), inhibits PIGF-associated stimulation by blocking the

PIGF-neuropilin-1 ligand–receptor interaction in capillary endothelial cells.¹²⁴ TB-403 can also interact and have an inhibitory effect with vascular endothelial growth factor receptor 1 (VEGFR1).¹²⁴ PIGF is expressed in MB PBT, produced by the cerebellar stroma via the SHH ligand.¹²⁵ Moreover, PIGF and neuropilin-1 (Nrp1) signaling play an important role in the growth and spread of MB.¹²⁴ In murine models with human MB xenograft and mimicking clinical symptoms, TB-403 inhibited primary tumor growth and spinal metastasis by interfering with PIGF and neuropilin-1 binding.¹²⁶ This preclinical study recorded that in the presence of TB-403, the mean mouse survival increased from 40 to >55 days. Regarding clinical data, a phase I dose escalation study of TB403 found that the most commonly observed treatment-emergent adverse events were fatigue, constipation, pyrexia, and dyspnea.¹²⁷ Available data also suggest that the VEGF pro-angiogenic signaling pathway inhibitors may increase plasma levels of pro-angiogenic factors such as PIGF, a determinant of drug-induced resistance to therapy.¹²⁴ The phase I trial of TB-403 in relapsed MB, neuroblastoma, Ewing Sarcoma, and alveolar Rhabdomyosarcoma indicated its good tolerance in the small population of heavily pretreated advanced solid tumor patients. In this trial, 15 subjects were given 4 dose levels (20, 50, 100, and 175 mg/kg), and the treatment caused a total of 75 adverse events (AEs) in 10 out of 15, but no fatal adverse events were observed during the project. However, serious adverse events were recorded in 3 out of 15 patients treated. The results of the study did not show any conclusive therapeutic response, with 63% of the relapsed MB patients experiencing stable disease conditions for 100 days.¹²⁸ TB-403 does not require BBB penetration as it exerts its inhibitory effect on ligand–receptor blocking. Nevertheless, investigation for the on-site effect of the antibody on the distal part of the brain by examining the BBB penetrating capability could improve the therapeutic future of TB403.

3.2 | Diffuse intrinsic pontine glioma (DIPG)

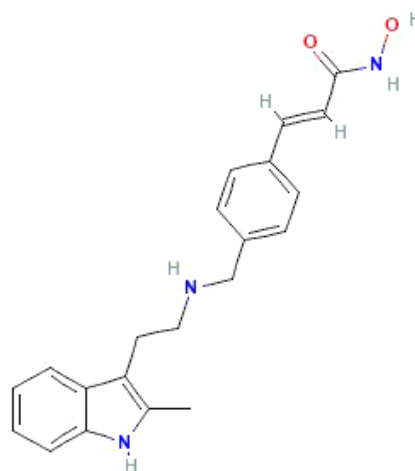
DIPG is a high-grade pediatric glioma, a malignant brainstem tumor, with a median survival of <1 year, while less than 10% of patients reported having overall survival >2 years.¹²⁹ The tumor's location makes it difficult for complete resection.¹³⁰ In children, DIPG accounts for 80% of brainstem tumors.^{131,132} Histological analysis reveals a close similarity between grade III anaplastic astrocytomas and grade IV glioblastoma.⁴⁵ In 50% of the patients, clinical symptoms include cranial nerve palsies, long tract signs,

cerebellar ataxia, and dysmetria.^{133,134} In DIPG, the most affected nerves are cranial nerves VI and VII, and these nerves' altered function is a symptomatic characteristic of DIPG.¹³³ Common and standard practices for DIPGs comprise a 54–59 Gy dose of fractionated radiation because of the interior location of the tumor.¹³⁵ Early approaches of monotherapy or combined chemotherapies have failed to work against DIPG cancer efficiently.^{136–138} It is believed that the oncogenic drivers for the DIPGs are the mutations in the histone protein, either by somatic-like mutations in H3K27M or H3K27 trimethylation.⁴⁷ Since the discoveries of histone protein mutations responsible for this disease, several molecular inhibitors for histone deacetylase and demethylase have been evaluated for potential therapeutic application.^{139–141} For DIPG, chemotherapeutic options are inefficient because of the intact BBB, which restricts the delivery of drugs to DIPG tumors.¹⁴² However, there is an indication of SHH-mediated signaling of lower BBB permeability in DIPG.¹⁴³ Regardless of the recent advances in identifying a target and specific drug, drug delivery failure across the BBB remains a significant challenge, and drug effectiveness against other tumors fails to inhibit DIPG.¹⁴⁴

3.2.1 | Panobinostat

Panobinostat is a histone deacetylase inhibitor that acts as a potent inhibitor of DIPG, and the epigenetic dysregulation is depicted in Figure 2A. It was first identified by Grasso, C.S et al. while performing chemical screenings against DIPG.^{139,145}

Chemical structure of Panobinostat. <https://pubchem.ncbi.nlm.nih.gov/compound/Panobinostat>



In the presence of panobinostat, there was a significant decrease in proliferation via the upregulation of genes MKI67 and CCND1.¹³⁹ The tumor inhibitory effect of panobinostat was validated in DIPG mouse and H3.3K27M

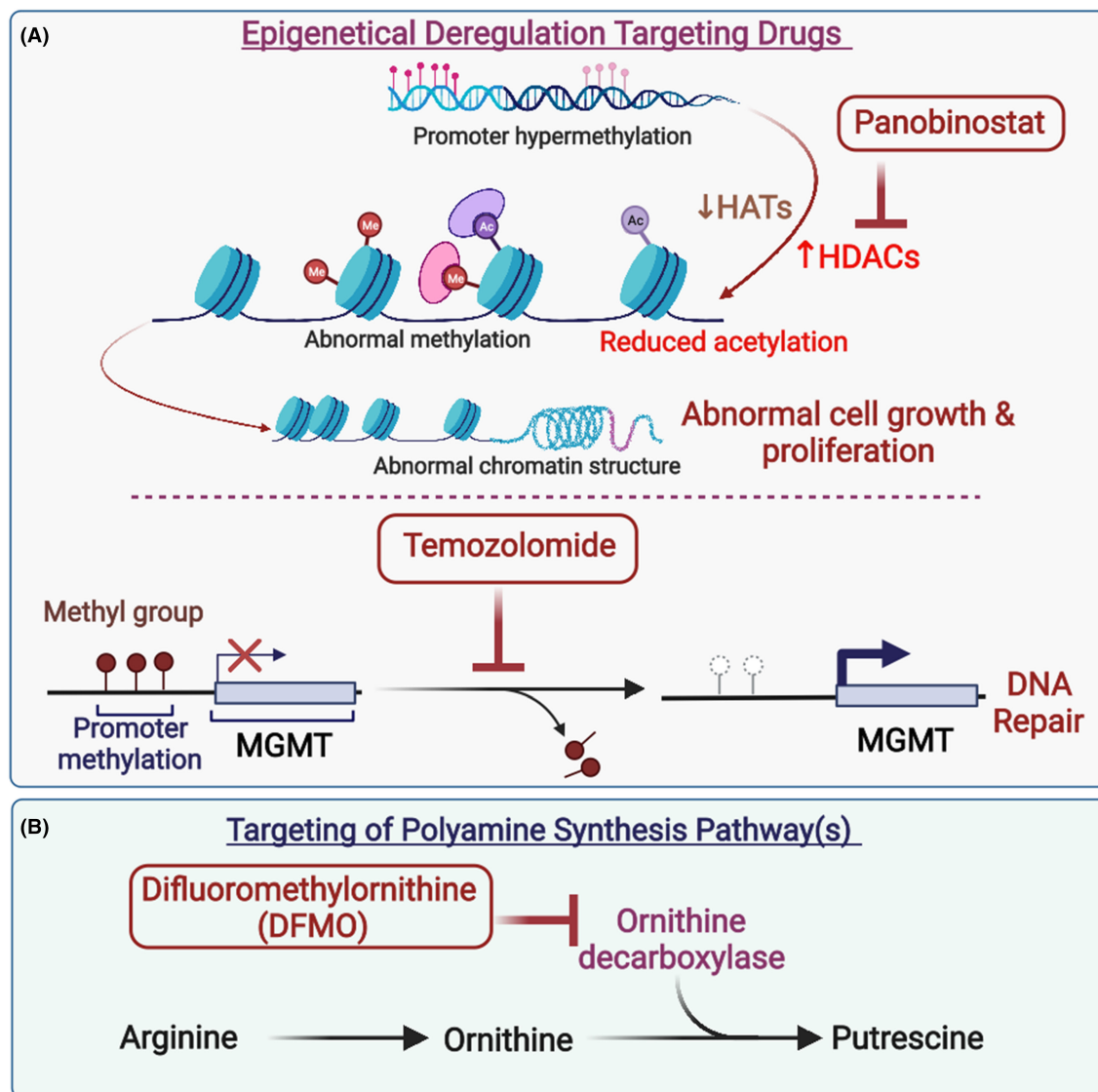


FIGURE 2 Major pathways or targets of (A) epigenetic drugs panobinostat (upper panel) and temozolomide (lower panel), and (B) polyamine synthesis targeting drug difluoromethylornithine. HATs, histone acetyltransferase; HDACs, histone deacetylase; MGMT, O6-methylguanine-DNA methyltransferase; DFMO, difluoromethylornithine. *Figure was generated utilizing [Biorender.com](#).

orthotopic xenograft models.^{146,147} It was shown that panobinostat improved the epigenetic effect on mesenchymal stem cells tumor necrosis factor-related apoptosis-inducing ligand (MSCs TRIAL), resulting in tumor growth arrest and an increase in overall survival by 5.5 days compared to control group.^{148,149} In vitro analysis based on western blotting showed dose-dependent enhancement in H3 acetylation and H3K27 trimethylation in panobinostat-treated cells expressing H3.3K27M mutation.¹³⁹ RNA-seq data from the study also supported the normalization of the K27M gene while decreasing oncogenic target gene expression in panobinostat-treated cells.¹³⁹ Further, pre-clinical studies on human cells and mouse DIPG have confirmed panobinostat as an efficient chemical agent against DIPG.¹⁴⁶ Preclinical studies for this inhibitor alone or in combination with other compounds have shown a

better survival rate in a synergistic approach in several studies.^{139,150}

Although the exact reason for the inefficient outcome of this drug when used alone is still not completely understood, poor BBB penetration has been proposed as a significant contributor to the diminished potency of panobinostat.^{151,152} An in vitro study by Hennika and colleagues tested this theory by administering mice with different regimens of panobinostat. They found that extended daily consecutive treatment in both genetic and orthotopic xenograft models was required to get adequate exposure in the brain, but this came with significant toxicity.¹⁴⁶ Efforts are being made to enhance the BBB penetration of panobinostat to achieve desired CNS concentrations. It was found that convection-enhanced delivery (CED) combined with positron emission

tomography (PET) might have utility in increasing BBB penetration.¹⁵³ A study by Tosi and colleagues utilized both CED and PET to modulate the CED infusions of panobinostat to ensure saturation of the tumor by drug.¹⁵³ They concluded that personalized image-guided drug delivery might be useful in potentiating CED-based treatment algorithms to support clinical translation of panobinostat for improvement in survival rates in pediatric diffuse midline glioma.

