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Review article

Current status and advances to improving drug delivery in diffuse intrinsic pontine glioma

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ABSTRACT

Diffuse midline glioma (DMG), including tumors diagnosed in the brainstem (diffuse intrinsic pontine glioma - DIPG), is the primary cause of brain tumor-related death in pediatric patients. DIPG is characterized by a median survival of <12 months from diagnosis, harboring the worst 5-year survival rate of any cancer. Corticosteroids and radiation are the mainstay of therapy; however, they only provide transient relief from the devastating neurological symptoms. Numerous therapies have been investigated for DIPG, but the majority have been unsuccessful in demonstrating a survival benefit beyond radiation alone. Although many barriers hinder brain drug delivery in DIPG, one of the most significant challenges is the blood-brain barrier (BBB). Therapeutic compounds must possess specific properties to enable efficient passage across the BBB. In brain cancer, the BBB is referred to as the blood-brain tumor barrier (BBTB), where tumors disrupt the structure and function of the BBB, which may provide opportunities for drug delivery. However, the biological characteristics of the brainstem's BBB/BBTB, both under normal physiological conditions and in response to DIPG, are poorly understood, which further complicates treatment. Better characterization of the changes that occur in the BBB/BBTB of DIPG patients is essential, as this informs future treatment strategies. Many novel drug delivery technologies have been investigated to bypass or disrupt the BBB/BBTB, including convection enhanced delivery, focused ultrasound, nanoparticle-mediated delivery, and intranasal delivery, all of which are yet to be clinically established for the treatment of DIPG. Herein, we review what is known about the BBB/BBTB and discuss the current status, limitations, and advances of conventional and novel treatments to improving brain drug delivery in DIPG.

1. Introduction

Brain tumors are one of the most devastating and fatal cancers diagnosed in the pediatric and adult population [1]. Among pediatric cancers, brain tumors are the leading cause of morbidity and mortality, representing approximately 40% of all cancer-related deaths [2,3]. Diffuse intrinsic pontine glioma (DIPG) is a rare type of brain tumor that originates in the pontine region of the brainstem and is the primary cause of brain tumor-related death in children [4]. The majority of children are diagnosed between the ages of 6 and 7 years, with a median survival of <12 months from diagnosis [5]. DIPG is considered an epigenetic cancer, characterized by the global loss of histone H3 trimethylation at lysine 27 (H3K27me3), which drives abnormal changes in gene expression and gliomagenesis [6,7]. Although these tumors frequently occur in the pons (i.e., DIPG), lesions may also occur in other midline locations such as the thalamus, midbrain, and spinal cord (i.e., diffuse midline gliomas – DMGs) [8]. As per the World Health Organization's (WHO) fifth classification of Central Nervous System (CNS) tumors (WHO CNS5), DIPG is a recognized subset of DMGs (formally termed "DMG, H3K27-altered"), given its diffuse nature, midline location, and shared loss of H3K27me3 [9]. However, for the purpose of this review, DIPGs originating in the pons of the brainstem forms the focus, rather than thalamic, midbrain, or spinal DMGs.

Over the past five decades, hundreds of pharmacological therapies

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have been investigated for DIPG. To date, all chemotherapy, targeted, and immunotherapy agents have been unsuccessful in demonstrating a survival benefit beyond radiation therapy [10,11] – except for the small molecule D2 dopamine receptor antagonist and mitochondrial protease (ClpP) agonist, ONC201 (dordaviprone), which recently demonstrated a transient benefit in early phase clinical trials for DIPG/DMG (NCT03416530 and NCT03134131). In the clinic, corticosteroids and radiation are the current mainstay of therapy, yet only provide limited efficacy by granting transient relief of symptoms. Therefore, there is an urgency for improved and novel treatment strategies for the clinical management of DIPG. Although there are numerous treatment barriers hindering the development and progression of effective therapies in DIPG, one of the most significant challenges affecting clinical translation is the blood-brain barrier (BBB) [8,12]. The BBB is a selectively permeable membrane that regulates the transport of molecules into the brain and is essential for maintaining brain homeostasis. It protects normal brain function by hindering the passage of most compounds across the physical and enzymatic barrier, including almost 98% of drugs [13]. Therefore, effective drug delivery into the brain is challenging.

Under certain pathological conditions of disease, the BBB is disrupted, resulting in heterogeneous changes in vasculature, function, and permeability [14,15]. In brain cancer, the BBB is referred to as the blood-brain tumor barrier (BBTB), where tumors compromise the integrity and structure of the healthy BBB, which, in some circumstances, may be advantageous for drug delivery. However, among different cancer types, there are functional and structural variations of BBTB disruption, which is a significant challenge to effective brain drug delivery [16,17]. Compared to other brain cancers, the extent of BBTB disruption in DIPG is thought to be minimal, however recent findings in human DIPG samples have challenged this perspective [18]. Over the last five years, a range of novel drug delivery technologies have been investigated to bypass or disrupt the BBTB and improve drug delivery for DIPG. These technologies have included convection enhanced delivery (CED), focused ultrasound (FUS), nanoparticle-mediated (NP) delivery, and intranasal (IN) delivery – all of which have similarly been investigated for numerous other CNS diseases and brain cancers [12,19]. This paper reviews what is known about the BBB/BBTB and discusses the current status, limitations, and advances of conventional and novel treatments to improving brain drug delivery in DIPG.

2. DIPG pathogenesis and molecular characteristics

DIPG is a rare pediatric high-grade glioma (HGG) that arises from the abnormal transformation of oligodendroglial precursor-like cells (OPC-like) in the brain [20,21]. Of all pediatric brainstem tumors, DIPG is the most common, comprising 75% of all cases [22,23]. DIPG tumors are diffuse, infiltrative of neighboring brain structures, and often possess leptomeningeal disease dissemination [24]. Leptomeningeal disease dissemination refers to DIPG cells that have spread to the tissue layers that cover the brain and the spinal cord, resulting in poorer survival rates for patients [24,25]. As the pontine region is responsible for maintaining a range of essential functions, such as respiration, balance, swallowing, sleep, motor function, sensation, and bladder control, various symptoms manifest as a result of tumor growth [26]. Classical symptoms of DIPG include cranial nerve deficits (such as facial asymmetry and diplopia), cerebellar dysfunction (such as dysarthria, dysmetria and ataxia), and long tract signs (such as spasticity and abnormal reflexes) [22]. Diagnosis is based on clinical presentation, imaging (including computerized tomography [CT] and magnetic resonance imaging [MRI]), and biopsy [27].

Overall, DIPG tumors are considered as WHO grade IV tumors (WHO CNS5) [23]. However, these tumors possess significant intertumoral and/or intratumoral heterogeneity, varying in their histological composition, genetic signatures, and protein expression [23,28–30]. This heterogeneity is a major obstacle in the treatment of DIPG, as it results in therapeutic variability, inconsistent treatment responses, and, ultimately, drug resistance [31]. However, the most profound and recurrent epigenetic alteration driving gliomagenesis is the H3K27M missense mutation, which is present in over 80% of cases [32–34]. H3K27M refers to the substitution of a methionine for a lysine at amino acid 27 (K27) in the genes encoding histone H3.1 (*HIST3H1B/C*) or H3.3 (*H3F3A*), representing approximately 12–19% and 65% of cases, respectively [35–38]. These "oncohistones" inhibit the function of polycomb repressive complex 2 (PRC2), resulting in hypomethylation of H3K27 and global changes in gene expression [39]. Overexpression of the enhancer of zeste homolog inhibitory protein (EZHIP) is another significant genomic alteration which is present in approximately 10–15% of cases, harboring what is termed a "H3-wildtype" molecular profile of DIPG [38]. EZHIP inhibits the EZH2 methyltransferase solely responsible for the deposition of trimethylated marks on H3K27, similarly resulting in H3K27 hypomethylation and changes in gene expression that is analogous to tumors harboring H3K27M mutations [40].

In addition to H3K27M and EZHIP alterations, there are many other "cooperating" mutations that affect the function of both proliferative and tumor suppressor genes. These mutations are highly heterogenous, both intratumorally and intertumorally, and occur to varying extents across different DIPG subtypes [38]. For example, another co-occurring mutation involved in DIPG pathogenesis arises in the activin A receptor type 1 (*ACVR1*) gene, which results in the upregulation of the bone morphogenic protein (BMP) signaling pathway, promoting tumor growth [38]. *ACVR1* mutations are present in approximately 20–32% of DIPG cases and often coincide with H3.1K27M histone mutations [41]. Mutations in tumor suppressor genes (e.g., tumor protein p53 [*TP53*], phosphatase and tensin homolog [*PTEN*], and protein phosphatase, Mg2+/Mn2+ dependent 1D [*PPM1D*]) and transcriptional regulators (e.g., MYC/MYCN proto-oncogene [*MYC/MYCN*] and ATRX chromatin remodeler [*ATRX*]) add further insult to the epigenetic abnormalities that drive cellular transformation of OPC-like cells in the brainstem of patients with DIPG [21,38,39]. Additionally, alterations in proliferative genes, including receptor tyrosine kinases (e.g., platelet-derived growth factor receptor A [*PDGFRA*], vascular endothelial growth factor receptor [*VEGFR*], and epidermal growth factor receptor [*EGFR*]) and cell cycle genes, including cyclin dependent kinases (e.g., 1, 4 and 6 [*CDK1/4/6*]), promote the rapid and uncontrolled proliferation of these OPC-like cells. Commonly, mutations in phosphoinositide 3-kinase (PI3K) genes (e.g., PI3K catalytic subunit alpha [*PIK3CA*] and PI3K regulatory subunit 1 [*PIK3R1*]) and serine/threonine-protein kinase genes (e.g., WEE1 G2 checkpoint kinase [*WEE1*], protein kinase-B [*AKT*], mammalian target of rapamycin [*mTOR*], and polo-like kinase 1 [*PLK1*]) influence downstream oncogenic signaling that underpin drug resistance, genomic instability, tumor survival, and productive metabolism that is universally present in DIPG [38,39,42]. Defects in DNA damage repair pathways (such as mismatch repair, nucleotide excision repair, base excision repair, non-homologous end-joining and homologous recombination) and altered expression of DNA repair enzymes (such as poly [ADP-ribose] polymerase-1 [PARP1]), have also been identified in DIPG, and similarly contribute to tumor progression and treatment resistance [41,43].

An awareness of the mutations and posttranslational environment in DIPG is important for the development of targeted therapeutics, however a comprehensive explanation of DIPG molecular genetics and epigenetics falls outside the scope of this review paper. Readers are directed to the reviews written by Buczkowicz & Hawkins (2015), Duchatel et al (2019), and Findlay et al (2022) for further details on the mutations and altered signaling pathways driving DIPG pathogenesis relevant to expediting future treatment strategies [38,39,41].

3. Current limitations of treatments in DIPG

When compared to other therapeutic areas, effective drug delivery for brain cancers has one of the poorest success rates, limited by the

potential for adverse effects (i.e., patients may be unable to tolerate the required dose and schedule of the cytotoxic agent), unique changes induced by tumor pathogenesis (i.e., irregular tumoral vasculature, changes in tissue stiffness, cerebral oedema, and increased interstitial pressure affecting drug-tumor penetration), and importantly, the BBB [12,14,22,44]. To enable passage across each layer of the BBB from the systemic circulation, drugs must possess certain physicochemical properties to traverse the specialized endothelial structure, evade efflux transporters, and bypass enzymatic degradation [22]. Therefore, the ability of drugs to reach their site of action in the brain, and do so at a concentration and for a duration that is tumoricidal, is greatly restricted by the BBB [12].

Additional factors influencing effective brain drug delivery include the route of administration, perfusion rate to the tumor, the availability of the receptor or target at the disease site, and the sensitivity of the tumor to the drug [22]. In DIPG, effective drug delivery is further confounded by: (i) the diffusely infiltrative growth pattern and location within critical brain regions, which requires highly functionalized drug delivery systems to penetrate tumor tissue and selectively target diseased cells; (ii) the potential for leptomeningeal disease dissemination, which can alter the molecular characteristics of the tumor and the ability of therapeutic agents to permeate the entire tumor site; (iii) the lack of tissue available, which has impaired the investigation of tumor biology and generation of suitable experimental models; and (iv) the intrinsic intertumoral and intratumoral heterogeneity, which requires a combinatorial therapeutic approach to target multiple disease pathways and evade treatment resistance [12,22,24,45–47]. Moreover, many treatments previously trialed for DIPG have been adapted from regimens implemented for adult and other pediatric HGGs, entrenched by the assumption that each disease shared similar pathogenesis, molecular profiles, and cellular origin [48]. For example, temozolomide, which is one of the few BBB-penetrant chemotherapy agents, was investigated for the treatment of DIPG based on its prior therapeutic efficacy in glioblastoma [49,50]. However, despite its ability to extend survival for glioblastoma patients, these results failed to translate for patients with DIPG [49,50]. Studies have since confirmed differences in DIPG gene expression and DNA copy number compared to other pediatric and adult HGGs, confirming DIPG to be distinct both biologically and in its developmental origin, requiring adapted treatment regimens to be abandoned and re-focused towards molecular pathways exclusive to DIPG [51–54].

3.1. Current standard of clinical care for DIPG

The current standard of clinical care for DIPG includes corticosteroids and radiation therapy. Corticosteroids (i.e., dexamethasone) are used to minimize peritumoral edema, control neurological symptoms, and improve quality of life [22]. Dexamethasone is the glucocorticoid of choice for brain diseases due to its superior CNS penetration, longer elimination half-life, and minimal mineralocorticoid activity [55]. There is a wide variation in dexamethasone dosing for DIPG, ranging from 0.15 mg/kg/day to 2.0 mg/kg/day, and is most commonly administered via the oral route, with seldom administration via the intravenous route [56]. However, dexamethasone is palliative, having no effect on overall survival, and its use is limited by significant side effects, including immunosuppression, mood disturbance, myopathy, peripheral oedema, growth retardation, hyperphagia, and gastrointestinal bleeding [55]. Alternatively, bevacizumab, an intravenously administered anti-VEGF monoclonal antibody, has been suggested to improve quality of life and reduce the need for steroid use in DIPG patients [57]. Although advantageous for mitigating steroid-induced side effects, the role of bevacizumab in the management of DIPG remains unclear, requiring further research into its steroid sparing ability for DIPG patients [57].