3.2.2 | Difluoromethylornithine (DFMO)

DFMO is a small molecule that irreversibly inhibits the polyamine synthesis pathway, inhibiting cell proliferation^{154,155}, illustrated in Figure 2B.

Chemical structure of DFMO. <https://pubchem.ncbi.nlm.nih.gov/compound/Difluoromethylornithine>

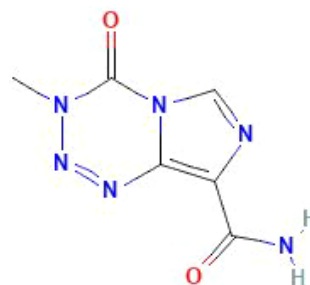


Polyamines are one of the major substrates in intracellular biosynthesis and catabolic pathways and are thus tightly regulated.^{156,157} DFMO inhibits the activity of ornithine decarboxylase1 (ODC1), which is required for the decarboxylation of ornithine into polyamine putrescine.^{158–160} ODC1, which has different activity levels in response to growth stimuli, is found to be upregulated in cancer.¹⁶¹ Though its effect on adult cancers is underwhelming,^{162,163} the use of DFMO in childhood cancer has potential given its activity in neuroblastoma cell lines.^{164,165} Recent data demonstrated hyperactivity of the polyamine pathway in DIPG preclinical in vitro and in vivo models.¹⁶⁶ Briefly, this study showed that DFMO, combined with polyamine transport inhibitor AMXT 1501, significantly increased the survival rate of mice to 160 days compared to 60 days for control group in the orthotopic DIPG model. Brain polyamines have also been shown to break down the integrity of BBB. Interestingly, given DFMO's inhibition of the synthesis of polyamines, it has been shown to decrease the postischemic breakdown of the BBB.¹⁶⁷ Consequently, BBB resistance to other synergistic molecules is risky in DIPG treatment.¹⁶⁷

3.2.3 | Temozolomide

Temozolomide is an orally bioavailable agent and has proven function against high-grade gliomas and has exhibited a better clinical effect than procarbazine (not discussed in this review), another potent tumor inhibitor.^{168–170}

Chemical structure of Temozolomide. <https://pubchem.ncbi.nlm.nih.gov/compound/Temozolomide>



Mechanistically, temozolomide causes a reduction in O⁶-methylguanine methyltransferase (MGMT), which is required for DNA repair (Figure 2A). This reduction in the enzyme results in an increased level of O⁶-methylguanine in DNA, leading to a higher cytotoxic effect.^{171,172} A phase III clinical trial in adults reported a higher survival rate of around 11% for radiotherapy combined with temozolomide when compared to radiotherapy alone.¹⁷³ Because of its good tolerance, temozolomide is prescribed for most cases of glioblastoma in patients.¹⁷⁴ Unfortunately, this drug's utilization post-radiotherapy has not shown benefit in pediatric DIPG cases.^{175–178} A comprehensive review compiling different trial studies pointed to no substantial difference in overall survival compared to the control treated with only radiotherapy.¹³²

One of the critical reasons for the ineffectiveness of this drug against DIPG is the BBB.¹⁷⁹ Despite the small size of temozolomide (194 Da) and associated lipophilicity, the detected concentration of the drug in brain tumor tissue is only about 17.8% of the plasma level with mean area under-concentration-time curve (AUC) for plasma level 17.1 and 2.7 µg/mL × h for brain.¹⁸⁰ The differential integrity of the BBB (different parts of the brain) was observed for the selective permeability of temozolomide in the pontine, the cortex, and CSF, suggesting a location-based phenotype for the BBB.^{181,182} A study by Ostermann et al. showed CSF levels of temozolomide in patients with newly diagnosed recurrent malignant gliomas were consistently in the 20% of plasma level range but could get up to 35% of the plasma levels when co-administered alongside radiation.¹⁸³ Controlled and targeted radiation can be used for a transient opening and modulation of the BBB neurovascular unit for better

drug penetration.¹⁸³ Studies are ongoing to improve BBB penetration for temozolomide by employing techniques like focused ultrasound, regadenoson (a vasodilating process), and nanoparticles to enhance penetration and inhibit transporters.^{184–187}

3.2.4 | Bevacizumab

Bevacizumab is a recombinant and humanized monoclonal antibody (mAb) with high specificity and affinity for VEGF^{188,189}; the mechanism is shown in Figure 3. VEGF is pivotal in tumor growth and metastasis in children.¹⁸⁹ Prior to the primary tumor resection, children with cancer have increased circulating VEGF levels.^{190,191} Clinical data show overexpression of VEGF-A and its receptor VEGFR2 in various brain tumors, including DIPG.^{189,192} Bevacizumab appears to be relatively safe for children with primary CNS tumors.¹⁹³ Non-randomized trials of bevacizumab in children diagnosed with PBT show varying levels of clinical improvements.^{158,194,195} Parekh et al demonstrated a 6-month progression-free survival of 38% in patients <21 years of age with WHO grade 3–4 gliomas who receive bevacizumab alone or in combination with CCNU1.⁹⁷ Hummel and colleagues demonstrated that bevacizumab-based therapies were feasible and safe in HGG and DIPG pediatric patients but did not improve survival in patients with DIPG.¹⁹⁵ Currently, for DIPG, trials are being conducted with VEGF-neutralizing mAb.^{196–198} A decrease of 65% in tumor size was observed

with bevacizumab in combination with temozolomide, but the study only included two patients, raising questions about this combination's clinical benefit.¹⁹⁹

The VEGF antagonist mAb does not require crossing the BBB as it can directly bind to VEGFR2; however, reaching the distal part of CNS will be more beneficial for treating DIPG.²⁰⁰ Currently, there are no clear data on the receptor-based transcytosis of mAb across the BBB. Since antibodies are relatively large (150 kDa), this will likely hinder mAb's ability to cross the BBB.²⁰¹

3.3 | Ependymoma

Ependymomas are tumors with a slow proliferating rate. In most cases, fractionated radiotherapy or surgery is the preferred treatment with limited knowledge of the benefits of chemotherapeutic regimes.^{202–204} A prominent marker of angiogenesis is the overexpression of VEGF.²⁰⁵ Approximately 190 ependymoma-diagnosed children's cases in the United States are reported yearly with <60% 10-year or more survival.^{43,50} The standard treatment regime includes near-total-resection (NTR) or gross total resection (GTR) followed by fractionated radiotherapy, with an exception in children under 3 years of age.²⁰⁶ Two clinical trials have tried to validate this resection methodology and confirmed survival rates ranging from 60% to 80%.^{206,207} Other clinical trials have demonstrated positive outcomes with combined therapy of alkylating agents like carboplatin, ormaplatin, and oxaliplatin with or without

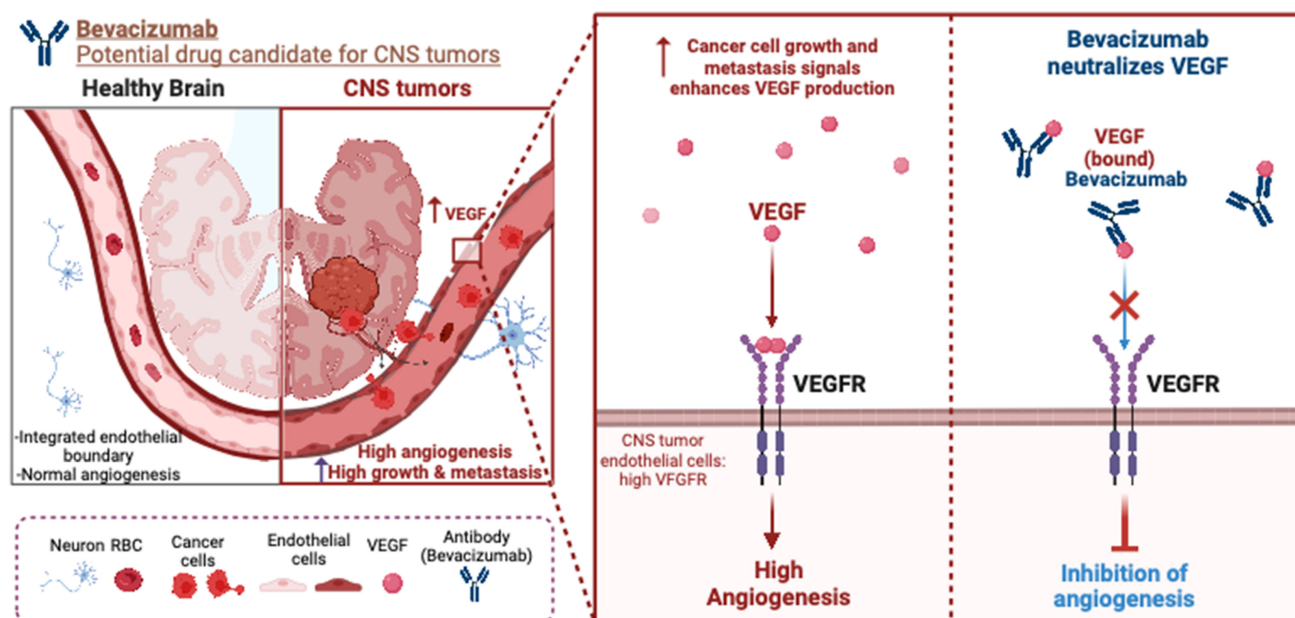


FIGURE 3 Bevacizumab is a humanized monoclonal antibody that inhibits angiogenesis by neutralizing vascular endothelial growth factor (VEGF), which is upregulated in tumor growth and metastasis of multiple types of CNS tumors. CNS, central nervous system; VEGFR, vascular endothelial growth factor receptor. *Figure was generated utilizing Biorender.com.

cisplatin.^{208,209} The progression-free survival of children treated with only GTR followed by radiotherapy compared to patients who received chemotherapy and NTR showed a comparable effect of around 58% and 67%, respectively.²⁰⁸ In addition to an increased progression-free survival rate, chemotherapy is also desired in situations where complete resection is impossible. A German HIT-REZ study enrolled 138 pediatric patients for evaluation of systemic chemotherapy and concluded no advantage of chemotherapy in recurrent ependymoma; however, resection followed by chemotherapy extended the survival rate by more than 1 year.²¹⁰ Another phase II trial for sunitinib enrolled 17 children with DIPG and 13 with ependymoma and found no significant anti-tumor activity of this drug alone.¹¹⁰ As such, multiple trials and research efforts are underway to find a better chemical agent for ependymoma inhibition.²¹¹

ABC transporters are located in tissues of the intestine, liver, kidneys, heart, lungs, brain, placenta, and testis and are highly expressed in tissue interfaces, specifically blood endothelial interfaces like the BBB.^{212,213} Reports have suggested the invariable occurrence of ABC receptors like multiple drug resistance 1 (MDR1) and CRP in different subtypes of ependymoma.^{214,215} The BBB-associated ABC transporters play a vital role in drug concentration across the BBB, and their primary function is to extrude both endogenous and exogenous molecules, including drugs.²¹⁶ Thus, a detailed, comprehensive analysis of the BBB on chemotherapy for ependymoma is needed.

3.3.1 | 5-azacytidine

5-azacytidine (AZA) was first discovered in 1960 as a pyrimidine analog with the ability to inhibit DNA methylation.²¹⁷ In an in vitro study, AZA was discovered to stimulate the differentiation of human glioblastoma cells while simultaneously reducing the expression of the G protein-coupled formylpeptide receptor (FPR), which acts as a mediator in the chemotaxis of phagocytic leukocytes. Additionally, AZA was observed to lower global methylation levels within glioblastoma cells, all the while activating the tumor suppressor.²¹⁸ A study involving rabbits and dogs examined the CSF levels of AZA. It demonstrated that AZA was able to penetrate the CNS through the blood-CSF barrier, with CSF levels reaching 27% and 58% of the plasma Cmax.²¹⁹ In a pilot clinical trial involving six children with recurrent posterior fossa ependymoma, AZA was administered at doses of 10 mg for 12 consecutive weekly infusions into the fourth ventricle tumor resection cavity. Notably, there were no observed neurological toxicities, and two out of five patients exhibited a decrease in the size of intraventricular lesions.²²⁰

3.3.2 | Pembrolizumab

Pembrolizumab is a humanized monoclonal antibody known for its high affinity for programmed cell death ligand 1 (PD-L1), which is found on antigen-presenting cells, including cancer cells. It functions by inhibiting the interaction between PD-L1 and the programmed cell death-1 (PD-1) receptor on cytotoxic T-lymphocytes, thereby enhancing the T-cell response against cancer cells.²²¹ Notably, PD-1 and PD-L1 are highly expressed in supratentorial ependymoma and posterior fossa ependymoma. In these cases, the PD-L1-PD-1 interaction serves to protect the host by restraining hyperactive T-effector cells. However, disrupting this interaction has shown promise in improving anti-tumor cytotoxic T-cell immunity.^{222–224} In advanced melanoma patients, treatment with pembrolizumab yielded an overall response rate of 33%. Furthermore, there was a 35% rate of progression-free survival for 12 months, and the median overall survival reached 23 months.²²⁵ Pembrolizumab has demonstrated effectiveness against various cancer types, as summarized in a report by the European Medicines Agency.²²⁶ Currently, an ongoing phase I clinical trial of pembrolizumab (Study ID-NCT02359565) is investigating its use in pediatric brain tumors, including ependymoma, with a focus on identifying side effects and determining the optimal dosing for patients under 18 years of age. However, despite its effectiveness as an immune checkpoint inhibitor (ICI), the success of pembrolizumab also depends on its ability to cross the BBB. Therefore, a phase II trial (Study ID-NCT05879120) is underway to explore the role of the BBB in influencing the potency of pembrolizumab in the treatment of recurrent glioblastoma in patients over 18 years of age.

4 | CONCLUSIONS AND FUTURE PROSPECTIVE

We focused this review on the major types of PBTs, their chemotherapeutic treatment, and the involvement of the BBB. PBTs represent a significant treatment challenge, and the success of even highly potent anti-PBT drugs can be limited by poor penetration through the BBB. The use of chemotherapy was initially as an adjuvant to surgical and radiotherapy-based treatments, but significant progress has been made in their usage in pediatric brain cancers. Chemotherapy can potentially become a standard treatment modality, thereby eliminating the need for radiotherapy and its associated long-term side effects. The failure of conventional anti-cancer drugs in treating PBTs is potentially due to the restrictive cellular barriers that isolate the CNS,

maintain homeostasis, and regulate the passage of molecules across the BBB.

Two promising research pathways dominate efforts to improve CNS drug delivery. One is focused on developing novel systemic drug delivery methods, and the other is on directly modulating the BBB to improve CNS drug bioavailability.^{81,82,227–229} PET image-guided HDAC inhibition (PETobinostat) is a recent drug delivery method that combines convection-enhanced delivery and image guidance is being applied to increase BBB penetration of panobinostat.¹⁵³

There is a need for a better delivery method to counter the BBB resistance for drugs like nimotuzumab, gefitinib, and erlotinib, which have been shown to have some beneficial outcomes in subsets of DIPG.^{230,231} In this regard, in vitro BBB models can be used to screen anti-tumor compounds in a timely manner and evaluate the BBB integrity modulation for desired drug penetration before clinical data are available.^{232–235} More research is needed in this area to optimize the bioavailability of anti-tumor agents across the BBB and augment PBT therapeutic options.

AUTHOR CONTRIBUTIONS

Johid Reza Malik: Conceptualization (equal); project administration (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Anthony T. Podany:** Project administration (equal); visualization (equal); writing – review and editing (equal). **Parvez Khan:** Writing – review and editing (equal). **Christopher L. Shaffer:** Writing – review and editing (equal). **Jawed A. Siddiqui:** Writing – review and editing (equal). **Janina Baranowska-Kortylewicz:** Writing – original draft (equal); writing – review and editing (equal). **Jennifer Le:** Writing – review and editing (equal). **Courtney V. Fletcher:** Writing – review and editing (equal). **Sadia Afruz Ether:** Writing – review and editing (equal). **Sean N. Avedissian:** Funding acquisition (lead); project administration (equal); supervision (equal); validation (equal); visualization (equal); writing – review and editing (equal).

FUNDING INFORMATION


We acknowledge support from the following grants from the National Institutes of Health: K23 MH125734 (to SNA) and K23 AI134307 (ATP). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CONFLICT OF INTEREST STATEMENT


All authors, no relevant conflicts.

ORCID

Johid Reza Malik  <https://orcid.org/0000-0001-5999-9187>

Anthony T. Podany  <https://orcid.org/0000-0002-8371-0379>


Parvez Khan  <https://orcid.org/0000-0002-8566-0248>

Christopher L. Shaffer  <https://orcid.org/0000-0002-7927-4914>

Jawed A. Siddiqui  <https://orcid.org/0000-0003-1287-3004>

Janina Baranowska-Kortylewicz  <https://orcid.org/0000-0002-2254-0791>

Jennifer Le  <https://orcid.org/0000-0002-6692-0884>

Courtney V. Fletcher  <https://orcid.org/0000-0002-3703-7849>

Sadia Afruz Ether  <https://orcid.org/0000-0002-1006-365X>

Sean N. Avedissian  <https://orcid.org/0000-0002-0935-6455>

REFERENCES

1. Packer RJ, Gajjar A, Vezina G, et al. Phase III study of craniospinal radiation therapy followed by adjuvant chemotherapy for newly diagnosed average-risk medulloblastoma. *J Clin Oncol*. 2006;24(25):4202–4208. doi:10.1200/JCO.2006.06.4980
2. Cohen AR. Brain tumors in children. *N Engl J Med*. 2022; 386(20):1922–1931. doi:10.1056/NEJMra2116344
3. Pollack IF. Brain tumors in children. *N Engl J Med*. 1994; 331(22):1500–1507. doi:10.1056/NEJM199412013312207
4. Wisoff JH, Sanford RA, Heier LA, Spoto R, Burger PC, Yates AJ, Holmes EJ, Kun LE. Primary neurosurgery for pediatric low-grade gliomas: a prospective multi-institutional study from the Children's Oncology Group. *Neurosurgery* 2011;68(6):1548–1554; discussion 54–5. doi:10.1227/NEU.0b013e31821a666e
5. Fang FY, Rosenblum JS, Ho WS, Heiss JD. New developments in the pathogenesis, therapeutic targeting, and treatment of pediatric medulloblastoma. *Cancers (Basel)*. 2022;14(9):2285. doi:10.3390/cancers14092285
6. Grewal MR, Spielman DB, Safi C, et al. Gross total versus subtotal surgical resection in the management of craniopharyngiomas. *Allergy Rhinol (Providence)*. 2020;11:2152656720964158. doi:10.1177/2152656720964158
7. Peters S, Frisch S, Stock A, et al. Proton beam therapy for pediatric tumors of the central nervous system-experiences of clinical outcome and feasibility from the KiProReg study. *Cancers (Basel)*. 2022;14(23):5863. doi:10.3390/cancers14235863
8. Vatner RE, Niemierko A, Misra M, et al. Endocrine deficiency as a function of radiation dose to the hypothalamus and pituitary in pediatric and young adult patients with brain tumors. *J Clin Oncol*. 2018;36(28):2854–2862. doi:10.1200/JCO.2018.78.1492
9. Paulino AC, Lobo M, Teh BS, et al. Ototoxicity after intensity-modulated radiation therapy and cisplatin-based chemotherapy in children with medulloblastoma. *Int J Radiat Oncol Biol Phys*. 2010;78(5):1445–1450. doi:10.1016/j.ijrobp.2009.09.031
10. Merchant TE, Conklin HM, Wu S, Lustig RH, Xiong X. Late effects of conformal radiation therapy for pediatric patients with low-grade glioma: prospective evaluation of cognitive,