Radiation therapy is similarly palliative, however, is the only approved therapy that somewhat alters the clinical course of DIPG, prolonging survival by approximately 3 months [58]. The standard treatment dose is 180–200 cGy fractions administered five days per week, up to a total dose of 54 to 60 Gy, targeting the tumor section and 1–2 cm of adjoining brainstem tissue [22]. Although tumor shrinkage can be significant, the response is usually temporary and can result in radiation necrosis, which is a common side effect causing neurological symptoms such as headache, drowsiness, ataxia, nausea, vomiting and cranial neuropathies [59]. Although approximately 75% of patients will demonstrate some improvement following radiation and corticosteroid therapy, neither options are curable and are significantly limited by side effects [22].

Owing to the delicate location and growth pattern of DIPG, tumor resection is not a recommended treatment option for children with DIPG [35]. As a consequence, there has been a lack of tissue available to molecularly characterize tumors, develop targeted therapies, and generate representative preclinical models [60]. However, advances in surgical biopsy procedures have allowed for the excision of tissue from brainstem tumors with an acceptable level of morbidity, which has improved the availability of tissue for research and analysis [61]. The implementation of tissue biopsies for DIPG patients, coupled with the progression of molecular profiling techniques, has enabled clinicians and researchers to better characterize tumors, identify new treatment targets, and implement individualized therapy against the expressed molecular subtype [35,62,63]. For example, a multicenter clinical trial conducted by Kline et al (2022) collected biopsy tissue for mRNA and whole-exome sequencing to guide individualized treatment strategies for patients with newly diagnosed DIPG (NCT02274987) [64]. This precision medicine approach enabled patients to receive treatment based on the molecular profile of their tumor, and although the trial was unsuccessful in producing a clinical benefit, it enabled the identification of clinically relevant biomarkers of DIPG tumors, supporting future therapeutic strategies [64]. However, despite the advances in tumor profiling and surgical techniques, biopsies still carry rare but serious risks, such as hemorrhage, oedema, infection, and seizures [65].

3.2. Pharmacological therapies for DIPG over the last 5 years

Numerous chemotherapy, targeted, and immunotherapy agents have been investigated in both experimental studies and clinical trials for the treatment of children with DIPG (Tables 1–4). Treatment strategies have included single-agent and multidrug regimens, in combination with and without standard of care radiation therapy [22,47,59,66]. More recently, DIPG therapies have gravitated away from traditional chemotherapy agents and towards targeted and immunotherapy approaches, in both experimental and clinical trials, as shown in Tables 1–4. Traditional chemotherapies are relatively nonselective agents that typically impair DNA synthesis and mitosis, with mechanisms of action unable to discriminate between diseased DIPG cells and healthy tissue [67]. Although these agents are often effective in inducing anti-tumor effects and apoptosis, off-target toxicity in healthy cells limits their application in clinical practice [68]. Targeted therapies differ from traditional chemotherapy agents as they specifically target proteins that control the proliferation, progression, and survival of DIPG cells, such as growth factor receptors, tyrosine kinase receptors, metabolic enzymes, DNA repair enzymes, and signaling molecules, thereby minimizing off-target toxicity and improving on-target efficacy [35,67]. Targeted therapies also include those directed against epigenetic pathways involved in the progression and survival of DIPG, acting on proteins involved in histone acetylation, histone methylation, and DNA methylation [35,69]. Unlike both traditional chemotherapy and targeted therapies, immunotherapy aims to enhance the intrinsic defenses of the immune system, primarily harnessing T-cells to induce potent anti-tumor effects [70]. There are various types of immunotherapy agents that have been investigated for DIPG, including chimeric antigen receptor (CAR) T-cell therapy, immune checkpoint inhibitors, adoptive cell transfer, oncolytic viruses, and vaccines [71]. Pharmacological

Table 1
Pharmacological therapies in experimental *in vivo* efficacy studies for DIPG over the last five years.

Treatment	Class	Treatment Mechanism	Route	Animal	Study Description	Major Findings	Reference
Paxalisib Metformin Enzastaurin	Targeted therapy	PI3K/AKT/mTOR inhibitor (paxalisib) Biguanide that decreases hepatic glucose production and increases peripheral glucose utilization. Mitochondrial complex I inhibitor and AMPK activator (metformin) PKCβ inhibitor (enzastaurin)	PO	Mouse	Examined the efficacy of paxalisib, metformin, enzastaurin, ribociclib, and vandetanib, as monotherapies, combination therapies, and/or with standard of care radiotherapy, using pontine PDX models injected with SU-DIPG-XIII-P*, HSJD-DIPG-007, RA-055, or UON-VIBE5 cells or pontine immunocompetent syngeneic allograft models injected with IUE-24 cells.	-Combination therapy with paxalisib (+ metformin) and enzastaurin synergistically prolonged survival in PDX and immunocompetent allograft models. -Combination paxalisib (+ metformin) and enzastaurin therapy was further potentiated by standard of care radiotherapy.	[274]
YF-PRJ8-1011	Targeted therapy	CDK4/6 inhibitor	PO	Mouse	Investigated the efficacy of YF-PRJ8-1011, compared with palbociclib and in combination with radiation, in orthotopic brainstem PDX mouse models injected with TT150630 or TT190326 cells.	-YF-PRJ8-1011 significantly inhibited tumor growth and prolonged survival compared with palbociclib. -YF-PRJ8-1011 in combination with radiation showed increased inhibition of tumor growth than radiotherapy alone.	[85]
ASO5	Targeted therapy	Oligonucleotide that degrades H3-3A^{K27M} mRNA	ICV	Mouse	Investigated the efficacy of ASO5 in an immunocompetent mouse model using transduced mutant human H3-3A^{K27M} cDNA (tumor bearing in the midline region) and in an immunocompromised orthotopic PDX mouse model injected with SU-DIPG-XIII cells into the fourth ventricle/pons.	- ASO5 decreased tumor growth and increased survival in both mouse models.	[90]
Alisertib	Targeted therapy	Aurora kinase inhibitor	PO	Mouse	Examined the efficacy of alisertib in a genetically engineered mouse model bearing a high-grade brainstem tumor and in a pontine PDX model injected with SU-DIPG-XIII-P* cells.	-Alisertib failed to demonstrate anti-tumor efficacy.	[98]
Cannabidiol	Targeted therapy	Reduces ID1 expression by increasing ROS	IP	Mouse	Investigated the efficacy of cannabidiol in a brainstem PDX model injected with SU-DIPG-XIII-P* cells and in an *in utero* H3.3/H3.1K27M genetically engineered model.	-Cannabidiol significantly improved survival in both PDX and genetically engineered models.	[81]
Lonidamine	Targeted therapy	Inhibits mitochondrially bound hexokinase, suppressing aerobic glycolytic activity	IP	Mouse	Examined the ability of lonidamine to overcome treatment resistance mechanisms induced by panobinostat and marizomib using midbrain tumor models xenografted with either SU-DIPG-XIII drug-naive or SU-DIPG-XIII panobinostat and marizomib-resistant cells.	-Lonidamine significantly increased survival in both models, however demonstrated an even greater survival benefit in the panobinostat and marizomib-resistant model.	[91]
ONC201 Paxalisib	Targeted therapy	D2 dopamine receptor antagonist and mitochondrial caseinolytic protease P agonist that impairs oxidative phosphorylation to induce cancer cell apoptosis (ONC201) PI3K/AKT/mTOR inhibitor (paxalisib)	PO	Mouse	Examined the efficacy of ONC201 and paxalisib, as monotherapies and combination therapy, using pontine xenograft models injected with SU-DIPG-VI, HSJD-DIPG-007, or SF8628 cells.	-Combination therapy with paxalisib and ONC201 significantly extended the survival of mice in the SU-DIPG-VI and SF8628 xenograft models compared with monotherapies, whereas the combination had a synergistic effect in the HSJD-DIPG-007 model compared with monotherapies.	[74]
Mycophenolate mofetil	Targeted therapy	Immunosuppressant that inhibits lymphocyte proliferation and antibody formation	IP	Mouse	Investigated the efficacy of mycophenolate mofetil in a pontine PDX mouse model injected with SF8628 cells.	-Mycophenolate mofetil delayed tumor growth in the pontine PDX model, however failed to demonstrate a survival benefit.	[117]
WP1066	Targeted therapy	STAT3 pathway inhibitor	PO	Mouse	Assessed the efficacy of WP1066 in pontine mouse PDX models injected with PED17 or DIPG-XIII cells.	-WP1066 resulted in either stasis or regression of tumor growth in the PED17 PDX model. - WP1066 did not improve	[118]

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Table 1 (*continued*)

Treatment	Class	Treatment Mechanism	Route	Animal	Study Description	Major Findings	Reference
						survival in the DIPG-XIII PDX model.	
GSK126	Targeted therapy	EZH2 inhibitor	IP	Mouse	Examined the efficacy of GSK126, as monotherapy and in combination with atorvastatin, in an orthotopic brainstem model implanted with SU-DIPG-IV cells.	-GSK126 monotherapy demonstrated significant tumor growth reduction. -Low dose GSK126 in combination with atorvastatin demonstrated enhanced tumor growth inhibition in comparison with monotherapies.	[86]
Vandetanib Everolimus	Targeted therapy	Multi-kinase inhibitor of VEGFR/RET/EGFR (vandetanib) mTOR inhibitor (everolimus)	IP PO	Mouse	Investigated the efficacy of IP vandetanib and PO everolimus, as monotherapies and in combination, using models implanted with either patient-derived HSJD-DIPG-007 cells or a Nestin-Tv-a/Trp53$^{fl/fl}$/ Hist1h3b^{K27M}/ Acvr1^{R206H} cell allograft derived from a genetically engineered mouse model.	-Combination vandetanib and everolimus therapy extended survival and reduced tumor burden in the HSJD-DIPG-007 model compared with monotherapy, however, failed to show significant survival improvements in the genetically engineered model.	[92]
BAY2402234	Targeted therapy	Dihydroorotate dehydrogenase inhibitor	PO	Mouse	Investigated the efficacy of BAY2402234 in a pontine xenograft mouse model injected with DIPG1 or SU- DIPG-XIII-P* cells.	-BAY2402234 significantly reduced tumor burden and prolonged survival in both mouse models.	[275]
Venetoclax	Chemotherapy	Inhibits the anti-apoptotic B cell lymphoma 2 protein, triggering cell death	IP	Mouse	Evaluated the efficacy of venetoclax and radiation, as monotherapies and as combination therapy, in pontine PDX mouse models injected with BT245 or SU-DIPG-XIII* cells.	-Venetoclax monotherapy failed to demonstrate anti-tumor effects -Radiation monotherapy demonstrated initial tumor regression prior to tumor relapse. -Combination venetoclax and radiation therapy demonstrated the greatest survival benefit and reduction in tumor burden.	[276]
Tazemetostat	Targeted therapy	EZH2 inhibitor	IP	Mouse	Investigated the efficacy of tazemetostat in a brainstem Np53f; ABC knock-out mouse model injected with RCAS-PDGF-B, CRE, and H3.3K27M virus producing DF1 cells.	-Tazemetostat failed to significantly impact survival.	[87]
Trametinib	Targeted therapy	Mitogen-activated extracellular signal-regulated kinase inhibitor	PO	Mouse	Investigated the efficacy of trametinib in a PDX pontine mouse model injected with ICR-B169 cells.	-Trametinib failed to demonstrate an overall survival benefit in the PDX mouse model.	[119]
RG7388	Targeted therapy	Mouse double minute 2 homolog inhibitor which acts to reactivate the p53 pathway	PO	Mouse	Examined the efficacy of RG7388 in a brainstem xenograft model injected with HSJD-DIPG-007 cells.	-RG7388 significantly reduce tumor progression and prolonged survival in the xenograft mouse model.	[88]
AMXT-1501 DFMO	Targeted therapy	Polyamine transport inhibitor (AMXT-1501) Polyamine synthesis inhibitor (DFMO)	PO SC	Mouse	Investigated the efficacy of AMXT 1501 and DFMO, as monotherapies, dual therapy, and triple therapy with radiation, in PDX brainstem models injected with SU-DIPG-VI, HSJD-DIPG-007, or RA055 cells.	-In the SU-DIPG-VI and HSJD-DIPG007 models, combination DFMO and AMXT 1501 significantly extended survival compared to monotherapy. -In the RA055 model, combination DFMO and AMXT 1501 significantly enhanced survival, which was extended further with the addition of radiation, compared to monotherapy and dual radiation therapy.	[76]
KL-1	Targeted therapy	Disrupts the function of super elongation complex, decreasing transcriptional elongation	IP	Mouse	Investigated the efficacy of KL-1 in pontine PDX models injected with SF8628 cells.	-KL-1 significantly suppressed tumor growth and prolonged survival in the PDX models.	[277]
CBL0137 Panobinostat	Targeted therapy	Chromatin transcription complex inhibitor (CBL0137) Histone deacetylase inhibitor (panobinostat)	IV IP	Mouse	Investigated the efficacy of IV CBL0137 and IP panobinostat, as monotherapies and combination therapy, using PDX models injected with HSJD-DIPG-007 or	-CBL0137 significantly prolonged survival in the SU-DIPG-VI model. -Combination CBL0137 and panobinostat significantly	[77]

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Table 1 (*continued*)