- endocrine, and hearing deficits. *J Clin Oncol*. 2009;27(22):3691-3697. doi:10.1200/JCO.2008.21.2738
11. Blethen KE, Sprowls SA, Arsiwala TA, et al. Effects of whole-brain radiation therapy on the blood-brain barrier in immunocompetent and immunocompromised mouse models. *Radiat Oncol*. 2023;18(1):22. doi:10.1186/s13014-023-02215-6
 12. Rubin P, Gash DM, Hansen JT, Nelson DF, Williams JP. Disruption of the blood-brain barrier as the primary effect of CNS irradiation. *Radiother Oncol*. 1994;31(1):51-60. doi:10.1016/0167-8140(94)90413-8
 13. Diserbo M, Agin A, Lamproglou I, et al. Blood-brain barrier permeability after gamma whole-body irradiation: an in vivo microdialysis study. *Can J Physiol Pharmacol*. 2002;80(7):670-678. doi:10.1139/y02-070
 14. Li YQ, Ballinger JR, Nordal RA, Su ZF, Wong CS. Hypoxia in radiation-induced blood-spinal cord barrier breakdown. *Cancer Res*. 2001;61(8):3348-3354.
 15. Ljubimova NV, Levitman MK, Plotnikova ED, Eidus LK. Endothelial cell population dynamics in rat brain after local irradiation. *Br J Radiol*. 1991;64(766):934-940. doi:10.1259/0007-1285-64-766-934
 16. Rubin DB, Drab EA, Ward WF. Physiological and biochemical markers of the endothelial cell response to irradiation. *Int J Radiat Biol*. 1991;60(1-2):29-32. doi:10.1080/09553009114551461
 17. Li YQ, Chen P, Haimovitz-Friedman A, Reilly RM, Wong CS. Endothelial apoptosis initiates acute blood-brain barrier disruption after ionizing radiation. *Cancer Res*. 2003;63(18):5950-5956.
 18. Peña LA, Fuks Z, Kolesnick RN. Radiation-induced apoptosis of endothelial cells in the murine central nervous system: protection by fibroblast growth factor and sphingomyelinase deficiency. *Cancer Res*. 2000;60(2):321-327.
 19. Khan FH, Reza MJ, Shao YF, et al. Role of exosomes in lung cancer: a comprehensive insight from immunomodulation to theragnostic applications. *Biochim Biophys Acta Rev Cancer*. 2022;1877(5):188776. doi:10.1016/j.bbcan.2022.188776
 20. Shalita C, Hanzlik E, Kaplan S, Thompson EM. Immunotherapy for the treatment of pediatric brain tumors: a narrative review. *Transl Pediatr*. 2022;11(12):2040-2056. doi:10.21037/tp-22-86
 21. Haanen JBAG, Carbone F, Robert C, et al. Management of toxicities from immunotherapy: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2017;28(suppl_4):iv119-iv142. doi:10.1093/annonc/mdx225
 22. Hoppe-Hirsch E, Renier D, Lellouch-Tubiana A, Sainte-Rose C, Pierre-Kahn A, Hirsch JF. Medulloblastoma in childhood: progressive intellectual deterioration. *Childs Nerv Syst*. 1990;6(2):60-65. doi:10.1007/BF00307922
 23. Loughton SJ, Merchant TE, Sklar CA, et al. Endocrine outcomes for children with embryonal brain tumors after risk-adapted craniospinal and conformal primary-site irradiation and high-dose chemotherapy with stem-cell rescue on the SJMB-96 trial. *J Clin Oncol*. 2008;26(7):1112-1118. doi:10.1200/JCO.2008.13.5293
 24. Mulhern RK, Merchant TE, Gajjar A, Reddick WE, Kun LE. Late neurocognitive sequelae in survivors of brain tumours in childhood. *Lancet Oncol*. 2004;5(7):399-408. doi:10.1016/S1470-2045(04)01507-4
 25. Silber JH, Radcliffe J, Peckham V, et al. Whole-brain irradiation and decline in intelligence: the influence of dose and age on IQ score. *J Clin Oncol*. 1992;10(9):1390-1396. doi:10.1200/JCO.1992.10.9.1390
 26. Nguyen T, Mueller S, Malbari F. Review: neurological complications from therapies for pediatric brain tumors. *Front Oncol*. 2022;12:853034. doi:10.3389/fonc.2022.853034
 27. Zhou Q, Xu Y, Zhou Y, Wang J. Promising chemotherapy for malignant pediatric brain tumor in recent biological insights. *Molecules*. 2022;27(9):2685. doi:10.3390/molecules27092685
 28. Power EA, Rechberger JS, Gupta S, Schwartz JD, Daniels DJ, Khatua S. Drug delivery across the blood-brain barrier for the treatment of pediatric brain tumors - an update. *Adv Drug Deliv Rev*. 2022;185:114303. doi:10.1016/j.addr.2022.114303
 29. Neumaier F, Zlatopolskiy BD, Neumaier B. Drug penetration into the central nervous system: pharmacokinetic concepts and in vitro model systems. *Pharmaceutics*. 2021;13(10):1542. doi:10.3390/pharmaceutics13101542
 30. Northcott PA, Robinson GW, Kratz CP, et al. Medulloblastoma. *Nat Rev Dis Primers*. 2019;5(1):11. doi:10.1038/s41572-019-0063-6
 31. Tabori U, Sung L, Hukin J, et al. Medulloblastoma in the second decade of life: a specific group with respect to toxicity and management: a Canadian Pediatric Brain Tumor Consortium Study. *Cancer*. 2005;103(9):1874-1880. doi:10.1002/cncr.21003
 32. de Bont JM, Holt B, Dekker AW, van der Does-van den Berg A, Sonneveld P, Pieters R. Significant difference in outcome for adolescents with acute lymphoblastic leukemia treated on pediatric vs adult protocols in The Netherlands. *Leukemia*. 2004;18(12):2032-2035. doi:10.1038/sj.leu.2403538
 33. Armstrong C, Sun LR. Neurological complications of pediatric cancer. *Cancer Metastasis Rev*. 2020;39(1):3-23. doi:10.1007/s10555-020-09847-0
 34. Hashizume R. Epigenetic targeted therapy for diffuse intrinsic pontine glioma. *Neurol Med Chir (Tokyo)*. 2017;57(7):331-342. doi:10.2176/nmc.ra.2017-0018
 35. Gajjar A, Pfister SM, Taylor MD, Gilbertson RJ. Molecular insights into pediatric brain tumors have the potential to transform therapy. *Clin Cancer Res*. 2014;20(22):5630-5640. doi:10.1158/1078-0432.CCR-14-0833
 36. Wu G, Diaz AK, Paugh BS, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nat Genet*. 2014;46(5):444-450. doi:10.1038/ng.2938
 37. Mynarek M, von Hoff K, Pietsch T, et al. Nonmetastatic medulloblastoma of early childhood: results from the prospective clinical trial HIT-2000 and an extended validation cohort. *J Clin Oncol*. 2020;38(18):2028-2040. doi:10.1200/JCO.19.03057
 38. Leary SES, Packer RJ, Li Y, et al. Efficacy of carboplatin and isotretinoin in children with high-risk medulloblastoma: a randomized clinical trial from the Children's Oncology Group. *JAMA Oncol*. 2021;7(9):1313-1321. doi:10.1001/jamaoncol.2021.2224
 39. Ostrom QT, Cioffi G, Waite K, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2014-2018. *Neuro Oncol*. 2021;23(12 Suppl 2):iii1-iii105. doi:10.1093/neuonc/noab200
 40. Khanna V, Achey RL, Ostrom QT, et al. Incidence and survival trends for medulloblastomas in the United States from 2001 to 2013. *J Neurooncol*. 2017;135(3):433-441. doi:10.1007/s11060-017-2594-6
 41. Pizer BL, Clifford SC. The potential impact of tumour biology on improved clinical practice for medulloblastoma:

- progress towards biologically driven clinical trials. *Br J Neurosurg*. 2009;23(4):364-375. doi:10.1080/02688690903121807
42. Northcott PA, Shih DJ, Peacock J, et al. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature*. 2012;488(7409):49-56. doi:10.1038/nature11327
 43. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol*. 2007;114(2):97-109. doi:10.1007/s00401-007-0243-4
 44. Ramaswamy V, Remke M, Bouffet E, et al. Risk stratification of childhood medulloblastoma in the molecular era: the current consensus. *Acta Neuropathol*. 2016;131(6):821-831. doi:10.1007/s00401-016-1569-6
 45. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131(6):803-820. doi:10.1007/s00401-016-1545-1
 46. Chatwin HV, Cruz Cruz J, Green AL. Pediatric high-grade glioma: moving toward subtype-specific multimodal therapy. *FEBS J*. 2021;288(21):6127-6141. doi:10.1111/febs.15739
 47. Castel D, Philippe C, Calmon R, et al. Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol*. 2015;130(6):815-827. doi:10.1007/s00401-015-1478-0
 48. Sturm D, Bender S, Jones DT, et al. Paediatric and adult glioblastoma: multifactorial (epi)genomic culprits emerge. *Nat Rev Cancer*. 2014;14(2):92-107. doi:10.1038/nrc3655
 49. Lewis PW, Müller MM, Koletsky MS, et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science*. 2013;340(6134):857-861. doi:10.1126/science.1232245
 50. Ostrom QT, de Blank PM, Kruchko C, et al. Alex's Lemonade Stand Foundation infant and childhood primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. *Neuro Oncol*. 2015;16(Suppl 10):x1-x36. doi:10.1093/neuonc/nou327
 51. McGuire CS, Sainani KL, Fisher PG. Incidence patterns for ependymoma: a surveillance, epidemiology, and end results study. *J Neurosurg*. 2009;110(4):725-729. doi:10.3171/2008.9.JNS08117
 52. Amirian ES, Armstrong TS, Aldape KD, Gilbert MR, Scheurer ME. Predictors of survival among pediatric and adult ependymoma cases: a study using surveillance, epidemiology, and end results data from 1973 to 2007. *Neuroepidemiology*. 2012;39(2):116-124. doi:10.1159/000339320
 53. Sun T, Warrington NM, Rubin JB. Why does Jack, and not Jill, break his crown? Sex disparity in brain tumors. *Biol Sex Differ*. 2012;3:3. doi:10.1186/2042-6410-3-3
 54. Pajtler KW, Mack SC, Ramaswamy V, et al. The current consensus on the clinical management of intracranial ependymoma and its distinct molecular variants. *Acta Neuropathol*. 2017;133(1):5-12. doi:10.1007/s00401-016-1643-0
 55. Viscusi ER, Viscusi AR. Blood-brain barrier: mechanisms governing permeability and interaction with peripherally acting μ -opioid receptor antagonists. *Reg Anesth Pain Med*. 2020;45(9):688-695. doi:10.1136/rapm-2020-101403
 56. Daneman R, Prat A. The blood-brain barrier. *Cold Spring Harb Perspect Biol*. 2015;7(1):a020412. doi:10.1101/cshperspect.a020412
 57. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. *Nat Med*. 2013;19(12):1584-1596. doi:10.1038/nm.3407
 58. Abbott NJ. Dynamics of CNS barriers: evolution, differentiation, and modulation. *Cell Mol Neurobiol*. 2005;25(1):5-23. doi:10.1007/s10571-004-1374-y
 59. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell*. 2011;21(2):193-215. doi:10.1016/j.devcel.2011.07.001
 60. Wolburg H, Noell S, Wolburg-Buchholz K, Mack A, Fallier-Becker P. Agrin, aquaporin-4, and astrocyte polarity as an important feature of the blood-brain barrier. *Neuroscientist*. 2009;15(2):180-193. doi:10.1177/1073858408329509
 61. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol*. 2010;119(1):7-35. doi:10.1007/s00401-009-0619-8
 62. Stamatovic SM, Johnson AM, Keep RF, Andjelkovic AV. Junctional proteins of the blood-brain barrier: new insights into function and dysfunction. *Tissue Barriers*. 2016;4(1):e1154641. doi:10.1080/21688370.2016.1154641
 63. Berndt P, Winkler L, Cording J, et al. Tight junction proteins at the blood-brain barrier: far more than claudin-5. *Cell Mol Life Sci*. 2019;76(10):1987-2002. doi:10.1007/s00018-019-03030-7
 64. Sasson E, Anzi S, Bell B, et al. Nano-scale architecture of blood-brain barrier tight-junctions. *eLife*. 2021;10:e63253. doi:10.7554/eLife.63253
 65. Furtado D, Björnmalm M, Ayton S, Bush AI, Kempe K, Caruso F. Overcoming the blood-brain barrier: the role of nanomaterials in treating neurological diseases. *Adv Mater*. 2018;30(46):e1801362. doi:10.1002/adma.201801362
 66. Bernacki J, Dobrowolska A, Nierwińska K, Małeck A. Physiology and pharmacological role of the blood-brain barrier. *Pharmacol Rep*. 2008;60(5):600-622.
 67. Engelhardt B, Sorokin L. The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. *Semin Immunopathol*. 2009;31(4):497-511. doi:10.1007/s00281-009-0177-0
 68. Nakagawa S, Deli MA, Kawaguchi H, et al. A new blood-brain barrier model using primary rat brain endothelial cells, pericytes and astrocytes. *Neurochem Int*. 2009;54(3-4):253-263. doi:10.1016/j.neuint.2008.12.002
 69. Kadry H, Noorani B, Cucullo L. A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids Barriers CNS*. 2020;17(1):69. doi:10.1186/s12987-020-00230-3
 70. Abbott NJ, Rönnebeck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*. 2006;7(1):41-53. doi:10.1038/nrn1824
 71. Wolburg H, Wolburg-Buchholz K, Fallier-Becker P, Noell S, Mack AF. Structure and functions of aquaporin-4-based orthogonal arrays of particles. *Int Rev Cell Mol Biol*. 2011;287:1-41. doi:10.1016/B978-0-12-386043-9.00001-3
 72. Löscher W, Potschka H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *NeuroRx*. 2005;2(1):86-98. doi:10.1602/neurorx.2.1.86
 73. Sun H, Dai H, Shaik N, Elmquist WF. Drug efflux transporters in the CNS. *Adv Drug Deliv Rev*. 2003;55(1):83-105. doi:10.1016/S0169-409X(02)00172-2
 74. Demeule M, Régina A, Jodoin J, et al. Drug transport to the brain: key roles for the efflux pump P-glycoprotein in the blood-brain barrier. *Vascul Pharmacol*. 2002;38(6):339-348. doi:10.1016/S1537-1891(02)00201-X
 75. Cisternino S, Mercier C, Bourasset F, Roux F, Scherrmann JM. Expression, up-regulation, and transport activity of the