Treatment	Class	Treatment Mechanism	Route	Animal	Study Description	Major Findings	Reference
					SU-DIPG-VI cells into the fourth ventricle/pons.	prolonged survival in both mouse models compared to monotherapy.	
ONC201 German sourced ONC201	Targeted therapy	D2 dopamine receptor antagonist and mitochondrial caseinolytic protease P agonist that impairs oxidative phosphorylation to induce cancer cell apoptosis (ONC201) Active angular isomer of ONC201 (German sourced ONC201)	PO	Mouse	Compared the efficacy of ONC201 with German sourced ONC201 using PDX models injected with SU-DIPG-VI or SU-DIPG-XIII-P* cells into the fourth ventricle/pons.	-ONC201 and German sourced ONC201 were equivalent in their ability to significantly prolong survival.	[75]
OKlahoma Nitrone-007 LDN-193189	Targeted therapy	Inhibits the expression of HIF-1 and VEGFR2 (OKlahoma Nitrone-007) Activin receptor-like kinase inhibitor (LDN-193189)	PO	Mouse	Examined the efficacy of OKlahoma Nitrone-007 compared with LDN-193189 using PDX mouse models injected with HSJD-DIPG-007 cells into the fourth ventricle.	-Both OKlahoma Nitrone-007 and LDN-193189 significantly reduced tumor volumes in the PDX models, with no significant difference found between the two treatments.	[93]
Everolimus Dasatinib	Targeted therapy	mTOR inhibitor (everolimus) Multi-kinase inhibitor, including PDGFRA (dasatinib)	PO	Mouse	Evaluated the efficacy of everolimus and dasatinib, as monotherapies and combination therapy, in mice harboring intrauterine electroporation generated high grade gliomas (mutant TP53, mutant PDGFRA, and H3K27M).	-Everolimus and dasatinib combination therapy significantly improved median survival compared to monotherapy.	[82]
E6201	Targeted therapy	Dual ACVR1 and mitogen-activated extracellular signal-regulated kinase 1/2 inhibitor	IP	Mouse	Investigated the efficacy of E6201 in a brainstem mouse model xenografted with SU-DIPG-XXXVI or HSJD-DIPG-007 cells.	-E6201 significantly prolonged survival in both mouse models.	[89]
Atuveciclib AZD4573	Targeted therapy	CDK9 inhibitors	PO IP	Mouse	Evaluated the efficacy of PO atuveciclib and IP AZD4573 as monotherapies in pontine xenograft mouse models injected with BT245 cells.	-Atuveciclib demonstrated a modest survival benefit in the PDX model. -AZD4573 demonstrated a greater overall survival benefit in the PDX model compared to atuveciclib.	[278]
Dichloroacetate Metformin	Targeted therapy	Pyruvate dehydrogenase kinase inhibitor (dichloroacetate) Biguanide that decreases hepatic glucose production and increases peripheral glucose utilization. Mitochondrial complex I inhibitor and AMPK activator (metformin)	PO	Mouse	Evaluated the efficacy of dichloroacetate and metformin, as monotherapies, dual therapy, and triple therapy in combination with radiation, in pontine xenograft models injected with HSJD-DIPG-007 cells.	-Dichloroacetate, metformin, and radiation triple therapy significantly prolonged survival, and demonstrated the longest survival benefit compared to all other treatment arms.	[279]
Panobinostat BGB324	Targeted therapy	Histone deacetylase inhibitor (panobinostat) Inhibitor of the AXL receptor tyrosine kinase (BGB324)	PO IP	Mouse	Evaluated the efficacy of PO BGB324 and two IP formulations of panobinostat (A and B), as monotherapies and in combination, in a pontine PDX model injected with HSJD-DIPG-07 cells or a DIPG murine UC-8D2 bearing allograft model.	-Combination BGB324 and panobinostat therapy significantly delayed tumor growth in both animal models. -Combination BGB324 and panobinostat A therapy in the HSJD-DIPG-07 animal model was poorly tolerated. -Combination BGB324 and panobinostat B therapy was better tolerated and demonstrated a significant increase in survival in the UC-8D2 model.	[280]
LDN-193189 LDN-214117	Targeted therapy	Activin receptor-like kinase inhibitors	PO	Mouse	Investigated the efficacy of LDN-193189 and LDN-214117 as monotherapies using xenograft models injected with HSJD-DIPG-007 or HSJD-GBM-001 cells into the fourth ventricle.	-No survival benefit was observed for either compound in the HSJD-GBM-001 model. -Both LDN-193189 and LDN-214117 significantly prolonged survival and decreased tumor cellularity in the HSJD-DIPG-007 model.	[94]
Palbociclib Erlotinib	Targeted therapy	CDK4/6 inhibitor (palbociclib)	PO	Mouse	Investigated the efficacy of palbociclib, as monotherapy and in combination with erlotinib, in	-Palbociclib monotherapy induced tumor shrinkage in all PDX models	[79]

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Table 1 (*continued*)

Treatment	Class	Treatment Mechanism	Route	Animal	Study Description	Major Findings	Reference
		EGFR inhibitor (erlotinib)			pontine xenograft models injected with TT150630, TT150728, or TT151201 cells.	-Palbociclib significantly prolonged survival in the TT150630 and TT150728 PDX models. -Combination palbociclib and erlotinib significantly prolonged survival in the TT150728 model.	
Panobinostat	Targeted therapy	Histone deacetylase inhibitor	IP	Mouse	Investigated the efficacy of IP panobinostat, as monotherapy and in combination with IV marizomib, PO BMS-754807, and PO selumetinib, in pontine PDX models injected with SU-DIPG-XIII-P* cells.	-Combination panobinostat and marizomib demonstrated the greatest survival benefit compared to all other treatments. -Selumetinib counteracted panobinostat's anti-tumor efficacy.	[78]
GSK-J4	Targeted therapy	Histone demethylase inhibitor	IP	Mouse	Investigated the efficacy of GSK-J4, as monotherapy and in combination with radiation therapy, in pontine PDX models injected with SF8628 cells.	-GSK-J4 in combination with radiation therapy was significantly more effective in reducing tumor growth rate and prolonging survival in the PDX models than single-agent therapy.	[281]
Panobinostat hAT-MSCs hAT-MSC.sTRAIL	Targeted therapy	Histone deacetylase inhibitor (panobinostat) Stem cell-based gene therapy (hAT-MSCs and hAT-MSC.sTRAIL)	IP IT	Mouse	Investigated the efficacy of IP panobinostat, as monotherapy and in combination with intra-tumoral hAT-MSCs and hAT-MSC.sTRAIL stem cell-based gene therapies, in pontine xenograft mouse models injected with DIPG-XIII cells.	-Panobinostat in combination with hAT-MSC.sTRAIL demonstrated the greatest survival benefit compared to all other treatment groups.	[282]
CUDC-907	Targeted therapy	Dual histone deacetylase and PI3K inhibitor	PO	Mouse	Investigated the efficacy of CUDC-907, as monotherapy and in combination with radiation, in pontine xenograft models injected with SF8628 cells.	-CUDC-907 and radiation monotherapy failed to increase survival significantly compared with control. -CUDC-907 in combination with radiation significantly increased survival compared with monotherapy.	[120]
OTSSP167	Targeted therapy	Maternal embryonic leucine zipper kinase inhibitor	PO	Mouse	Investigated the efficacy of OTSSP167 in pontine PDX models injected with JHH-DIPG-01 or VUMC-DIPG-F cells.	-OTSSP167 demonstrated significant inhibition of tumor growth in the JHH-DIPG-01 xenograft model. -OTSSP167 significantly prolonged survival in the VUMC-DIPG-F xenograft model.	[283]
PENAO Temsirolimus	Targeted therapy	Adenine nucleotide translocase inhibitor (PENAO) mTOR inhibitor (temsirolimus)	IP	Mouse	Investigated the efficacy of temsirolimus and PENAO, as monotherapies and as combination therapy, in PDX models xenografted with HSJD-DIPG-007 cells in the fourth ventricle.	-PENAO monotherapy failed to significantly improve median time to progression. -Temsirolimus monotherapy marginally prolonged survival -Combination PENAO and temsirolimus failed to significantly improve overall survival.	[95]
GD2-CAR NK-92 therapy	Immunotherapy	CAR-engineered natural killer cells designed to produce anti-tumor effects against disialoganglioside GD2-expressing glioma cells	ICV	Mouse	Investigated the efficacy of GD2-CAR NK-92 therapy in brainstem xenograft models injected with TT150630 (high GD2 expression) or TT190326 cells (low GD2 expression).	-GD2-CAR NK-92 significantly inhibited tumor growth and prolonged survival in the TT150630 model. -GD2-CAR NK-92 demonstrated limited anti-tumor efficacy in the TT190326 model.	[109]
B7-H3 CAR T-cell therapy	Immunotherapy	Human T-cells that have been re-engineered to mediate antitumor activity against B7-H3-expressing glioma cells	IC	Mouse	Investigated the efficacy of IC B7-H3 CAR T-cell therapy in cerebral xenograft models injected with U87 cells.	-B7-H3 CAR T-cell therapy significantly prolonged survival in the mouse xenograft model.	[103]
HER2 CAR T-cell therapy	Immunotherapy	Human T-cells that have been re-engineered to mediate antitumor activity against HER2-expressing glioma cells	IV	Mouse	Investigated the efficacy of HER2 CAR T-cell therapy in intracranial PDX models injected with SU-DIPG36 cells.	-HER2 CAR T-cell therapy demonstrated a significant reduction in tumor burden in the PDX model.	[73]
Delta-24-ACT	Immunotherapy	Oncolytic adenovirus genetically modified to express the costimulatory 4-1BBL ligand to further co-	IT	Mouse	Investigated the efficacy of Delta-24-ACT in immunocompetent pontine	-Delta-24-ACT significantly extended survival in both models. -Delta-24-ACT demonstrated	[104]

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Table 1 (*continued*)

Treatment	Class	Treatment Mechanism	Route	Animal	Study Description	Major Findings	Reference
		stimulate anti-tumor immune responses			allograft models implanted with NP53 or XFM cells.	significantly better efficacy than standard radiotherapy treatment in the NP53 model.	
$\alpha_v\beta_3$ CAR T-cell therapy	Immunotherapy	Human T-cells that have been re-engineered to mediate antitumor activity against $\alpha_v\beta_3$ integrin complex-expressing glioma cells	IT	Mouse	Investigated the efficacy $\alpha_v\beta_3$ CAR T-cells in PDX pontine models injected with SU-DIPG-36 cells.	- $\alpha_v\beta_3$ CAR T-cell therapy significantly reduced tumor burden, slowed tumor relapse, and extended survival.	[110]
GD2 CAR T-cell therapy Linsitinib	Immunotherapy Targeted therapy	T-cells that have been re-engineered to mediate antitumor activity against disialoganglioside GD2-expressing glioma cells (GD2-CAR T-cell therapy) Dual insulin-like growth factor 1 receptor and insulin receptor inhibitor (linsitinib)	IV PO	Mouse	Investigated the efficacy IV GD2-CAR T-cell therapy and PO linsitinib, as monotherapies and in combination, in pontine xenograft models injected with SU-DIPG-VI cells.	-Linsitinib monotherapy failed to demonstrate anti-tumor efficacy -Single-agent GD2-CAR T-cell therapy demonstrated a sustained reduction in tumor burden at higher doses. -Combination therapy of GD2-CAR T-cell therapy and linsitinib demonstrated a sustained anti-tumor effect at a lower GD2-CAR T-cell therapy dose.	[111]
CRAd.S.pK7 loaded mesenchymal stem cells	Immunotherapy	Oncolytic adenovirus that is delivered to DIPG tumor tissue by mesenchymal stem cells	IC IN	Mouse	Evaluated the efficacy of IN/IC CRAd.S.pK7 loaded mesenchymal stem cells, as monotherapy and in combination with radiation, in pontine PDX models injected with SF8628 cells.	-IC administration of CRAd.S.pK7 loaded mesenchymal stem cells failed to show a survival benefit as monotherapy. -IC administration of CRAd.S.pK7 loaded mesenchymal stem cells in combination with radiation therapy significantly improved survival compared to monotherapy. -IN administration of CRAd.S.pK7 loaded mesenchymal stem cells in combination with radiation therapy failed to show a survival benefit.	[113]
Ad-CD40L Ad-GFP rAd-Δ24-luc rAd-Δ24-CD40L	Immunotherapy	Oncolytic adenoviruses targeting the cluster of differentiation (CD)40 ligand	IT	Mouse	Evaluated the efficacy of Ad-CD40L, Ad-GFP, rAd-Δ24-luc, and rAd-Δ24-CD40L in immune-competent brainstem allograft mouse models implanted with GL261 or CT2A cells.	-High doses of Ad-CD40L significantly resulted in long-term cures compared with Ad-GFP, however required supportive care to avoid significant toxicity and death. -rAd-Δ24-CD40L was better tolerated than Ad-CD40L and significantly improved survival in both mouse models when compared with rAd-Δ24-luc. -rAd-Δ24-CD40L resulted in at least 50% cure rates in both mouse models.	[72]
Thymidine kinase and fms-like tyrosine kinase 3 ligand (TK/Flt3L) gene therapy	Immunotherapy	Immune-stimulatory gene therapy delivered by adenoviruses	IT	Mouse	Investigated the efficacy of TK/Flt3L immune-stimulatory gene therapy in a genetically engineered, immunocompetent mACVR1 pontine model.	-TK/Flt3L therapy significantly prolonged median survival compared to standard of care radiation therapy.	[284]
Delta-24-RGD	Immunotherapy	Oncolytic adenovirus	IT	Mouse	Investigated the efficacy of Delta-24-RGD in immunodeficient pontine xenograft models (injected with TP80 and TP54 cells) and immune-competent orthotopic mouse models (implanted with NP53 or XFM cells).	-Delta-24-RGD significantly increased the overall survival in both immunodeficient and immunocompetent animal models.	[105]
Delta-24-RGD	Immunotherapy	Oncolytic adenovirus	IT	Mouse	Investigated the efficacy of Delta-24-RGD and radiation therapy in pontine xenograft models injected with TP54 cells.	-Delta-24-RGD in combination with radiation therapy was feasible and demonstrated a significant increase in survival when compared to radiotherapy alone.	[112]
GD2 CAR T-cell therapy	Immunotherapy	T-cells that have been re-engineered to mediate antitumor activity against	IV	Mouse	Investigated the efficacy of GD2-CAR T-cell therapy in pontine PDX models injected with SU-DIPG-VI or SU-DIPG-XIII-P cells.	-GD2-targeted CAR T-cell therapy reduced tumor burden in both animal models.	[80]

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Table 1 (*continued*)

Treatment	Class	Treatment Mechanism	Route	Animal	Study Description	Major Findings	Reference
		disialoganglioside GD2-expressing glioma cells				-GD2-targeted CAR T-cell therapy increased survival in the SU-DIPG-XIII-P* model.	
T-cell receptor (TCR)-transduced T-cells	Immunotherapy	Specifically targets the H3.3K27M epitope expressed by H3.3K27M^{+}HLA-A*0201^{+} tumor cells	IV	Mouse	Investigated the efficacy of TCR-transduced T-cells in intracranial xenograft models injected with U87H3.3K27M-expressing cells.	-Adoptive transfer of TCR-transduced T-cells demonstrated a significant reduction in tumor burden.	[96]

Abbreviations: Activin A receptor type 1 (ACVR1); Adenosine monophosphate–activated protein kinase (AMPK); By mouth (PO); Chimeric antigen receptor (CAR); Cyclin-dependent kinase (CDK); Difluoromethylornithine (DFMO); Diffuse intrinsic pontine glioma (DIPG); Enhancer of zeste homolog 2 (EZH2); Epidermal growth factor receptor (EGFR); Disialoganglioside GD2 (GD2); Human adipose tissue-derived mesenchymal stem cells (hAT-MSGs); Human adipose tissue-derived mesenchymal stem cells expressing tumor necrosis factor-related apoptosis-inducing ligand (hAT-MSC.sTRAIL); Human epidermal growth factor receptor 2 (HER2); Hypoxia inducible factor 1 (HIF-1); Inhibitor of DNA binding 1 (ID1); Intra-cranial (IC); Intracerebroventricular (ICV); Intraperitoneal (IP); Intra-tumoral (IT); Intravenous (IV); Mammalian target of rapamycin (mTOR); Natural killer cells (NK); Patient-derived xenograft (PDX); Patient derived growth factor beta (PDGF-B); Phosphatidylinositol-3 kinase (PI3K); Platelet derived growth factor receptor alpha (PDGFRA); Protein kinase-B (AKT); Protein kinase-Cβ (PKCβ); Reactive oxygen species (ROS); Rearranged during transfection (RET); Replication competent avian sarcoma-leucosis (RCAS); Signal transducer and activator of transcription 3 (STAT3); Subcutaneous (SC); Tumor necrosis factor ligand superfamily member 9 (4-1BBL); Tumor protein p53 (TP53); Vascular endothelial growth factor (VEGF); Vascular endothelial growth factor receptor (VEGFR); Vascular endothelial growth factor receptor 2 (VEGFR2).

agents investigated in experimental studies and clinical trials over the last five years for DIPG are summarized in Table 1 and Table 2, respectively.

To better represent the human disease state and evaluate the biological barriers affecting brain drug delivery in DIPG, Table 1 was restricted to *in vivo* studies and orthotopic experimental models. Despite the lack of effective treatments clinically available for DIPG, the majority of experimental studies reported statistically significant improvements in tumor regression and/or overall survival when compared with controls, monotherapies, and/or standard of care radiation therapy, demonstrating a range of pharmacological treatments, particularly targeted and immunotherapy agents, to possess therapeutic efficacy in DIPG. A study conducted by Wongthida et al (2020), who evaluated the efficacy of oncolytic adenoviruses targeting the cluster of differentiation (CD)40 ligand, even demonstrated a cure rate of at least 50% in murine models [72]. Many experimental pharmacological agents (Table 1) have progressed to clinical trials (Table 2), demonstrating promise in the development of new therapies for DIPG [73–80]. However, there are a range of factors which may underpin the lack of clinical success yielded by experimental therapies, impacting the overall translatability of preclinical data. These factors include the: (i) type of animal model; (ii) tumor location; (iii) type of cells utilized for tumor generation; (iv) route of administration; (v) extent of survival benefit; (vi) efficacy in combination with standard of care radiation therapy and other pharmacological therapies; and (vii) treatment safety and tolerability.

Firstly, the type of animal model should be considered when interpreting the translatability of study results. For example, generating orthotopic tumors by injecting tumor cells or genetically engineered vectors intracranially may induce mechanical damage and inflammation at the BBB, potentially resulting in increased permeability and enhanced efficacy of the therapeutic agent *in vivo*, which may not translate in clinical practice. However, tumor generation by *in utero* electroporation, demonstrated in the studies conducted by Messinger et al (2023) and Miklja et al (2020), has the potential to generate spontaneous orthotopic DIPG tumors in murine models without disrupting the BBB, which may mitigate the limitations imposed by injecting tumor cells or vectors intracranially [81–83]. Another important consideration when evaluating the animal model, is the location in which the tumor is generated. Ideally, for DIPG experimental studies, orthotopic tumors should be established in the pons, or at a minimum, in the brainstem, to recapitulate human DIPG characteristics [84]. Although the majority of studies generated tumors in the pons, a range of tumor locations were observed in the experimental studies, including brainstem [72,76,81,85–89], midline region [90], midbrain [91], fourth ventricle [92–95], and unknown tumor locations [73,82,96]. Moreover, all studies included in Tables 1 and 3, except for Louis et al (2018) and Power et al (2023), exclusively utilized DIPG murine models [97,98]. Patient-derived xenograft and genetically engineered mouse models of DIPG/DMG are the current gold standard for evaluating preclinical therapies, as they are able to recapitulate the molecular and histopathological features of the human disease [84,99]. Additionally, mice are one of the most frequently used animals to model the BBB, sharing many biological features to that of the human BBB [100]. However, interspecies differences between humans and mice cannot be denied, such as differences in their anatomical size, which may impact the translation of experimental data to the clinic [101]. A recent commentary by Koschmann et al (2024) described the future possibility of generating DIPG/DMG models in larger species, such as rats (which were utilized by Louis et al [2018] and Power et al [2023]), swine, or ferrets, in order to better recapitulate the physiology of humans, and hopefully, improve the translation of preclinical data [97,98,102].

Significant variability in the type, origin, and aggressiveness of cells used for tumor generation is also apparent across the DIPG studies. For example, the U87 cell line, xenografted by Vitanza et al (2023), was originally derived from a patient with glioblastoma, which differs from DIPG in its pathogenesis, molecular profile, and cellular origin [51,103]. Wongthida et al (2020) implanted murine derived cells, which were derived from diseases dissimilar to DIPG, with GL261 originating from a glioblastoma model and CT2A originating from a subcutaneous, non-metastatic glioma model [72]. XFM and NP53 cell lines allografted in the studies conducted by Laspidea et al (2022) and Martinez-Velez et al (2019) are also of murine origin, however, were derived from a genetically modified model harboring a murine brainstem glioma [104,105]. Where possible, cell lines used to generate DIPG tumors for *in vivo* experimental studies should originate from the same disease and be derived from human cells in order to support clinical translation [106].

However, xenografting human DIPG cells requires an immunocompromised model in order to prevent graft rejection, which is problematic when investigating the efficacy and safety of immunotherapy agents [107]. Ideally, immunodeficient animals should be humanized prior to engraftment, either with human peripheral blood mononuclear cells or CD34^{+} immune cells, to facilitate more representative immune responses in the host, allowing for better interpretations of experimental immunotherapy interventions [108]. This should be noted when observing the results obtained by the immunotherapy studies shown in Table 1, as the majority of studies used immunodeficient models without prior humanization [73,80,96,103,109–113]. However, more recently, a study conducted by du Chatinier (2022) et al generated immunocompetent DMG mouse models by orthotopically implanting primary murine tumor cells, that were generated by brainstem-targeted intrauterine electroporation, into syngeneic mice. These models were able to recapitulate the growth pattern, morphology, and immunologic

Table 2
Pharmacological therapies in clinical trials for DIPG over the last five years (Ref: clinicaltrials.gov).

Treatment	Class	Treatment Mechanism	Study Phase	Status	Study Size	Age	Study Description	Clinical Trial Identifier	Year
Ribociclib Everolimus	Targeted therapy	CDK4/6 inhibitor (ribociclib) mTOR inhibitor (everolimus)	2	Not yet recruiting	100	1–39 years	To evaluate the efficacy of PO ribociclib and everolimus in patients with newly diagnosed high-grade glioma, including DIPG.	NCT05843253	2023
AMXT-1501 DFMO	Targeted therapy	Polyamine transport inhibitor (AMXT-1501) Polyamine synthesis inhibitor (DFMO)	1 / 2	Recruiting	56	≥ 12 years	To determine the safety and maximum tolerated dose of PO AMXT-1501 in combination with IV DFMO for patients with advanced solid tumors or DIPG.	NCT05500508	2022
Lutathera	Targeted therapy	Radiolabelled somatostatin analogue that binds to type-2A somatostatin receptors, inducing anti-tumor effects through the release of lutetium-177	1 / 2	Recruiting	65	≤ 4 years	To evaluate to safety and efficacy of IV lutathera in patients with progressive/ recurrent high grade CNS tumors, including DIPG.	NCT05278208	2022
ONC201	Targeted therapy	D2 dopamine receptor antagonist and mitochondrial caseinolytic protease P agonist that impairs oxidative phosphorylation to induce cancer cell apoptosis	3	Recruiting	409	≥ 6 months	To evaluate the efficacy and safety of PO ONC201 and radiation in comparison with PO everolimus and radiation in DIPG.	NCT05476939	2022
ONC201 Paxalisib	Targeted therapy	D2 dopamine receptor antagonist and mitochondrial caseinolytic protease P agonist that impairs oxidative phosphorylation to induce cancer cell apoptosis (ONC201) PI3K/AKT/mTOR inhibitor (paxalisib)	2	Active, not recruiting	143	2–39 years	To evaluate the efficacy of PO ONC201 and PO paxalisib therapy with concurrent radiation for patients with DMGs including DIPG.	NCT05009992	2021
Selinexor	Targeted therapy	Exportin-1 (XPO1) inhibitor	1 / 2	Suspended	210	1–21 years	To evaluate the toxicity, side effects, and maximum tolerated dose of PO selinexor with concurrent radiation therapy for patients with newly diagnosed DIPG or other high-grade gliomas.	NCT05099003	2021
BXQ-350	Targeted therapy	Anti-neoplastic agent targeting sphingolipid metabolism resulting in cancer cell death	1	Recruiting	22	1–30 years	To assess the safety and maximum tolerated dose of IV BXQ-350 in combination with radiation therapy for patients with newly diagnosed DIPG or DMG.	NCT04771897	2021
CBL0137	Targeted therapy	Inhibits NF-κB transcription and activates TP53	1 / 2	Recruiting	95	1–30 years	To evaluate the maximum tolerated dose, safety, and efficacy of IV CBL0137 for patients with solid tumors or lymphoma, including DIPG.	NCT04870944	2021
NKTR-214 Nivolumab	Targeted therapy	IL-2 pathway agonist (NKTR-214) PD-1 inhibitor (nivolumab)	1 / 2	Terminated	15	≤ 30 years	To evaluate the safety, tolerability, and efficacy of nivolumab and NKTR-214 in patients with recurrent/ refractory malignancies, including DIPG.	NCT04730349	2021
Nimotuzumab	Targeted therapy	EGFR inhibitor	3	Recruiting	48	3–15 years	To evaluate the safety and efficacy of IV nimotuzumab with concurrent radiochemotherapy using intensity modulated radiotherapy and PO temozolamide for patients with newly diagnosed DIPG.	NCT04532229	2020
Larotrectinib	Targeted therapy	Tropomysoin receptor kinase inhibitor	1	Recruiting	15	≤ 21 years	To evaluate the efficacy and safety of PO larotrectinib in patients with NTRK fusion-positive high-grade gliomas, including DIPG.	NCT04655404	2020

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Table 2 (*continued*)

Treatment	Class	Treatment Mechanism	Study Phase	Status	Study Size	Age	Study Description	Clinical Trial Identifier	Year
Marizomib Panobinostat	Targeted therapy	Proteasome inhibitor (marizomib) Histone deacetylase inhibitor (panobinostat)	1	Terminated	4	≤ 21 years	To evaluate the tolerability, safety, and efficacy of IV marizomib given as monotherapy and in combination with PO panobinostat for patients with DIPG.	NCT04341311	2020
9-ING-41	Targeted therapy	GSK-3β inhibitor	1	Active, not recruiting	68	≤ 22 years	To evaluate the safety and efficacy of IV 9-ING-41 as monotherapy or in combination with various chemotherapy agents in patients with advanced malignancies, including DIPG.	NCT04239092	2020
Abemaciclib	Targeted therapy	CDK4/6 inhibitor	1 / 2	Recruiting	117	≤ 21 years	To evaluate the safety and efficacy of PO abemaciclib given in combination with a range of anti-cancer treatments for patients with relapsed/refractory solid tumors, including DIPG.	NCT04238819	2020
Bevacizumab	Targeted therapy	VEGF inhibitor	2	Recruiting	40	3–18 years	To evaluate the efficacy and safety of low dose IV bevacizumab in combination with standard radiotherapy and ultra-low dose radiation therapy in patients with DIPG.	NCT04250064	2020
Fimepinostat	Targeted therapy	Dual inhibitor of histone deacetylase and PI3K	1	Active, not recruiting	30	3–39 years	To evaluate the BBB penetration, safety, and efficacy of PO fimepinostat in combination with standard of care surgery (e.g., biopsy) for patients with newly diagnosed DIPG and other brain tumors.	NCT03893487	2019
Indoximod	Targeted therapy	IDO pathway inhibitor	2	Recruiting	140	3–21 years	To evaluate the efficacy of PO indoximod in combination with various chemotherapy and radiation regimens for patients with newly diagnosed DIPG or other progressive brain cancers.	NCT04049669	2019
ONC201	Targeted therapy	D2 dopamine receptor antagonist and mitochondrial caseinolytic protease P agonist that impairs oxidative phosphorylation to induce cancer cell apoptosis	1	Active, not recruiting	130	2–18 years	To determine a phase 2 dose of PO ONC201 either as a monotherapy or in combination with radiation in patients with pediatric H3K27M gliomas, including DIPG.	NCT03416530	2018
PTC596	Targeted therapy	Small-molecule tubulin-binding agent that interrupts microtubule assembly, inducing cell cycle arrest and apoptosis	1	Active, not recruiting	64	1–21 years	To evaluate the maximum tolerated dose and safety of PO PTC596 in combination with radiation therapy for patients with newly diagnosed high-grade glioma, including DIPG.	NCT03605550	2018
Panobinostat Everolimus	Targeted therapy	Histone deacetylase inhibitor (panobinostat) mTOR inhibitor (everolimus)	2	Withdrawn	0	2–30 years	To evaluate the efficacy of PO panobinostat and everolimus for patients with gliomas harboring H3.1 or H3.3K27M mutations, including DIPG.	NCT03632317	2018
Paxalisib (GDC-0084)	Targeted therapy	PI3K/AKT/mTOR inhibitor	1	Complete	27	2–21 years	Evaluated the tolerability, safety, and maximum tolerated dose of PO paxalisib (GDC-0084) for patients with DIPG or other H3K27M-mutant gliomas following radiation therapy.	NCT03696355	2018
Savolitinib (Volitinib)	Targeted therapy	c-MET tyrosine kinase inhibitor	1	Recruiting	50	6–21 years	To evaluate the safety and maximum tolerated dose of PO savolitinib for patients	NCT03598244	2018

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Table 2 (*continued*)