- multidrug-resistance protein Abcg2 at the mouse blood-brain barrier. *Cancer Res.* 2004;64(9):3296-3301. doi:10.1158/0008-5472.can-03-2033
76. Agarwal S, Hartz AM, Elmquist WF, Bauer B. Breast cancer resistance protein and P-glycoprotein in brain cancer: two gatekeepers team up. *Curr Pharm Des.* 2011;17(26):2793-2802. doi:10.2174/138161211797440186
 77. Griffith JJ, Rathi S, Zhang W, Drewes LR, Sarkaria JN, Elmquist WF. Addressing BBB heterogeneity: a new paradigm for drug delivery to brain tumors. *Pharmaceutics.* 2020;12(12):1205. doi:10.3390/pharmaceutics12121205
 78. Welby JP, Kaptzan T, Wohl A, et al. Current murine models and new developments in H3K27M diffuse midline gliomas. *Front Oncol.* 2019;9:92. doi:10.3389/fonc.2019.00092
 79. Phoenix TN, Patmore DM, Boop S, et al. Medulloblastoma genotype dictates blood brain barrier phenotype. *Cancer Cell.* 2016;29(4):508-522. doi:10.1016/j.ccell.2016.03.002
 80. Arvanitis CD, Ferraro GB, Jain RK. The blood-brain barrier and blood-tumour barrier in brain tumours and metastases. *Nat Rev Cancer.* 2020;20(1):26-41. doi:10.1038/s41568-019-0205-x
 81. Dong X. Current strategies for brain drug delivery. *Theranostics.* 2018;8(6):1481-1493. doi:10.7150/thno.21254
 82. Patel MM, Patel BM. Crossing the blood-brain barrier: recent advances in drug delivery to the brain. *CNS Drugs.* 2017;31(2):109-133. doi:10.1007/s40263-016-0405-9
 83. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx.* 2005;2(1):3-14. doi:10.1602/neurorx.2.1.3
 84. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol.* 2004;1(4):337-341. doi:10.1016/j.ddtec.2004.11.007
 85. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J Comb Chem.* 1999;1(1):55-68. doi:10.1021/cc9800071
 86. Gibbs SA, Proserpio P, Francione S, et al. Clinical features of sleep-related hypermotor epilepsy in relation to the seizure-onset zone: a review of 135 surgically treated cases. *Epilepsia.* 2019;60(4):707-717. doi:10.1111/epi.14690
 87. Packer RJ, Goldwein J, Nicholson HS, et al. Treatment of children with medulloblastomas with reduced-dose craniospinal radiation therapy and adjuvant chemotherapy: a Children's Cancer Group Study. *J Clin Oncol.* 1999;17(7):2127-2136. doi:10.1200/JCO.1999.17.7.2127
 88. Evans AE, Jenkin RD, Spoto R, et al. The treatment of medulloblastoma. Results of a prospective randomized trial of radiation therapy with and without CCNU, vincristine, and prednisone. *J Neurosurg.* 1990;72(4):572-582. doi:10.3171/jns.1990.72.4.0572
 89. Packer RJ, Sutton LN, Elterman R, et al. Outcome for children with medulloblastoma treated with radiation and cisplatin, CCNU, and vincristine chemotherapy. *J Neurosurg.* 1994;81(5):690-698. doi:10.3171/jns.1994.81.5.0690
 90. Packer RJ, Siegel KR, Sutton LN, et al. Efficacy of adjuvant chemotherapy for patients with poor-risk medulloblastoma: a preliminary report. *Ann Neurol.* 1988;24(4):503-508. doi:10.1002/ana.410240405
 91. Tait DM, Thornton-Jones H, Bloom HJ, Lemerle J, Morris-Jones P. Adjuvant chemotherapy for medulloblastoma: the first multi-centre control trial of the International Society of Paediatric Oncology (SIOP I). *Eur J Cancer.* 1990;26(4):464-469.
 92. Rutkowski S, von Hoff K, Emser A, et al. Survival and prognostic factors of early childhood medulloblastoma: an international meta-analysis. *J Clin Oncol.* 2010;28(33):4961-4968. doi:10.1200/JCO.2010.30.2299
 93. Grundy RG, Wilne SH, Robinson KJ, et al. Primary postoperative chemotherapy without radiotherapy for treatment of brain tumours other than ependymoma in children under 3 years: results of the first UKCCSG/SIOP CNS 9204 trial. *Eur J Cancer.* 2010;46(1):120-133. doi:10.1016/j.ejca.2009.09.013
 94. Rutkowski S, Gerber NU, von Hoff K, et al. Treatment of early childhood medulloblastoma by postoperative chemotherapy and deferred radiotherapy. *Neuro Oncol.* 2009;11(2):201-210. doi:10.1215/15228517-2008-084
 95. Rutkowski S, Bode U, Deinlein F, et al. Treatment of early childhood medulloblastoma by postoperative chemotherapy alone. *N Engl J Med.* 2005;352(10):978-986. doi:10.1056/NEJMoa042176
 96. Cohen BH, Geyer JR, Miller DC, et al. Pilot study of intensive chemotherapy with peripheral hematopoietic cell support for children less than 3 years of age with malignant brain tumors, the CCG-99703 phase I/II study. A report from the Children's Oncology Group. *Pediatr Neurol.* 2015;53(1):31-46. doi:10.1016/j.pediatrneurol.2015.03.019
 97. von Bueren AO, von Hoff K, Pietsch T, et al. Treatment of young children with localized medulloblastoma by chemotherapy alone: results of the prospective, multicenter trial HIT 2000 confirming the prognostic impact of histology. *Neuro Oncol.* 2011;13(6):669-679. doi:10.1093/neuonc/nor025
 98. Dhall G, Grodman H, Ji L, et al. Outcome of children less than three years old at diagnosis with non-metastatic medulloblastoma treated with chemotherapy on the "Head Start" I and II protocols. *Pediatr Blood Cancer.* 2008;50(6):1169-1175. doi:10.1002/pbc.21525
 99. Oyharcabal-Bourden V, Kalifa C, Gentet JC, et al. Standard-risk medulloblastoma treated by adjuvant chemotherapy followed by reduced-dose craniospinal radiation therapy: a French Society of Pediatric Oncology Study. *J Clin Oncol.* 2005;23(21):4726-4734. doi:10.1200/JCO.2005.00.760
 100. Lannering B, Rutkowski S, Doz F, et al. Hyperfractionated versus conventional radiotherapy followed by chemotherapy in standard-risk medulloblastoma: results from the randomized multicenter HIT-SIOP PNET 4 trial. *J Clin Oncol.* 2012;30(26):3187-3193. doi:10.1200/JCO.2011.39.8719
 101. Verlooy J, Mosseri V, Bracard S, et al. Treatment of high risk medulloblastomas in children above the age of 3 years: a SFOP study. *Eur J Cancer.* 2006;42(17):3004-3014. doi:10.1016/j.ejca.2006.02.026
 102. Taylor RE, Bailey CC, Robinson KJ, et al. Outcome for patients with metastatic (M2-3) medulloblastoma treated with SIOP/UKCCSG PNET-3 chemotherapy. *Eur J Cancer.* 2005;41(5):727-734. doi:10.1016/j.ejca.2004.12.017
 103. Kieran MW, Chisholm J, Casanova M, et al. Phase I study of oral sonidegib (LDE225) in pediatric brain and solid tumors and a phase II study in children and adults with relapsed medulloblastoma. *Neuro Oncol.* 2017;19(11):1542-1552. doi:10.1093/neuonc/nox109
 104. Rock EP, Goodman V, Jiang JX, et al. Food and Drug Administration drug approval summary: sunitinib malate