Treatment	Class	Treatment Mechanism	Study Phase	Status	Study Size	Age	Study Description	Clinical Trial Identifier	Year
							with recurrent/refractory primary CNS tumors, including DIPG.		
Palbociclib	Targeted therapy	CDK4/6 inhibitor	1 / 2	Active, not recruiting	128	2–20 years	To evaluate the safety, maximum tolerated dose, and efficacy of PO palbociclib given in combination with a range of chemotherapy agents in patients with recurrent/ refractory solid tumors, including DIPG.	NCT03709680	2018
CLR 131	Targeted therapy	Radiolabelled therapeutic agent that exploits a tumor-specific phospholipid uptake mechanism, inducing anti-tumor effects through the release of iodine-131	1	Active, not recruiting	30	2–25 years	To evaluate the safety and efficacy of IV CLR 131 for patients with lymphoma or relapsed/refractory tumors, including DIPG.	NCT03478462	2018
APX005M	Targeted	Cluster of differentiation (CD)40 agonist that inhibits tumor growth and triggers apoptosis	1	Active, not recruiting	32	1–21 years	To evaluate the safety and maximum tolerated dose of APX005M for patients with newly diagnosed DIPG or recurrent/refractory CNS tumors.	NCT03389802	2018
SC-CAR4BRAIN CAR T-cell therapy	Immunotherapy	Administration of re-engineered autologous T-cells to mediate antitumor activity against a combination of B7-H3, EGFR806, HER2, and IL13-zetakine expressing tumor cells	1	Recruiting	72	1–26 years	To evaluate the maximum tolerated dose, safety, and feasibility of IV SC-CAR4BRAIN CAR T-cell therapy for patients with DIPG, DMG, or recurrent/ refractory CNS tumors.	NCT05768880	2023
iC9-GD2-CAR T-cell therapy	Immunotherapy	Administration of iC9 genetically modified autologous T-cells to mediate antitumor activity against disialoganglioside GD2-expressing glioma cells	1	Recruiting	54	6 months – 30 years	To evaluate the efficacy and safety of IV iC9-GD2-CAR T-cell therapy for patients with relapsed/refractory CNS tumors, including DIPG.	NCT05298995	2022
Ad-TD-nsIL12	Immunotherapy	Oncolytic virus	1	Recruiting	18	1–18 years	To evaluate the safety, tolerability, and side effects of intra-tumoral Ad-TD-nsIL12 in patients with primary DIPG.	NCT05717712	2023
Ad-TD-nsIL12	Immunotherapy	Oncolytic virus	1	Recruiting	18	1–18 years	To evaluate the safety, tolerability, and side effects of intra-tumoral Ad-TD-nsIL12 in patients with progressive DIPG.	NCT05717699	2023
AloCELYVIR	Immunotherapy	Oncolytic virus	1 / 2	Recruiting	12	1–21 years	To evaluate the efficacy and safety of AloCELYVIR in combination with radiation therapy for patients with newly diagnosed DIPG, or as monotherapy for patients with relapsed/progressive medulloblastoma.	NCT04758533	2021
TTRNA-DCs TTRNA-xALT	Immunotherapy	Adoptive cell-based therapies derived from autologous dendritic cells (TTRNA-DCs) and autologous T-cells (TTRNA-xALT) with immunostimulatory and anti-tumor effects	1	Recruiting	24	1–30 years	To evaluate the maximum tolerated dose, safety, and feasibility of TTRNA-DCs and TTRNA-xALT immunotherapy products for patients with newly diagnosed DIPG or recurrent neuroblastoma.	NCT04837547	2021
SurVaxM vaccine	Immunotherapy	Anti-tumor vaccine targeting the survivin protein, which is highly expressed in certain pediatric malignancies	1	Recruiting	35	1–21 years	To evaluate the tolerability, safety, and effects of SC SurVaxM for patients with newly diagnosed DIPG and other CNS malignancies.	NCT04978727	2021
Histone H3.3-K27M vaccine	Immunotherapy	Activates neoantigen specific T-cells and triggers cytotoxic T-cell immune responses to eradicate H3.3-K27M-expressing DIPG cells	1	Recruiting	30	≥ 5 years	To determine the safety and efficacy of the SC histone H3.3-K27M neoantigen vaccine in combination with standard therapy for patients with newly diagnosed DIPG.	NCT04749641	2021

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Table 2 (*continued*)

Treatment	Class	Treatment Mechanism	Study Phase	Status	Study Size	Age	Study Description	Clinical Trial Identifier	Year
rHSC-DIPGVax vaccine Balstilimab Zalifrelimab	Immunotherapy	Immunostimulatory heat shock protein vaccine containing neoantigenic peptides native to DIPG and DMG tumors (rHSC-DIPGVax) Immune checkpoint inhibitor directed against PD-1 (balstilimab) Immune checkpoint inhibitor directed against CTLA-4 (zalifrelimab)	1	Recruiting	36	1–18 years	To assess the safety, tolerability, and efficacy of rHSC-DIPGVax in combination with balstilimab and zalifrelimab for patients with newly diagnosed DIPG or DMG post radiation therapy.	NCT04943848	2021
PEP-CMV vaccine	Immunotherapy	Immunostimulatory peptide vaccine directed against the pp65 CMV antigen expressed in malignant tumors	2	Not yet recruiting	120	3–25 years	To evaluate the safety and efficacy of the intradermally administered PEP-CMV vaccine for patients with recurrent medulloblastoma, newly diagnosed DIPG, or other newly diagnosed high-grade gliomas following intradermal tetanus-diphtheria vaccination and PO temozolomide therapy.	NCT05096481	2021
Dendritic cell vaccination	Immunotherapy	Dendritic cells loaded with tumor-specific antigens activate native antigen-specific T-cells that selectively target and eliminate antigen-expressing tumor cells	1 / 2	Active, not recruiting	10	1–17 years	To evaluate the safety and feasibility of intradermally administered dendritic cell vaccination in combination with either temozolomide-based chemoradiation or standard anti-glioma treatment for patients with DIPG or other high-grade gliomas.	NCT04911621	2021
Dendritic cell vaccination	Immunotherapy	Dendritic cells loaded with tumor-specific antigens activate native antigen-specific T-cells that selectively target and eliminate antigen-expressing tumor cells	1	Unknown	10	1–75 years	To evaluate the safety and efficacy of dendritic cell vaccination injected near tumor lymphoid tissue in combination with cyclophosphamide and bevacizumab for patients with DIPG or glioblastoma who have received standard anti-glioma treatment.	NCT03914768	2019
C7R-GD2 CAR T-cell therapy	Immunotherapy	Administration of C7R genetically modified autologous T-cells to mediate antitumor activity against disialoganglioside GD2-expressing glioma cells	1	Recruiting	34	1–21 years	To evaluate the dose limiting toxicity and anti-tumor response of IV/ICV C7R-GD2 CAR T-cell therapy in combination with fludarabine and cyclophosphamide for patients with GD2-expressing brain tumors, including DIPG.	NCT04099797	2019
GD2 CAR T-cell therapy	Immunotherapy	Administration of re-engineered autologous T-cells to mediate antitumor activity against disialoganglioside GD2-expressing glioma cells	1	Recruiting	54	2–30 years	To evaluate the safety, feasibility, and maximum tolerate dose of IV/ICV GD2-CAR T-cells for patients with H3K27M-mutant DIPG or spinal H3K27M-mutant DMG following treatment with fludarabine and cyclophosphamide.	NCT04196413	2019
B7-H3 CAR T-cell therapy	Immunotherapy	Administration of re-engineered autologous T-cells to mediate antitumor activity against B7-H3-expressing tumor cells	1	Recruiting	90	1–26 years	To evaluate the safety and feasibility of administering B7-H3-specific CAR T-cell therapy into the ventricular system or tumor resection cavity for patients with CNS tumors, including DIPG.	NCT04185038	2019
TTRNA-DC vaccination TTRNA-xALT	Immunotherapy	Adoptive cell-based therapies derived from autologous dendritic cells (TTRNA-DC vaccination)	1	Active, not recruiting	21	3–30 years	To evaluate the maximum tolerated dose, safety, and feasibility of various immunotherapy products in	NCT03396575	2018

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Table 2 (*continued*)

Treatment	Class	Treatment Mechanism	Study Phase	Status	Study Size	Age	Study Description	Clinical Trial Identifier	Year
		and autologous T-cells (TTRNA-xALT) with immunostimulatory and anti-tumor effects					combination with different chemotherapy regimens for patients with brainstem gliomas, including DIPG.		
Cemiplimab (REGN2810)	Immunotherapy	Immune checkpoint inhibitor directed against PD-1	1 / 2	Terminated	57	≤ 25 years	To evaluate the safety and efficacy of IV cemiplimab as monotherapy and in combination with radiation therapy for patients with newly diagnosed DIPG and other gliomas.	NCT03690869	2018
Gemcitabine	Chemotherapy	Nucleoside analogue that inhibits DNA synthesis and induces apoptosis in cancer cells	1	Withdrawn	0	3–17 years	To determine the presence of gemcitabine in tumor tissue and quantify the intratumoral gemcitabine concentration following IV drug administration for patients with newly diagnosed DMG, including DIPG.	NCT04051047	2019

Abbreviations: Blood-brain barrier (BBB); B7 homolog 3 protein (B7-H3); By mouth (PO); Chimeric antigen receptor (CAR); Cyclin-dependent kinase (CDK); Cytomegalovirus (CMV); Central nervous system (CNS); Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4); Dendritic cells (DCs); Diffuse intrinsic pontine glioma (DIPG); Diffuse midline glioma (DMG); Epidermal growth factor receptor (EGFR); Disialoganglioside GD2 (GD2); Glycogen synthase kinase-3 beta (GSK-3β); Human epidermal growth factor receptor 2 (HER2); Indoleamine 2,3-dioxygenase (IDO); Intracerebroventricular (ICV); Interleukin-2 (IL-2); Interleukin-13 (IL-13); Inducible caspase 9 (iC9); Intravenous (IV); Mammalian target of rapamycin (mTOR); Mesenchymal epithelial transition factor (c-MET); Neurotrophic tyrosine receptor kinase (NTRK); Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); Phosphatidylinositol-3 kinase (PI3K); Platelet derived growth factor receptor alpha (PDGFRA); Programmed cell death 1 protein (PD-1); Protein kinase-B (AKT); Rearranged during transfection (RET); Subcutaneous (SC); Total tumor RNA (TTRNA); Tumor protein p53 (TP53); Vascular endothelial growth factor (VEGF); Vascular endothelial growth factor receptor (VEGFR).

characteristics of human DMG, which may help to mitigate the issues surrounding preclinical immunotherapy studies in the future [114].

Another consideration that likely impacts therapeutic efficacy *in vivo* and clinical translation are the timepoints in which cell lines were originally derived from DIPG patients. In comparison to biopsy-derived tissue, autopsy-derived models are likely to be more aggressive and have prior exposure to treatment, which may alter the epigenetic and genetic landscape of the DIPG model [84]. For example, SU-DIPG-IV, SU-DIPG-VI, and JHH-DIPGI represent autopsy-derived cells, all of which have had prior radiation and/or pharmacological exposure [20].

Various routes of drug administration were employed across the studies included in Table 1, ranging from relatively non-invasive routes, such as oral, intranasal, intravenous, intraperitoneal, and subcutaneous, to invasive routes, including intratumoral, intracerebral, and intracerebroventricular [115]. Although intratumoral, intracerebral, and intracerebroventricular routes of administration all share the advantage of bypassing the BBB to facilitate direct drug delivery, they are significantly limited by their invasiveness, complex injection technique, and morbidity risks [115]. Non-invasive routes of drug administration, such as oral, intravenous, and intraperitoneal, are also hindered by off-target effects, exposure to systemic degradation pathways, and the BBB, which greatly restricts the ability of drugs to penetrate the tumor site [115,116]. Moreover, the safety and tolerability of interventions must also be assessed in order to determine their clinical translation, which is yet to be completed for many studies included in Table 1.

In regard to efficacy findings, pharmacological agents were mostly investigated in single cell line generated tumor models, and although this is likely due to feasibility and financial reasons, it may not truly reflect the heterogeneity of DIPG. Moreover, efficacy of investigated agents should also be studied in combination therapy, either with current standard of care radiation therapy and/or other pharmacological agents, given the diffusely infiltrative, heterogeneous, and resistive nature of the disease [12,22,46,47]. Overall, combination pharmacological therapy was investigated by ~40% of the experimental studies included in Table 1. Although survival was extended in the majority of studies, a lack of curative approaches emerged from the data. This is likely due to the inherent intractability of the disease, compounded by tumor resistance mechanisms, tumor heterogeneity, and importantly, lack of drug penetration across the BBB [95,98,117–120].

4. Biological barriers in the brain to effective drug delivery

The three main barriers formed between the cerebrovasculature and the brain parenchyma influencing brain drug delivery include the blood-brain barrier (BBB), the blood-cerebrospinal fluid barrier (BCSFB), and the arachnoid barrier (Fig. 1) [121,122]. The BBB forms a structural and enzymatic transport barrier between the cerebral capillaries and the brain parenchyma. A range of transporters and metabolizing enzymes are expressed by the BBB, functioning to protect the brain from neurotoxins, supply the brain with essential nutrients, and regulate drug, ion, neurotransmitter, macromolecule transport [122]. Of the three barriers separating the vasculature from the brain tissue, the BBB is the main regulator of blood and CNS material exchange, and as a result, the BBB forms the focus of this review. However, the BCSFB and arachnoid barrier also influence brain drug delivery and should be considered when designing brain drug delivery systems.

The BCSFB, also known as the second barrier, functions as both a physical barrier and biochemical barrier between the systemic circulation and cerebrospinal fluid (CSF) [123]. This barrier is established by the choroid plexus, which is a highly vascularized network of fenestrated and thin-walled capillaries located in the lateral, third, and fourth ventricles of the brain, as depicted in Fig. 1A. CSF is primarily produced by the choroid plexus and resides in the ventricular compartments and subarachnoid spaces. Conventionally, CSF in the brain descends via a network of flow tracts from the choroid plexus through the ventricular system to the subarachnoid spaces, eventually reabsorbing into the peripheral bloodstream or lymphatic system [124]. The choroid plexus is essential for maintaining CNS homeostasis, as it regulates the exchange of ions, molecules, metabolites, and drugs from the systemic circulation into the CSF, which in turn influences the composition of brain interstitial fluid [123,125,126]. However, compared to the BBB, the BCSFB is more permeable to substance transport given the fenestrated nature of the epithelial cells, meaning that drug entry into the CSF from the blood (i.e., across the BCSFB) does not mirror drug permeation across the BBB

Table 3
Novel drug delivery technologies in experimental *in vivo* studies for DIPG over the last five years.