- for the treatment of gastrointestinal stromal tumor and advanced renal cell carcinoma. *Oncologist*. 2007;12(1):107-113. doi:10.1634/theoncologist.12-1-107
105. Yang F, Jove V, Xin H, Hedvat M, Van Meter TE, Yu H. Sunitinib induces apoptosis and growth arrest of medulloblastoma tumor cells by inhibiting STAT3 and AKT signaling pathways. *Mol Cancer Res*. 2010;8(1):35-45. doi:10.1158/1541-7786.MCR-09-0220
106. Haznedar JO, Patyna S, Bello CL, et al. Single- and multiple-dose disposition kinetics of sunitinib malate, a multitargeted receptor tyrosine kinase inhibitor: comparative plasma kinetics in non-clinical species. *Cancer Chemother Pharmacol*. 2009;64(4):691-706. doi:10.1007/s00280-008-0917-1
107. Houk BE, Bello CL, Kang D, Amantea M. A population pharmacokinetic meta-analysis of sunitinib malate (SU11248) and its primary metabolite (SU12662) in healthy volunteers and oncology patients. *Clin Cancer Res*. 2009;15(7):2497-2506. doi:10.1158/1078-0432.CCR-08-1893
108. Sobańska K, Karbownik A, Szałek E, et al. The influence of the time-of-day administration of sunitinib on the penetration through the blood-brain and blood-aqueous humour barriers in rabbits. *Eur Rev Med Pharmacol Sci*. 2016;20(1):166-173.
109. Szałek E, Karbownik A, Sobańska K, et al. The penetration of sunitinib through the blood-brain barrier after the administration of ciprofloxacin. *Acta pol Pharm*. 2014;71(4):691-697.
110. Wetmore C, Daryani VM, Billups CA, et al. Phase II evaluation of sunitinib in the treatment of recurrent or refractory high-grade glioma or ependymoma in children: a children's Oncology Group Study ACNS1021. *Cancer Med*. 2016;5(7):1416-1424. doi:10.1002/cam4.713
111. Li XN, Shu Q, Su JM, Perlaky L, Blaney SM, Lau CC. Valproic acid induces growth arrest, apoptosis, and senescence in medulloblastomas by increasing histone hyperacetylation and regulating expression of p21Cip1, CDK4, and CMYC. *Mol Cancer Ther*. 2005;4(12):1912-1922. doi:10.1158/1535-7163.MCT-05-0184
112. Su JM, Li XN, Thompson P, et al. Phase I study of valproic acid in pediatric patients with refractory solid or CNS tumors: a children's oncology group report. *Clin Cancer Res*. 2011;17(3):589-597. doi:10.1158/1078-0432.CCR-10-0738
113. Chateauxvieux S, Morceau F, Dicato M, Diederich M. Molecular and therapeutic potential and toxicity of valproic acid. *J Biomed Biotechnol*. 2010;2010:479364. doi:10.1155/2010/479364
114. Terbach N, Shah R, Kelemen R, et al. Identifying an uptake mechanism for the antiepileptic and bipolar disorder treatment valproic acid using the simple biomedical model Dictyostelium. *J Cell Sci*. 2011;124(Pt 13):2267-2276. doi:10.1242/jcs.084285
115. Naora K, Shen DD. Mechanism of valproic acid uptake by isolated rat brain microvessels. *Epilepsy Res*. 1995;22(2):97-106. doi:10.1016/0920-1211(95)00034-8
116. Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet*. 2003;42(1):59-98. doi:10.2165/00003088-200342010-00003
117. Yang L, Lin IH, Ting CT, Tsai TH. Modulation of the transport of valproic acid through the blood-brain barrier in rats by the *Gastrodia elata* extracts. *J Ethnopharmacol*. 2021;278:114276. doi:10.1016/j.jep.2021.114276
118. Institute NNC. NCI dictionary of cancer terms. carboplatin. Accessed May 01, 2023. <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/carboplatin>
119. Elliott PJ, Hayward NJ, Huff MR, Nagle TL, Black KL, Bartus RT. Unlocking the blood-brain barrier: a role for RMP-7 in brain tumor therapy. *Exp Neurol*. 1996;141(2):214-224. doi:10.1006/exnr.1996.0156
120. Matsukado K, Inamura T, Nakano S, Fukui M, Bartus RT, Black KL. Enhanced tumor uptake of carboplatin and survival in glioma-bearing rats by intracarotid infusion of bradykinin analog, RMP-7. *Neurosurgery*. 1996;39(1):125-133; discussion 33-4. doi:10.1097/00006123-199607000-00025
121. Robinson GW, Orr BA, Wu G, et al. Vismodegib exerts targeted efficacy against recurrent sonic hedgehog-subgroup medulloblastoma: results from phase II pediatric brain tumor consortium studies PBTC-025B and PBTC-032. *J Clin Oncol*. 2015;33(24):2646-2654. doi:10.1200/JCO.2014.60.1591
122. Li Y, Song Q, Day BW. Phase I and phase II sonidegib and vismodegib clinical trials for the treatment of paediatric and adult MB patients: a systematic review and meta-analysis. *Acta Neuropathol Commun*. 2019;7(1):123. doi:10.1186/s40478-019-0773-8
123. Tylawsky DE, Kiguchi H, Vaynshteyn J, et al. P-selectin-targeted nanocarriers induce active crossing of the blood-brain barrier via caveolin-1-dependent transcytosis. *Nat Mater*. 2023;22(3):391-399. doi:10.1038/s41563-023-01481-9
124. Fischer C, Jonckx B, Mazzone M, et al. Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell*. 2007;131(3):463-475. doi:10.1016/j.cell.2007.08.038
125. Jain RK, Xu L. alphaPlGF: a new kid on the antiangiogenesis block. *Cell*. 2007;131(3):443-445. doi:10.1016/j.cell.2007.10.023
126. Snuderl M, Batista A, Kirkpatrick ND, et al. Targeting placental growth factor/neuropilin 1 pathway inhibits growth and spread of medulloblastoma. *Cell*. 2013;152(5):1065-1076. doi:10.1016/j.cell.2013.01.036
127. Lassen U, Nielsen DL, Sørensen M, et al. A phase I, dose-escalation study of TB-403, a monoclonal antibody directed against PlGF, in patients with advanced solid tumours. *Br J Cancer*. 2012;106(4):678-684. doi:10.1038/bjc.2011.609
128. Saulnier-Sholler G, Duda DG, Bergendahl G, et al. A phase I trial of TB-403 in relapsed medulloblastoma, neuroblastoma, Ewing sarcoma, and alveolar rhabdomyosarcoma. *Clin Cancer Res*. 2022;28(18):3950-3957. doi:10.1158/1078-0432.CCR-22-1169
129. Jackson S, Patay Z, Howarth R, et al. Clinico-radiologic characteristics of long-term survivors of diffuse intrinsic pontine glioma. *J Neurooncol*. 2013;114(3):339-344. doi:10.1007/s11060-013-1189-0
130. Jansen MH, Lagerweij T, Sewing AC, et al. Bevacizumab targeting diffuse intrinsic pontine glioma: results of 89Zr-bevacizumab PET imaging in brain tumor models. *Mol Cancer Ther*. 2016;15(9):2166-2174. doi:10.1158/1535-7163.MCT-15-0558
131. Duffner PK, Cohen ME, Freeman AI. Pediatric brain tumors: an overview. *CA Cancer J Clin*. 1985;35(5):287-301. doi:10.3322/canjclin.35.5.287
132. Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. *Lancet Oncol*. 2006;7(3):241-248. doi:10.1016/S1470-2045(06)70615-5
133. Fisher PG, Breiter SN, Carson BS, et al. A clinicopathologic reappraisal of brain stem tumor classification. Identification of pilocytic astrocytoma and fibrillary astrocytoma as distinct entities. *Cancer*. 2000;89(7):1569-1576.
134. Albright AL, Guthkelch AN, Packer RJ, Price RA, Rourke LB. Prognostic factors in pediatric brain-stem gliomas. *J Neurosurg*. 1986;65(6):751-755. doi:10.3171/jns.1986.65.6.0751

135. Cohen KJ, Jabado N, Grill J. Diffuse intrinsic pontine gliomas-current management and new biologic insights. Is there a glimmer of hope? *Neuro Oncol.* 2017;19(8):1025-1034. doi:10.1093/neuonc/nox021
136. Vanan MI, Eisenstat DD. DIPG in children – what can we learn from the past? *Front Oncol.* 2015;5:237. doi:10.3389/fonc.2015.00237
137. Frazier JL, Lee J, Thomale UW, Noggle JC, Cohen KJ, Jallo GI. Treatment of diffuse intrinsic brainstem gliomas: failed approaches and future strategies. *J Neurosurg Pediatr.* 2009;3(4):259-269. doi:10.3171/2008.11.PEDS08281
138. Grimm SA, Chamberlain MC. Brainstem glioma: a review. *Curr Neurol Neurosci Rep.* 2013;13(5):346. doi:10.1007/s11910-013-0346-3
139. Grasso CS, Tang Y, Truffaux N, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med.* 2015;21(6):555-559. doi:10.1038/nm.3855
140. Piunti A, Hashizume R, Morgan MA, et al. Therapeutic targeting of polycomb and BET bromodomain proteins in diffuse intrinsic pontine gliomas. *Nat Med.* 2017;23(4):493-500. doi:10.1038/nm.4296
141. Morales La Madrid A, Hashizume R, Kieran MW. Future clinical trials in DIPG: bringing epigenetics to the clinic. *Front Oncol.* 2015;5:148. doi:10.3389/fonc.2015.00148
142. Sajesh B, On N, Donald M, et al. DIPG-18. Sonic hedgehog (SHH) signalling promotes blood brain barrier (BBB) integrity in diffuse intrinsic pontine glioma (DIPG). *Neuro-Oncology.* 2019;21(Suppl 2):ii72. doi:10.1093/neuonc/noz036.039
143. Chapouly C, Guimbal S, Hollier PL, Renault MA. Role of hedgehog signaling in vasculature development, differentiation, and maintenance. *Int J Mol Sci.* 2019;20(12):3076. doi:10.3390/ijms20123076
144. Veringa SJ, Biesmans D, van Vuurden DG, et al. In vitro drug response and efflux transporters associated with drug resistance in pediatric high grade glioma and diffuse intrinsic pontine glioma. *PloS One.* 2013;8(4):e61512. doi:10.1371/journal.pone.0061512
145. Bradner JE, West N, Grachan ML, et al. Chemical phylogenetics of histone deacetylases. *Nat Chem Biol.* 2010;6(3):238-243. doi:10.1038/nchembio.313
146. Hennika T, Hu G, Olaciregui NG, et al. Pre-clinical study of panobinostat in xenograft and genetically engineered murine diffuse intrinsic pontine glioma models. *PloS One.* 2017;12(1):e0169485. doi:10.1371/journal.pone.0169485
147. Pinder RM, Brogden RN, Speight TM, Avery GS. Sodium valproate: a review of its pharmacological properties and therapeutic efficacy in epilepsy. *Drugs.* 1977;13(2):81-123. doi:10.2165/00003495-197713020-00001
148. Jung PY, Ryu H, Rhee KJ, et al. Adipose tissue-derived mesenchymal stem cells cultured at high density express IFN- β and TRAIL and suppress the growth of H460 human lung cancer cells. *Cancer Lett.* 2019;440–441:202-210. doi:10.1016/j.canlet.2018.10.017
149. Choi SA, Lee C, Kwak PA, et al. Histone deacetylase inhibitor panobinostat potentiates the anti-cancer effects of mesenchymal stem cell-based sTRAIL gene therapy against malignant glioma. *Cancer Lett.* 2019;442:161-169. doi:10.1016/j.canlet.2018.10.012
150. Anastas JN, Zee BM, Kalin JH, et al. Re-programing chromatin with a bifunctional LSD1/HDAC inhibitor induces therapeutic differentiation in DIPG. *Cancer Cell.* 2019;36(5):528-44.e10. doi:10.1016/j.ccell.2019.09.005
151. Singleton WGB, Bienemann AS, Woolley M, et al. The distribution, clearance, and brainstem toxicity of panobinostat administered by convection-enhanced delivery. *J Neurosurg Pediatr.* 2018;22(3):288-296. doi:10.3171/2018.2.PEDS17663
152. Brinthen S, De Wit E, Dimopoulos MA. New agents in multiple myeloma: an examination of safety profiles. *Clin Lymphoma Myeloma Leuk.* 2017;17(7):391-407.e5. doi:10.1016/j.clml.2017.05.003
153. Tosi U, Kommidi H, Adeuyan O, et al. PET, image-guided HDAC inhibition of pediatric diffuse midline glioma improves survival in murine models. *Sci Adv.* 2020;6(30):eabb4105. doi:10.1126/sciadv.abb4105
154. Rea G, Bocedi A, Cervelli M. Question: what is the biological function of the polyamines? *IUBMB Life.* 2004;56(3):167-169. doi:10.1080/15216540410001673996
155. Wallace HM, Fraser AV, Hughes A. A perspective of polyamine metabolism. *Biochem J.* 2003;376(Pt 1):1-14. doi:10.1042/BJ20031327
156. Pegg AE. Mammalian polyamine metabolism and function. *IUBMB Life.* 2009;61(9):880-894. doi:10.1002/iub.230
157. Wallace HM. The physiological role of the polyamines. *Eur J Clin Invest.* 2000;30(1):1-3. doi:10.1046/j.1365-2362.2000.00585.x
158. Murray-Stewart TR, Woster PM, Casero RA. Targeting polyamine metabolism for cancer therapy and prevention. *Biochem J.* 2016;473(19):2937-2953. doi:10.1042/BCJ20160383
159. Pommergaard HC, Burcharth J, Rosenberg J, Raskov H. Topical combination of diclofenac, calcipotriol, and difluoromethylornithine has beneficial effects comparable to 5-fluorouracil for the treatment of non-melanoma skin cancer in mice. *J Chemother.* 2014;26(2):105-110. doi:10.1179/1973947813Y.0000000117
160. Christov KT, Moon RC, Lantvit DD, et al. Prostate intraepithelial neoplasia in Noble rats, a potential intermediate endpoint for chemoprevention studies. *Eur J Cancer.* 2004;40(9):1404-1411. doi:10.1016/j.ejca.2003.11.037
161. Shantz LM, Levin VA. Regulation of ornithine decarboxylase during oncogenic transformation: mechanisms and therapeutic potential. *Amino Acids.* 2007;33(2):213-223. doi:10.1007/s00726-007-0531-2
162. Wolff AC, Armstrong DK, Fetting JH, et al. A phase II study of the polyamine analog N1, N11-diethylnorspermine (DENSpm) daily for five days every 21 days in patients with previously treated metastatic breast cancer. *Clin Cancer Res.* 2003;9(16 Pt 1):5922-5928.
163. Paridaens R, Uges DR, Barbet N, et al. A phase I study of a new polyamine biosynthesis inhibitor, SAM486A, in cancer patients with solid tumours. *Br J Cancer.* 2000;83(5):594-601. doi:10.1054/bjoc.2000.1305
164. Jessa S, Blanchet-Cohen A, Krug B, et al. Stalled developmental programs at the root of pediatric brain tumors. *Nat Genet.* 2019;51(12):1702-1713. doi:10.1038/s41588-019-0531-7
165. Samal K, Zhao P, Kendzicky A, et al. AMXT-1501, a novel polyamine transport inhibitor, synergizes with DFMO in inhibiting neuroblastoma cell proliferation by targeting both ornithine decarboxylase and polyamine transport. *Int J Cancer.* 2013;133(6):1323-1333. doi:10.1002/ijc.28139
166. Khan A, Gamble LD, Upton DH, et al. Dual targeting of polyamine synthesis and uptake in diffuse intrinsic

- pontine gliomas. *Nat Commun.* 2021;12(1):971. doi:10.1038/s41467-021-20896-z
167. Schmitz MP, Combs DJ, Dempsey RJ. Difluoromethylornithine decreases postischemic brain edema and blood-brain barrier breakdown. *Neurosurgery.* 1993;33(5):882-887; discussion 7-8. doi:10.1227/00006123-199311000-00016
 168. Brada M, Hoang-Xuan K, Rampling R, et al. Multicenter phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. *Ann Oncol.* 2001;12(2):259-266. doi:10.1023/a:1008382516636
 169. Yung WK, Albright RE, Olson J, et al. A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *Br J Cancer.* 2000;83(5):588-593. doi:10.1054/bjoc.2000.1316
 170. Yung WK, Prados MD, Yaya-Tur R, et al. Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. Temodal Brain Tumor Group. *J Clin Oncol.* 1999;17(9):2762-2771. doi:10.1200/JCO.1999.17.9.2762
 171. Hegi ME, Liu L, Herman JG, et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. *J Clin Oncol.* 2008;26(25):4189-4199. doi:10.1200/JCO.2007.11.5964
 172. Tolcher AW, Gerson SL, Denis L, et al. Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br J Cancer.* 2003;88(7):1004-1011. doi:10.1038/sj.bjc.6600827
 173. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 2009;10(5):459-466. doi:10.1016/S1470-2045(09)70025-7
 174. Weller M, van den Bent M, Hopkins K, et al. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. *Lancet Oncol.* 2014;15(9):e395-e403. doi:10.1016/S1470-2045(14)70011-7
 175. Chassot A, Canale S, Varlet P, et al. Radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *J Neurooncol.* 2012;106(2):399-407. doi:10.1007/s11060-011-0681-7
 176. Cohen KJ, Heideman RL, Zhou T, et al. Temozolomide in the treatment of children with newly diagnosed diffuse intrinsic pontine gliomas: a report from the Children's oncology group. *Neuro Oncol.* 2011;13(4):410-416. doi:10.1093/neuonc/noq205
 177. Sharp JR, Bouffet E, Stempak D, et al. A multi-Centre Canadian pilot study of metronomic temozolomide combined with radiotherapy for newly diagnosed paediatric brainstem glioma. *Eur J Cancer.* 2010;46(18):3271-3279. doi:10.1016/j.ejca.2010.06.115
 178. Jalali R, Raut N, Arora B, et al. Prospective evaluation of radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *Int J Radiat Oncol Biol Phys.* 2010;77(1):113-118. doi:10.1016/j.ijrobp.2009.04.031
 179. Song KH, Harvey BK, Borden MA. State-of-the-art of microbubble-assisted blood-brain barrier disruption. *Theranostics.* 2018;8(16):4393-4408. doi:10.7150/thno.26869
 180. Portnow J, Badie B, Chen M, Liu A, Blanchard S, Synold TW. The neuropharmacokinetics of temozolomide in patients with resectable brain tumors: potential implications for the current approach to chemoradiation. *Clin Cancer Res.* 2009;15(22):7092-7098. doi:10.1158/1078-0432.CCR-09-1349
 181. Warren KE. Beyond the blood:brain barrier: the importance of central nervous system (CNS) pharmacokinetics for the treatment of CNS tumors, including diffuse intrinsic pontine glioma. *Front Oncol.* 2018;8:239. doi:10.3389/fonc.2018.00239
 182. McCully CM, Pastakia D, Bacher J, et al. Model for concomitant microdialysis sampling of the pons and cerebral cortex in rhesus macaques (*Macaca mulatta*). *Comp Med.* 2013;63(4):355-360.
 183. Ostermann S, Csajka C, Buclin T, et al. Plasma and cerebrospinal fluid population pharmacokinetics of temozolomide in malignant glioma patients. *Clin Cancer Res.* 2004;10(11):3728-3736. doi:10.1158/1078-0432.CCR-03-0807
 184. de Gooijer MC, de Vries NA, Buckle T, et al. Improved brain penetration and antitumor efficacy of temozolomide by inhibition of ABCB1 and ABCG2. *Neoplasia.* 2018;20(7):710-720. doi:10.1016/j.neo.2018.05.001
 185. Kumari S, Ahsan SM, Kumar JM, Kondapi AK, Rao NM. Overcoming blood brain barrier with a dual purpose temozolomide loaded lactoferrin nanoparticles for combating glioma (SERP-17-12433). *Sci Rep.* 2017;7(1):6602. doi:10.1038/s41598-017-06888-4
 186. Ellingson BM, Chung C, Pope WB, Boxerman JL, Kaufmann TJ. Pseudoprogression, radionecrosis, inflammation or true tumor progression? Challenges associated with glioblastoma response assessment in an evolving therapeutic landscape. *J Neurooncol.* 2017;134(3):495-504. doi:10.1007/s11060-017-2375-2
 187. Wei KC, Chu PC, Wang HY, et al. Focused ultrasound-induced blood-brain barrier opening to enhance temozolomide delivery for glioblastoma treatment: a preclinical study. *PloS One.* 2013;8(3):e58995. doi:10.1371/journal.pone.0058995
 188. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol.* 2009;27(28):4733-4740. doi:10.1200/JCO.2008.19.8721
 189. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev.* 2004;25(4):581-611. doi:10.1210/er.2003-0027
 190. Blann AD, Li JL, Li C, Kumar S. Increased serum VEGF in 13 children with Wilms' tumour falls after surgery but rising levels predict poor prognosis. *Cancer Lett.* 2001;173(2):183-186. doi:10.1016/s0304-3835(01)00666-8
 191. Holzer G, Obermair A, Koschat M, Preyer O, Kotz R, Trieb K. Concentration of vascular endothelial growth factor (VEGF) in the serum of patients with malignant bone tumors. *Med Pediatr Oncol.* 2001;36(6):601-604. doi:10.1002/mpo.1136
 192. Shibuya M. Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. *J Biochem Mol Biol.* 2006;39(5):469-478. doi:10.5483/bmbrep.2006.39.5.469
 193. Fangusaro J, Gururangan S, Poussaint TY, et al. Bevacizumab (BVZ)-associated toxicities in children with recurrent central nervous system tumors treated with BVZ and irinotecan (CPT-11): a Pediatric Brain Tumor Consortium Study (PBTC-022). *Cancer.* 2013;119(23):4180-4187.