Treatment	Class	Treatment Mechanism	Technology	Animal	Study Description	Major Findings	Reference
Panobinostat	Targeted therapy	Histone deacetylase inhibitor	FUS	Mouse	Investigated the efficacy of IP panobinostat and magnetic resonance guided-FUS using pontine PDX models injected with BT245 cells.	-Combined panobinostat and FUS reduced tumor volume and significantly increased mean survival.	[285]
Olaparib	Targeted therapy	PARP1 inhibitor	FUS	Mouse	Investigated the efficacy of IP olaparib, radiation therapy, and FUS in various treatment combinations using intracranial PDX models injected with HSJD-DIPG-007 cells.	-Combined radiation therapy, olaparib and FUS delayed local tumor growth, however failed to produce a survival benefit.	[234]
Doxorubicin	Chemotherapy	Anthracycline that intercalates DNA base pairs, inhibits topoisomerase II, and produces free radicals	FUS	Mouse	Investigated the efficacy of IV doxorubicin, as monotherapy and in combination with magnetic resonance guided-FUS, in brainstem xenograft models injected with SU-DIPG-17 cells.	-No survival benefit was demonstrated in any of the treatment groups.	[233]
Doxorubicin	Chemotherapy	Anthracycline that intercalates DNA base pairs, inhibits topoisomerase II, and produces free radicals	FUS NP	Mouse	Investigated the efficacy of IV-administered free and liposomal doxorubicin formulations, as monotherapies and in combination with FUS, in pontine PDX models injected with HSJD-DIPG-07 cells.	-Free and liposomal doxorubicin formulations in combination with FUS failed to demonstrate any treatment benefit in the mouse models.	[193]
Irinotecan	Chemotherapy	Topoisomerase I inhibitor	NP IN CED	Rat	Investigated the efficacy of IV, CED, and IN nanoliposomal irinotecan (CPT-11) in pontine xenograft models injected with GS2 cells.	-IV, CED, and IN delivery of nanoliposomal irinotecan inhibited significantly increased survival.	[97]
SN-38	Chemotherapy	Active metabolite of irinotecan, which is a topoisomerase I inhibitor	IN NP	Mouse	Investigated the efficacy of IN nanoliposomal SN-38 in pontine xenograft models injected with SF8628 cells.	-Nanoliposomal SN-38 inhibited tumor growth and significantly prolonged survival in the xenograft models.	[241]
Panobinostat PPM1D-siRNA	Targeted therapy	Histone deacetylase inhibitor (panobinostat) Targets and silences the *PPM1D* gene, reducing tumor growth and inducing pro- apoptotic effects (PPM1D-siRNA)	NP	Mouse	Investigated the efficacy of various IV nanoplatforms loaded with panobinostat and PPM1D-siRNA in xenograft brainstem models injected with DIPG cells.	-cRGD surface-modified macrophage exosomes demonstrated a greater tumor suppressing effect and survival benefit compared to the other nanoplatforms. -cRGD surface-modified macrophage exosomes demonstrated similar tumor suppression and survival benefit to the group receiving high dose IP panobinostat.	[242]
Doxorubicin	Chemotherapy	Anthracycline that intercalates DNA base pairs, inhibits topoisomerase II, and produces free radicals	NP	Mouse	Investigated the efficacy of IV passionfruit-like gold nanoarchitectures loaded with doxorubicin in a brainstem xenograft model injected with HSJD-DIPG-007 cells.	-Doxorubicin loaded nanoarchitectures failed to demonstrate a survival benefit in the xenograft models.	[243]
Vincristine	Chemotherapy	Vinca alkaloid that inhibits microtubule formation	NP	Mouse	Investigated the efficacy of various IV nanoformulations loaded with vincristine in pontine PDX models injected with DIPG-XIII-P cells.	-The sequential targeting in crosslinking (STICK) nanoparticle formulation loaded with vincristine significantly suppressed tumor growth and almost doubled the survival time compared with three other vincristine-loaded nanoplatforms. -High-dose IV vincristine failed to demonstrate a survival benefit, however when loaded into the STICK nanoparticle formulation, it prolonged survival and produced 2 long-term survivors.	[157]

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Table 3 (*continued*)

Treatment	Class	Treatment Mechanism	Technology	Animal	Study Description	Major Findings	Reference
Emtansine	Chemotherapy	Cytotoxic microtubule inhibitor	NP CED	Mouse	Investigated the efficacy of an emtansine-loaded peptide nanofiber precursor platform administered via CED in pontine PDX models injected with SU-DIPG-IV or SF8628 cells.	-The emtansine-loaded nanoplatform significantly suppressed tumor growth compared to free drug in both PDX models. -The emtansine-loaded nanoplatform significantly prolonged survival compared to free drug in the SU-DIPG-IV model.	[244]
Alisertib	Targeted therapy	Aurora kinase inhibitor	CED	Rat Mouse	Examined the efficacy of alisertib administered by via CED in a pontine PDX rat model injected with DIPG-XIII-P* cells and a genetically engineered mouse model bearing a high-grade brainstem tumor.	-Singular CED administration of alisertib failed to demonstrate an improvement in overall survival in the PDX rat model. -Continuous CED administration of alisertib significantly improved survival in both rodent models.	[98]
Mycophenolate mofetil	Targeted therapy	Immunosuppressant that inhibits lymphocyte proliferation and antibody formation	CED	Mouse	Investigated the efficacy of mycophenolate mofetil administered by CED in a pontine PDX model injected with SF8628 DIPG cells.	-CED administered mycophenolate mofetil inhibited tumor growth and significantly extended survival of mice bearing pontine SF8628 xenografts.	[117]
GB-13	Targeted therapy	Peptide–toxin conjugate that binds to IL-13Rα2	CED	Mouse	Examined the efficacy of GB-13 administered via CED in PDX pontine mouse models injected with PED17 (IL-13Rα2-high) or SU-DIPG-XIII-P (IL-13Rα2-low) cells.	-GB-13 administered via CED significantly reduced tumor burden and prolonged survival in the IL-13Rα2-upregulated (PED17) PDX model. -GB-13 administered via CED did not impact tumor volume or survival in the IL-13Rα2-down regulated (SU-DIPG-XIII-P) PDX model.	[286]
Panobinostat BGB324	Targeted therapy	Histone deacetylase inhibitor (panobinostat) Inhibitor of the AXL receptor tyrosine kinase (BGB324)	CED	Mouse	Evaluated the efficacy of panobinostat administered via single CED infusion, as monotherapy and in combination with PO BGB324, in a pontine PDX model injected with HSJD-DIPG-07 cells.	-Panobinostat and BGB324 combination therapy was well-tolerated and demonstrated a modest increase in median survival, whereas monotherapy failed to demonstrate a significant survival benefit.	[280]
EPZ-6438	Targeted therapy	EZH2 inhibitor	CED	Mouse	Evaluated the efficacy of EPZ-6438, administered by via single CED infusion or IP injection, in a pontine xenograft model injected with SF8628 cells.	-EPZ-6438 administered via CED inhibited tumor growth and significantly prolonged survival when compared to IP administration of EPZ-6438. -IP administration of EPZ6438 failed to demonstrate any effect on tumor growth or survival.	[287]
Tariquidar Dexamethasone Dasatinib	Targeted therapy	P-gp inhibitor (tariquidar) Potent glucocorticoid (dexamethasone) Multi-kinase inhibitor, including PDGFRA (dasatinib)	CED	Mouse	Evaluated the efficacy of IP dexamethasone and IP tariquidar to augment the CED of dasatinib in genetically engineered brainstem mouse models injected with DF1 cells transfected with RCAS plamids expressing PDGF-B, Cre, and H3K27M.	-Treatment with tariquidar and dexamethasone prior to CED of dasatinib demonstrated a greater increase in survival in the animal model compared with single-agent CED of dasatinib.	[288]
ZSTK474 Trametinib	Targeted therapy	PI3K inhibitor (ZSTK474) Mitogen-activated extracellular signal-regulated kinase inhibitor (trametinib)	CED	Mouse	Investigated the efficacy of ZSTK474 in combination with trametinib administered via two CED infusions in *Nestin*-Tv-a;$p53^{fl/fl}$ genetically engineered mouse models harboring tumors in the brainstem.	-ZSTK474 and trametinib demonstrated a significant survival benefit in the genetically engineered mouse models compared with the control.	[289]
Corin	Targeted therapy	Dual inhibitor of histone deactylase and lysine specific demethylase 1.	CED	Mouse	Investigated the efficacy of Corin administered via CED in pontine xenograft models injected with HSJD-DIPG-007,	-Corin administered via CED reduced tumor volume in all PDX models.	[290]

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Table 3 (*continued*)

Treatment	Class	Treatment Mechanism	Technology	Animal	Study Description	Major Findings	Reference
WP1066	Targeted therapy	STAT3 pathway inhibitor	Intracerebral Osmotic Pump	Mouse	SU-DIPG-XIII, or SU-DIPG-XIII-P* cells. Evaluated the efficacy of WP1066 administered intratumorally with an ALZET® osmotic pump using a pontine PDX model injected with DIPG-XIII cells.	-WP1066 administered via the Alzet osmotic pump significantly prolonged survival relative to the control in the PDX mouse model.	[118]

Abbreviations: By mouth (PO); Convection enhanced delivery (CED); Diffuse intrinsic pontine glioma (DIPG); Enhancer of zeste homolog 2 (EZH2); Focused ultrasound (FUS); Intranasal delivery (IN); Intraperitoneal (IP); Intravenous (IV); Nanoparticle (NP); Patient derived growth factor beta (PDGF-B); Patient-derived xenograft (PDX); P-glycoprotein (P-gp); Phosphatidylinositol-3 kinase (PI3K); Platelet derived growth factor receptor alpha (PDGFRA); Poly (ADP-ribose) polymerase-1 (PARP1); Protein phosphatase magnesium-dependent 1 delta (PPM1D); Signal transducer and activator of transcription 3 (STAT3); Small interfering RNA (siRNA).

[126]. When therapeutic agents enter the CSF, either from the systemic circulation (i.e., across the BCSFB or arachnoid barrier) or via direct intrathecal injection, they must then diffuse across the ventricular ependyma or pia mater and glia limitans to enter the brain parenchyma, all of which are significantly more permeable to the passage of substances than the BBB [127,128]. However, once present in the CSF compartment, drugs are rapidly removed by convection and bulk flow through CSF flow tracts, which is further compounded by the slow diffusion rate of drugs from the CSF into the CNS interstitial space [126,129]. This is an important consideration when administering drugs directly into the CSF via the intrathecal route, as although it may be successful in bypassing the BBB, rapid CSF clearance may significantly hinder therapeutic efficacy. The presence of efflux pumps and enzymes at this interface also functions to clear toxic substances and drugs that have been taken up by epithelium at the BCSFB [130]. Drug efflux pumps present in the choroid plexus include P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistant proteins (MRPs) 1, 2, 4 and 5 [131–136]. Numerous drug metabolizing enzymes are similarly expressed at the choroid plexus, including monoamine oxidases (MOAs), UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), epoxide hydrolases (EHs), glutathione S-transferases (GSTs), monooxygenases, and cytochrome P450 (CYP) enzymes [133–135,137,138].

Finally, the arachnoid barrier is composed of a multi-layered avascular epithelium that forms a barrier between the CSF-filled subarachnoid space and the fenestrated capillaries located in the dura mater (Fig. 1B) [139]. This barrier has a regulatory role in mediating the transport of substances between the subarachnoid space and the dura mater, however, due to its avascularity and relatively limited surface area compared to other brain barriers, the arachnoid barrier has not been considered a significant regulator for substance exchange between the systemic circulation and the CNS [122,140]. However, the recent discovery of efflux transporters and CYP drug-metabolizing enzymes in the arachnoid barrier has demonstrated that it may also influence the delivery of therapeutic agents from the CSF into the brain parenchyma [127,139]. A study conducted by Yasuda et al (2013) characterized arachnoid barrier cells using microarray analysis to investigate the expression of efflux transporters and CYP metabolizing enzymes in mouse and human arachnoidal tissue [139]. Their results demonstrated the expression of efflux transporters (including P-gp and BCRP) and CYP enzymes (including CYP1B1 and CYP4A*)* in arachnoid barrier cells, suggesting the arachnoid barrier to influence both the entry of systemically administered therapies from the fenestrated dural capillaries into the CSF and the removal of intrathecally administered drugs from the CSF [139].

4.1. Blood-brain barrier (BBB) structure

The BBB is notoriously difficult to penetrate owing to the tight structure of the brain capillaries. Cerebral microvasculature of the brain parenchyma is composed of three cellular components separating blood from the brain interstitial fluid: endothelial cells, pericytes, and astrocytes. These three cell types, together with microglia, neurons, and the basement membrane, are the main components comprising the BBB neurovascular unit (Fig. 1C) [141]. Compared to peripheral vasculature, brain capillary endothelial cells are highly specialized, and it is these specializations that limit the diffusion and transcytosis of molecules and proteins into the brain [142,143]. The structure of brain capillary endothelial cells and their limited rates of pinocytic activity, increased numbers of mitochondria, and comparatively high expression of efflux transporters greatly restricts BBB permeability [143]. The negative surface charge of the microvascular endothelial surface at the BBB provides an additional electrostatic barrier regulating the penetration of molecules and drugs into the CNS [144]. Brain capillary endothelial cells lack fenestrations and are compactly joined by tight junctions (TJs) and adherens junctions, which are complex protein structures involved in endothelial cell-cell adhesion, intracellular cytoskeleton dynamics, signaling pathways, and transcriptional regulation [145]. These adhesive structures limit the paracellular penetration of drugs, ions, molecules, and other polar substances through the BBB [145,146]. Brain endothelium is surrounded by a continuous basement membrane that is embedded with pericytes and adjoined by astrocytic end-feet, sporadically interconnected by microglia and neurons in the brain parenchyma [142]. Pericytes are vascular mural cells that coordinate a range of responses vital for CNS function, including phagocytic clearance, vascular development and maintenance, barrier permeability, TJ regulation, and cerebral blood flow, and are also suggested to provide structural support to the neurovascular unit [142,147]. Astrocytes similarly regulate a range of BBB functions, including structural (i.e., by maintaining the integrity of TJs), transport (i.e., by modulating the expression of efflux pumps such as P-gp), and metabolic (i.e., by activating enzyme systems) barrier features [143,148].

4.2. Blood-brain barrier (BBB) transport

Considering the specialized cellular structure of the BBB, it is evident that the translocation of compounds from the blood into the brain parenchyma occurs only through specific transcellular (i.e., through cells) or paracellular pathways (i.e., between adjacent cells), as shown in Fig. 2 [13,14]. Unlike the paracellular pathway, which mainly involves passive diffusion, the transcellular pathway involves transport mechanisms such as passive diffusion, transporter-mediated transport, and transcytosis. However, the mechanism by which molecules move across the BBB is dependent upon the physicochemical characteristics of the molecule and the direction of transport. Only small molecules that are highly lipophilic (e.g., with a log P of 2.5) with molecular weights of $<$400 Da can passively diffuse across the BBB, which impedes the passage of macromolecular therapeutics such as proteins, peptides, and antibodies [13,149]. Minimizing hydrogen bond donor capacity (i.e., $<$ 3), topological polar surface area (i.e., $<$ 90 Å), and pKa (i.e., low pKa values prevent excretion by efflux pumps) are additional physicochemical features known to improve BBB penetration [149]. To further

Table 4
Novel drug delivery technologies in clinical trials for DIPG over the last five years (Ref: clinicaltrials.gov).

Treatment	Class	Treatment Mechanism	Technology	Study Phase	Status	Study Size	Age	Study Description	Clinical Trial Identifier	Year
Etoposide	Chemotherapy	Topoisomerase II inhibitor	FUS	1	Recruiting	10	4–21 years	To evaluate the safety and feasibility of opening the BBB with FUS prior to PO etoposide treatment for patients with DIPG or H3K27M-mutant DMG.	NCT05762419	2023
Doxorubicin	Chemotherapy	Anthracycline that intercalates DNA base pairs, inhibits topoisomerase II, and produces free radicals	FUS	1 / 2	Recruiting	10	5–21 years	To evaluate the feasibility, safety, and efficacy of BBB disruption using magnetic resonance guided-FUS for patients with DIPG undergoing IV doxorubicin therapy.	NCT05630209	2022
Doxorubicin	Chemotherapy	Anthracycline that intercalates DNA base pairs, inhibits topoisomerase II, and produces free radicals	FUS	1 / 2	Recruiting	3	5–18 years	To evaluate the feasibility, safety, and efficacy of BBB disruption using magnetic resonance guided-FUS for patients with DIPG undergoing IV doxorubicin therapy.	NCT05615623	2022
SONALA-001	Chemotherapy	An intravenous formulation of aminolevulinic acid (5-ALA) which reacts with energy delivered by magnetic resonance guided-FUS to induce necrosis and apoptosis in glioma cells	FUS	2	Recruiting	40	$\geq$ 5 years	To evaluate the safety, maximum tolerated dose, and efficacy of IV SONALA-001 in combination with magnetic resonance guided-FUS in patients diagnosed with DIPG.	NCT05123534	2021
Panobinostat	Targeted therapy	Histone deacetylase inhibitor	FUS	1	Active, not recruiting	3	4–21 years	To evaluate the safety, efficacy, and feasibility of opening the BBB with FUS prior to PO panobinostat treatment for patients with progressive DMG including DIPG.	NCT04804709	2021
Omburtamab	Targeted therapy	Targets the cluster of differentiation (CD)276 protein expressed on tumor cells	CED	1	Withdrawn	0	3–21 years	To evaluate the safety and efficacy of iodine-131 conjugated omburtamab (131I-omburtamab) administered via CED in patients with DIPG that have not progressed following radiation therapy.	NCT05063357	2021
Panobinostat	Targeted therapy	Histone deacetylase inhibitor	CED NP	1	Completed	9	3–18 years	To evaluate the safety and maximum tolerated dose of a panobinostat nanoparticle formulation (MTX110) and gadolinium administered by CED for treating patients with newly diagnosed DMGs, including DIPG.	NCT04264143	2020
Panobinostat	Targeted therapy	Histone deacetylase inhibitor	CED NP	1 / 2	Completed	7	2–21 years	Evaluated the safety, tolerability, and efficacy of a panobinostat nanoparticle formulation (MTX110) administered by CED for patients with newly diagnosed DIPG.	NCT03566199	2018

Abbreviations: Blood-brain barrier (BBB); By mouth (PO); Convection enhanced delivery (CED); Diffuse intrinsic pontine glioma (DIPG); Diffuse midline glioma (DMG); Focused ultrasound (FUS); Intravenous (IV); Nanoparticle delivery (NP).

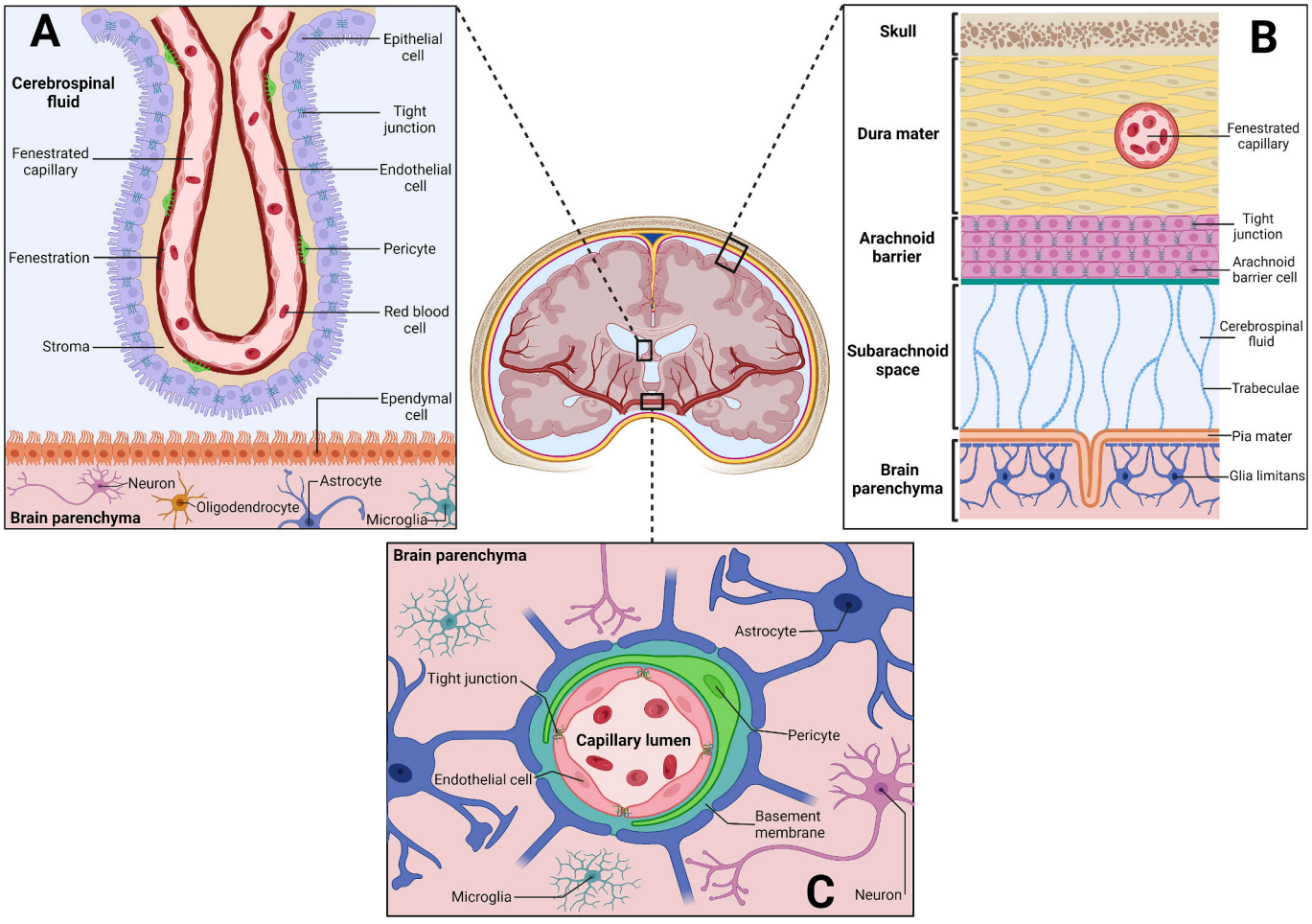

Fig. 1. Biological barriers in the brain to effective drug delivery. The blood-brain barrier (BBB), blood-cerebrospinal fluid barrier (BCSFB), and the arachnoid barrier form the three main biological barriers separating the systemic circulation from the brain parenchyma. They each play a role in influencing brain drug delivery. (A) Blood-cerebrospinal fluid barrier (BCSFB). (B) Arachnoid barrier. (C) Blood-brain barrier (BBB).

enhance CNS drug delivery, ideal therapeutic agents should be unionized, non-polar, have low protein/tissue binding, and not be a substrate for efflux transporters, such as P-gp, MRP, or BCRP [149]. Drug pharmacokinetics (i.e., bioavailability, metabolism, degradation and clearance) also affects BBB drug delivery and should be considered when delivering drugs to the CNS [12]. These physiological obstacles of the BBB means that majority of drugs are unable to overcome this barrier, where many molecules fail to fulfil the physicochemical and pharmacokinetic requirements for permeation [13,150].

4.2.1. *Passive transport*

Passive diffusion is a non-saturable and non-competitive mechanism that does not require energy expenditure or carrier proteins to move substances across cell membranes [151]. Passive diffusion of molecules can occur transcellularly or paracellularly (Fig. 2A), depending on the physicochemical properties of the substance. Transcellular diffusion of molecules is dependent on their lipophilicity, where a higher oil/water partition coefficient correlates to higher BBB penetration [152]. Usually, small lipophilic substances can diffuse freely across plasma membranes along their concentration gradient. For the paracellular pathway, small hydrophilic molecules generally utilize concentration gradients to penetrate the BBB by passive diffusion. However, due to endothelial TJs and their regulation of transient relaxation, the paracellular flux of molecules across the BBB is limited under normal physiological conditions [152].

4.2.2. *Transporter-mediated passage*

Specialized solute carrier and efflux transporters expressed by the BBB capillary endothelial cells also regulate the ability of substances to penetrate the brain parenchyma from the systemic circulation (Fig. 2B) [13,14,153]. Solute carrier transporters are the largest family of transmembrane transporters and play a critical role in maintaining brain homeostasis, regulating the exchange of drugs, nutrients (e.g. glucose), nucleosides, amino acids, ions, and metabolites across physiological membranes [154–156]. Solute carrier transport is powered by either a concentration or electrochemical gradient, not requiring ATP expenditure, and can be uniporter (i.e., transports a single solute), symporter (i.e., simultaneously transports two solutes in the same direction), or antiporter (i.e., simultaneously transports two solutes in opposite directions) transport systems [152]. There are 60 families of solute carrier transporters expressed in the brain, with members of the SLC7A (e.g., amino acid transporters), SLCO (e.g., organic anion transporters), and SLC22A (e.g., organic cation transporters) families particularly expressed at the BBB. Key transporters present at the BBB include glucose transporter 1 (GLUT1) (transports monosaccharides), cationic amino-acid transporter type 1 (CAT1) (transports cationic amino acids), large neutral amino-acid transporter type 1 (LAT1) (transports neutral amino acids), glutathione transporters (GDH) (transports glutathione), monocarboxylate transporters 1 and 2 (MCT1 and MCT2) (transports monocarboxylic acid), and equilibrative nucleoside transporter 1 (ENT1) (transports nucleosides and nucleobases) [154,155]. Examples of solute carrier transporters that have been targeted for brain drug

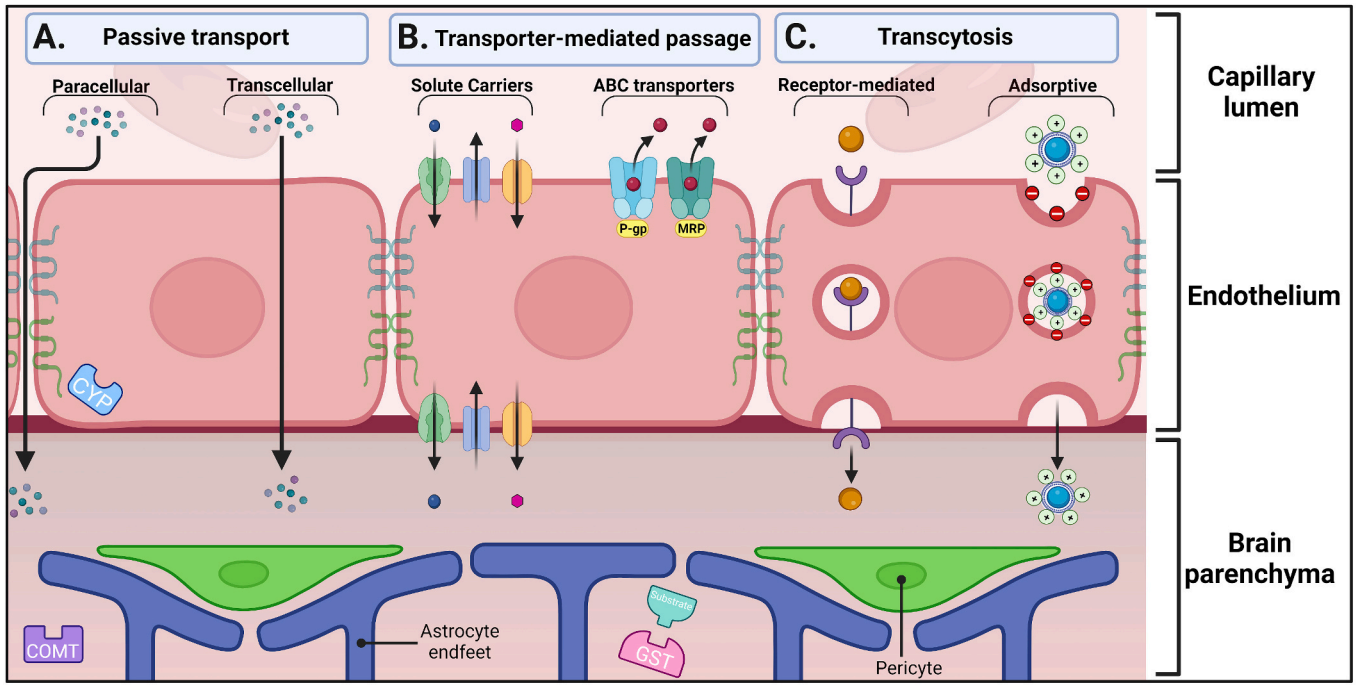

Fig. 2. Blood-brain barrier (BBB) transport and metabolism. Transport of substances from the systemic circulation into the brain occurs only through specialized transcellular or paracellular pathways. The pathway and mechanism by which molecules move across the BBB endothelium is dependent upon the physicochemical properties of the molecule and the transport direction. Metabolic enzymes located in vascular endothelial cells and the brain parenchyma (e.g., CYP enzymes) are additional obstacles impeding substances from entering the brain, given their ability to render drugs inactive or alter their physicochemical characteristics. (A) Passive transport of molecules can occur either between cells (i.e., paracellularly) or through cells (i.e., transcellularly), moving from an area of high concentration to low concentration (i.e., diffusion). (B) Transporter-mediated passage of molecules across the BBB endothelium involves both solute carrier transporters (e.g., members of the SLC7A, SLCO, or SLC22A families) and efflux pumps (e.g., ATP-binding cassette proteins). (C) Transcytosis facilitates the movement of substances from the blood into the brain through either receptor-mediated (i.e., ligand binding) or adsorptive (i.e., electrostatic interaction) transport mechanisms. *Abbreviations:* ATP-binding cassette (ABC); Catechol-O-methyltransferase (COMT); Cytochrome P450 enzyme (CYP); Glutathione S-transferases (GSTs); Multidrug resistant protein (MRP); P-glycoprotein (P-gp).

delivery include GLUT1 [157,158], LAT1 [159,160], and organic cation/carnitine transporter (OCTN2) [161,162]. When designing targeted drug delivery systems, the BBB membrane localization (i.e., apical and/or basolateral), direction of transport (i.e., from blood-to-brain or from brain-to-blood), and expression intensity (i.e., compared with other peripheral tissues) of the solute carrier must be considered in order to facilitate appropriate CNS penetration and avoid off-target effects [163].