194. Parekh C, Jubran R, Erdreich-Epstein A, et al. Treatment of children with recurrent high grade gliomas with a bevacizumab containing regimen. *J Neurooncol*. 2011;103(3):673-680. doi:10.1007/s11060-010-0444-x
195. Narayana A, Kunnakkat S, Chacko-Mathew J, et al. Bevacizumab in recurrent high-grade pediatric gliomas. *Neuro Oncol*. 2010;12(9):985-990. doi:10.1093/neuonc/noq033
196. Hummel TR, Salloum R, Drissi R, et al. A pilot study of bevacizumab-based therapy in patients with newly diagnosed high-grade gliomas and diffuse intrinsic pontine gliomas. *J Neurooncol*. 2016;127(1):53-61. doi:10.1007/s11060-015-2008-6
197. Zaky W, Wellner M, Brown RJ, Blüml S, Finlay JL, Dhall G. Treatment of children with diffuse intrinsic pontine gliomas with chemoradiotherapy followed by a combination of temozolomide, irinotecan, and bevacizumab. *Pediatr Hematol Oncol*. 2013;30(7):623-632. doi:10.3109/08880018.2013.829895
198. Gururangan S, Chi SN, Young Poussaint T, et al. Lack of efficacy of bevacizumab plus irinotecan in children with recurrent malignant glioma and diffuse brainstem glioma: a Pediatric Brain Tumor Consortium study. *J Clin Oncol*. 2010;28(18):3069-3075. doi:10.1200/JCO.2009.26.8789
199. Aguilera DG, Mazewski C, Hayes L, et al. Prolonged survival after treatment of diffuse intrinsic pontine glioma with radiation, temozolamide, and bevacizumab: report of 2 cases. *J Pediatr Hematol Oncol*. 2013;35(1):e42-e46. doi:10.1097/MPH.0b013e318279aed8
200. Chacko AM, Li C, Pryma DA, Brem S, Coukos G, Muzykantov V. Targeted delivery of antibody-based therapeutic and imaging agents to CNS tumors: crossing the blood-brain barrier divide. *Expert Opin Drug Deliv*. 2013;10(7):907-926. doi:10.1517/17425247.2013.808184
201. Boado RJ, Zhou QH, Lu JZ, Hui EK, Pardridge WM. Pharmacokinetics and brain uptake of a genetically engineered bifunctional fusion antibody targeting the mouse transferrin receptor. *Mol Pharm*. 2010;7(1):237-244. doi:10.1021/mp900235k
202. Pollack IF, Agnihotri S, Broniscer A. Childhood brain tumors: current management, biological insights, and future directions. *J Neurosurg Pediatr*. 2019;23(3):261-273. doi:10.3171/2018.10.PEDS18377
203. Wells EM, Packer RJ. Pediatric brain tumors. *Continuum (Minneapolis)*. 2015;21(2 Neuro-oncology):373-396. doi:10.1212/01.CON.0000464176.96311.d1
204. Karajannis M, Allen JC, Newcomb EW. Treatment of pediatric brain tumors. *J Cell Physiol*. 2008;217(3):584-589. doi:10.1002/jcp.21544
205. Wagemakers M, Sie M, Hoving EW, Molema G, de Bont ES, den Dunnen WF. Tumor vessel biology in pediatric intracranial ependymoma. *J Neurosurg Pediatr*. 2010;5(4):335-341. doi:10.3171/2009.11.PEDS09260
206. Merchant TE, Li C, Xiong X, Kun LE, Boop FA, Sanford RA. Conformal radiotherapy after surgery for paediatric ependymoma: a prospective study. *Lancet Oncol*. 2009;10(3):258-266. doi:10.1016/s1470-2045(08)70342-5
207. Merchant TE, Bendel AE, Sabin ND, et al. Conformal radiation therapy for pediatric ependymoma, chemotherapy for incompletely resected ependymoma, and observation for completely resected, supratentorial ependymoma. *J Clin Oncol*. 2019;37(12):974-983. doi:10.1200/JCO.18.01765
208. Garvin JH, Selch MT, Holmes E, et al. Phase II study of pre-irradiation chemotherapy for childhood intracranial ependymoma. Children's Cancer Group protocol 9942: a report from the Children's Oncology Group. *Pediatr Blood Cancer*. 2012;59(7):1183-1189. doi:10.1002/pbc.24274
209. Duffner PK, Horowitz ME, Krischer JP, et al. Postoperative chemotherapy and delayed radiation in children less than three years of age with malignant brain tumors. *N Engl J Med*. 1993;328(24):1725-1731. doi:10.1056/NEJM199306173282401
210. Adolph JE, Fleischhack G, Gaab C, et al. Systemic chemotherapy of pediatric recurrent ependymomas: results from the German HIT-REZ studies. *J Neurooncol*. 2021;155(2):193-202. doi:10.1007/s11060-021-03867-8
211. Jünger ST, Timmermann B, Pietsch T. Pediatric ependymoma: an overview of a complex disease. *Childs Nerv Syst*. 2021;37(8):2451-2463. doi:10.1007/s00381-021-05207-7
212. DeGorter MK, Xia CQ, Yang JJ, Kim RB. Drug transporters in drug efficacy and toxicity. *Annu Rev Pharmacol Toxicol*. 2012;52:249-273. doi:10.1146/annurev-pharmtox-010611-134529
213. Gottesman MM, Ambudkar SV. Overview: ABC transporters and human disease. *J Bioenerg Biomembr*. 2001;33(6):453-458. doi:10.1023/a:1012866803188
214. Ginguen  C, Champier J, Maallem S, et al. P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) localize in the microvessels forming the blood-tumor barrier in ependymomas. *Brain Pathol*. 2010;20(5):926-935. doi:10.1111/j.1750-3639.2010.00389.x
215. Chou PM, Barquin N, Gonzalez-Crussi F, Ridaura Sanz C, Tomita T, Reyes-Mugica M. Ependymomas in children express the multidrug resistance gene: immunohistochemical and molecular biologic study. *Pediatr Pathol Lab Med*. 1996;16(4):551-561.
216. Gomez-Zepeda D, Taghi M, Scherrmann JM, Decleves X, Menet MC. ABC transporters at the blood-brain interfaces, their study models, and drug delivery implications in gliomas. *Pharmaceutics*. 2019;12(1):20. doi:10.3390/pharmaceutics12010020
217. Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacitidine and decitabine. *Int J Cancer*. 2008;123(1):8-13. doi:10.1002/ijc.23607
218. Huang J, Chen K, Gong W, et al. Regulation of the leucocyte chemoattractant receptor FPR in glioblastoma cells by cell differentiation. *Carcinogenesis*. 2009;30(2):348-355. doi:10.1093/carcin/bgn266
219. Chabot GG, Rivard GE, Momparler RL. Plasma and cerebrospinal fluid pharmacokinetics of 5-Aza-2'-deoxycytidine in rabbits and dogs. *Cancer Res*. 1983;43(2):592-597.
220. Sandberg DI, Yu B, Patel R, et al. Infusion of 5-Azacitidine (5-AZA) into the fourth ventricle or resection cavity in children with recurrent posterior Fossa Ependymoma: a pilot clinical trial. *J Neurooncol*. 2019;141(2):449-457. doi:10.1007/s11060-018-03055-1
221. Jia L, Zhang Q, Zhang R. PD-1/PD-L1 pathway blockade works as an effective and practical therapy for cancer immunotherapy. *Cancer Biol Med*. 2018;15(2):116-123. doi:10.20892/j.issn.2095-3941.2017.0086
222. Nambirajan A, Malgulwar PB, Sharma A, et al. Clinicopathological evaluation of PD-L1 expression and cytotoxic T-lymphocyte infiltrates across intracranial molecular subgroups of ependymomas: are these tumors potential

- candidates for immune check-point blockade? *Brain Tumor Pathol.* 2019;36(4):152-161. doi:10.1007/s10014-019-00350-1
223. Nam SJ, Kim YH, Park JE, et al. Tumor-infiltrating immune cell subpopulations and programmed death ligand 1 (PD-L1) expression associated with clinicopathological and prognostic parameters in ependymoma. *Cancer Immunol Immunother.* 2019;68(2):305-318. doi:10.1007/s00262-018-2278-x
 224. Hwang K, Koh EJ, Choi EJ, et al. PD-1/PD-L1 and immune-related gene expression pattern in pediatric malignant brain tumors: clinical correlation with survival data in Korean population. *J Neurooncol.* 2018;139(2):281-291. doi:10.1007/s11060-018-2886-5
 225. Ribas A, Hamid O, Daud A, et al. Association of pembrolizumab with tumor response and survival among Patients with Advanced Melanoma. *JAMA.* 2016;315(15):1600-1609. doi:10.1001/jama.2016.4059
 226. European Medicines Agency. Keytruda. <https://www.ema.europa.eu/en/medicines/human/EPAR/keytruda>
 227. Azad TD, Pan J, Connolly ID, Remington A, Wilson CM, Grant GA. Therapeutic strategies to improve drug delivery across the blood-brain barrier. *Neurosurg Focus.* 2015;38(3):E9. doi:10.3171/2014.12.FOCUS14758
 228. Zhan C, Lu W. The blood-brain/tumor barriers: challenges and chances for malignant gliomas targeted drug delivery. *Curr Pharm Biotechnol.* 2012;13(12):2380-2387. doi:10.2174/138920112803341798
 229. Pardridge WM. Blood-brain barrier delivery. *Drug Discov Today.* 2007;12(1-2):54-61. doi:10.1016/j.drudis.2006.10.013
 230. Massimino M, Bode U, Biassoni V, Fleischhack G. Nimotuzumab for pediatric diffuse intrinsic pontine gliomas. *Expert Opin Biol Ther.* 2011;11(2):247-256. doi:10.1517/14712598.2011.546341
 231. Geoerger B, Hargrave D, Thomas F, et al. Innovative therapies for children with cancer pediatric phase I study of erlotinib in brainstem glioma and relapsing/refractory brain tumors. *Neuro Oncol.* 2011;13(1):109-118. doi:10.1093/neuonc/noq141
 232. Williams-Medina A, Deblock M, Janigro D. Models of the blood-brain barrier: tools in translational medicine. *Front Med Technol.* 2020;2:623950. doi:10.3389/fmedt.2020.623950
 233. Ohshima M, Kamei S, Fushimi H, Mima S, Yamada T, Yamamoto T. Prediction of drug permeability using in vitro blood-brain barrier models with human induced pluripotent stem cell-derived brain microvascular endothelial cells. *Biores Open Access.* 2019;8(1):200-209. doi:10.1089/biores.2019.0026
 234. Bagchi S, Chhibber T, Lahooti B, Verma A, Borse V, Jayant RD. In-vitro blood-brain barrier models for drug screening and permeation studies: an overview. *Drug Des Devel Ther.* 2019;13:3591-3605. doi:10.2147/DDDT.S218708
 235. Banerjee J, Shi Y, Azevedo HS. In vitro blood-brain barrier models for drug research: state-of-the-art and new perspectives on reconstituting these models on artificial basement membrane platforms. *Drug Discov Today.* 2016;21(9):1367-1386. doi:10.1016/j.drudis.2016.05.020
 236. Sirachainan N, Pakakasama S, Anurathapan U, et al. Outcome of newly diagnosed high risk medulloblastoma treated with carboplatin, vincristine, cyclophosphamide and etoposide. *J Clin Neurosci.* 2018;56:139-142. doi:10.1016/j.jocn.2018.06.028
 237. Pribnow A, Jonchere B, Liu J, et al. Combination of ribociclib and gemcitabine for the treatment of medulloblastoma. *Mol Cancer Ther.* 2022;21(8):1306-1317. doi:10.1158/1535-7163.MCT-21-0598
 238. Grasso CS, Tang Y, Truffaux N, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nat Med.* 2015;21(7):827. doi:10.1038/nm0715-827a
 239. Blumenthal DT, Yalon M, Vainer GW, et al. Pembrolizumab: first experience with recurrent primary central nervous system (CNS) tumors. *J Neurooncol.* 2016;129(3):453-460. doi:10.1007/s11060-016-2190-1
 240. Mo F, Pellerino A, Soffietti R, Rudà R. Blood-brain barrier in brain tumors: biology and clinical relevance. *Int J Mol Sci.* 2021;22(23):12654. doi:10.3390/ijms222312654
 241. Gao X, Yue Q, Liu Y, et al. Image-guided chemotherapy with specifically tuned blood brain barrier permeability in glioma margins. *Theranostics.* 2018;8(11):3126-3137. doi:10.7150/thno.24784
 242. Wei X, Meel MH, Breur M, Bugiani M, Hulleman E, Phoenix TN. Defining tumor-associated vascular heterogeneity in pediatric high-grade and diffuse midline gliomas. *Acta Neuropathol Commun.* 2021;9(1):142. doi:10.1186/s40478-021-01243-1
 243. Duda-Szymańska J, Papierz W. Morphological analysis of vascular density in ependymomas. *Folia Neuropathol.* 2007;45(3):115-119.

How to cite this article: Malik JR, Podany AT, Khan P, et al. Chemotherapy in pediatric brain tumor and the challenge of the blood-brain barrier. *Cancer Med.* 2023;00:1-22. doi:10.1002/cam4.6647