Efflux pumps, namely ATP-binding cassette (ABC) proteins, are increasingly acknowledged to influence CNS drug delivery and elimination. ABC proteins belong to a large superfamily of BBB membrane-associated transporters, including 49 transporters grouped into seven sub-families from ABCA to ABCG [152]. They are active transporters that utilize the energy gained from ATP hydrolysis to transport substances across cell membranes against concentration gradients. Drugs, steroids, phospholipids, amino acids, ions, polysaccharides, and xenobiotics are some of the various substances that ABC transporters unidirectionally transport across the BBB [152]. ABC transporters are highly expressed in brain endothelium and predominantly function to efflux unwanted compounds from the brain, with P-gp, BCRP, and MRP proteins 1 and 2 being regarded as key BBB transporters bestowing resistance to targeted drug therapies [155,164,165].

4.2.3. *Transcytosis*

Strategies for increasing drug delivery across the BBB include modifications that exploit active transport mechanisms such as transcytosis pathways [149]. Transcytosis is a key physiological mechanism facilitating the transport of substances through the brain endothelium to the brain parenchyma. Unlike solute carrier transport, transcytosis is ideal for macromolecular transport, facilitating the passage of large or hydrophilic compounds across the BBB [156,166]. Transcytosis involves a substance either binding to a surface receptor (i.e., receptor-mediated transcytosis) or interacting with the negatively charged membrane surface (i.e., adsorptive transcytosis) to travel from the apical endothelial membrane through to the basolateral membrane, as depicted in Fig. 2C. Following ligand receptor binding or adsorptive electrostatic interaction with the cell membrane, transcytosis involves three key steps: (i) endocytosis; (ii) intracellular vesicular trafficking; and (iii) exocytosis [153]. There are two main vesicular routes for transcytosis: clathrin-mediated and caveolin-mediated transcytosis. Clathrin-mediated endocytosis occurs in clathrin-enriched areas of the cell membrane. Clathrin coated vesicles merge with the cell membrane, forming an early and late endosome which eventually merges with a lysosome to facilitate cargo degradation [167]. Caveolin-mediated endocytosis occurs in “caveolin lipid rafts” formed from invaginations of the plasma membrane. These rafts either merge with endosomes resulting in lysosome cargo degradation or are trafficked to intracellular organelles [167]. BBB receptors involved in transcytosis that have previously been targeted for drug delivery include the transferrin receptor (TFRC) [168,169], low-density lipoprotein receptor (LDLR) [170,171], insulin receptor (INSR) [172,173], insulin-like growth factor receptor (IGFR) [174,175], and diphtheria toxin receptor (DTR) [176,177].

4.3. *Blood-brain barrier (BBB) metabolism*

Metabolic enzymes located in vascular endothelial cells and the brain parenchyma are further obstacles against substances entering the brain (Fig. 2) [13,14]. Drug metabolizing enzymes may result in

treatment failure by rendering drugs chemically inactive and/or modifying BBB permeability characteristics such as polarity [178]. Examples of drug metabolizing enzymes that have been detected in BBB microvessels include CYP enzymes (e.g., CYP1B1, CYP2U1, CYP2D6, CYP2J2, CYP2E1 and CYP2R1), GSTs (e.g., GSTO1, GSTP1, GSTM2, GSTM3, and GSTM5), histamine *N*-methyltransferase (HNMT), thiopurine *S*-methyltransferase (TPMT), and catechol-*O*-methyltransferase (COMT) [178–182]. In addition to this, a range of enzymes have also been found in the brain parenchyma, including CYP enzymes (e.g., CYP46A1, CYP1B1, CYP2D6, CYP2E1, CYP2J2, and CYP2U1), GSTs (e.g., GSTP1, GSTM2, GSTM3, and GST4), COMT, and sulphotransferase (SULT1A4) [178,179,183]. Moreover, the location and expression of drug metabolizing enzymes is highly heterogenous, meaning that different cortical regions and cell types exert various metabolic effects [179]. An awareness of drug metabolizing enzymes found in the BBB and brain parenchyma is important for the delivery of effective therapies, however an exhaustive list of these enzymes and their substrates, locations, and expression intensity is outside the focus of this paper. Readers are directed to the reviews written by Agúndez et al (2014) and Silva-Adaya et al (2021) for further information on brain drug metabolism [178,179].

5. The blood-brain tumor barrier (BBTB) and tumor immune microenvironment (TIME) in DIPG

Although the general function, molecular composition, and structure of the BBB is similar across much of the CNS, the BBB is heterogeneous. Variability in the morphology, cellular composition, and microvascular density of the neurovascular unit has been documented across different cerebral regions, with certain areas possessing a highly permeable BBB (e.g., the circumventricular organs) and others a more robust BBB (e.g., the brainstem) [184–186]. In addition to the inherent heterogeneity of the healthy BBB, in response to different pathological diseases, the BBB undergoes dynamic changes and adaptations, ranging from transient alterations in BBB permeability to chronic barrier dysregulation [15]. In brain cancer, the BBB is referred to as the BBTB, as tumors typically compromise the function, integrity, and structure of the cells that form the healthy BBB [187]. The BBTB can be composed of both existing and newly generated tumor microvasculature, ranging from continuous non-fenestrated endothelium (i.e., "healthy" vasculature) to fenestrated and discontinuous endothelium (i.e., "leaky" vasculature) [188]. Reduced expression of TJs, loss of astrocyte end-feet, varied pericyte distribution, neuronal connection dysfunction, and basement membrane degradation

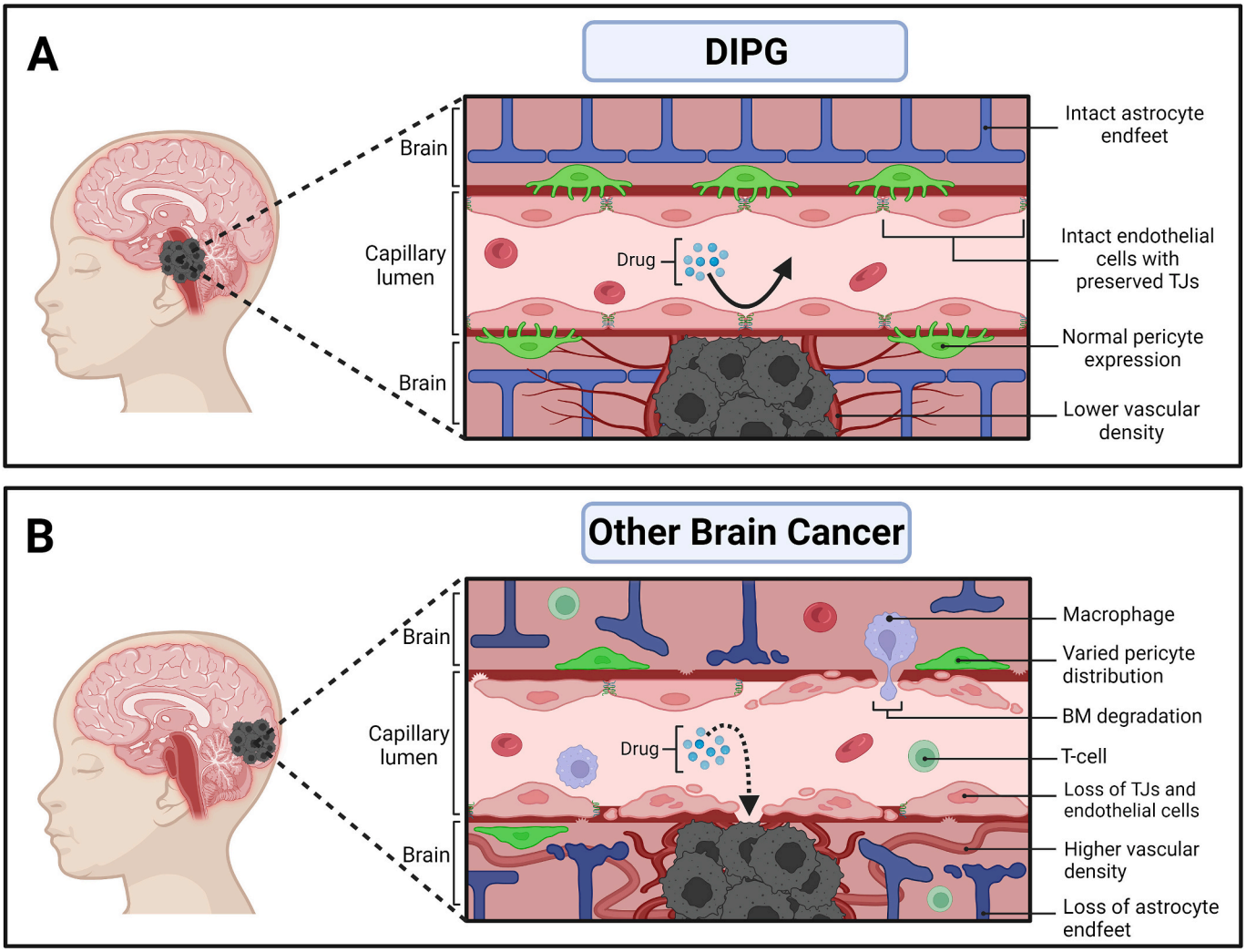

Fig. 3. Blood-brain tumor barrier (BBTB) and tumor immune microenvironment (TIME) in DIPG. (A) The BBTB is thought to remain intact in DIPG, with minimal disruption to the function, integrity, and structure of the cells that form the healthy BBB. However, findings in human DIPG samples have suggested that the BBTB in DIPG may exhibit features of leaky vasculature and impaired barrier function similar to other brain cancers. (B) Features of BBTB disruption commonly present in brain cancer, such as reduced expression of tight junctions, loss of astrocyte end-feet and endothelial cells, varied pericyte distribution, basement membrane degradation, and the presence of immune/inflammatory cells. *Abbreviations:* Basement membrane (BM); Diffuse intrinsic pontine glioma (DIPG); Tight junctions (TJs).

further characterize barrier changes present in brain cancer, as summarized in Fig. 3B [17,189].

BBTB dysfunction affects brain drug delivery, where tumor-induced inflammation and "leakiness" may enhance the passage of drug compounds across brain barriers, providing a potential exploitable mechanism for localized delivery [190]. However, among different cancer types, tumors of the same origin or histology, and within the same tumor microenvironment, changes in barrier vasculature and permeability are highly heterogeneous, which can limit homogeneous drug distribution [17,191]. Furthermore, tumor growth may impede the binding and penetration of drugs via the transcytosis pathway, and high intratumoral pressures likely affect drug retention in the brain parenchyma [192,193]. Although more significant for nodular brain tumors rather than infiltrative, solid stress and stiffness induced by tumor growth can reduce peritumoral vascular perfusion, which can further affect the ability of therapeutic agents to permeate the tumor site [194].

Compared to other primary brain cancers, such as glioblastoma and medulloblastoma, little is known about the exact structure and function of the BBTB in DIPG, which is likely due to its rarity and inoperable location. However, the accepted consensus among majority of the literature is that the BBTB remains intact in DIPG, as depicted in Fig. 3A. This was originally based on diagnostic imaging results, given the lack of tumor enhancement generated by contrast agents [12,22]. Further support of this hypothesis is a study conducted by Wei et al (2021), which performed histological and molecular analysis of patient-derived xenograft and *in utero* electroporation orthotopic murine models to examine the tumor vasculature of DIPG [195]. The results demonstrated the BBTB in DIPG to possess normal pericyte coverage, consistent expression of claudin-5 and CD-31 adhesion proteins, normal GLUT1 expression, and an absence of plasmalemma vesicle associated protein expression (PVLAP), which is an endothelial marker associated with increased fenestration, angiogenesis, and hyperpermeability [99,195,196]. These findings suggest minimal disruption in the vascular architecture and function of the BBTB in DIPG compared to the healthy BBB, which may underpin the current lack of therapeutic success [99,195,197].

However, findings in human DIPG samples have challenged this perspective. A study conducted by El-Khouly et al (2021) reported structural changes in the BBTB of both biopsy ($n = 4$) and autopsy ($n = 6$) human DIPG samples when compared to age-matched healthy pontine samples ($n = 20$) [18]. Immunohistochemistry revealed the extravasation of intravascular proteins (pre-albumin, fibrinogen, and immunoglobulin G) and the expression of claudin-5 and zonula occludens-1 (TJ proteins), laminin (basement membrane constituent), and platelet-derived growth factor receptor-B (pericyte marker) to be lower in both biopsy and autopsy DIPG patient samples, suggesting an impaired and "leaky" BBTB in DIPG. This study also demonstrated a significant reduction in the vascular density of the DIPG autopsy samples when compared to the healthy controls. As therapeutic agents typically depend on vascular perfusion to enable tumoral drug distribution, a lower BBTB vascular density may counteract any drug delivery benefits facilitated by barrier "leakiness" [18]. This reduction in vascular density could also explain the lack of therapeutic efficacy seen in DIPG, rather than an intact BBTB as previously theorized. However, whether early-stage disease (i.e., at time of biopsy) possesses reduced vascular density is yet to be confirmed.

Moreover, a study conducted by Veringa et al (2013) investigated the *in vitro* expression of P-gp, MRP-1, and BCRP-1 BBB efflux transporters in primary DIPG cell cultures using immunohistochemical staining [198]. The results demonstrated the presence of all three efflux transporters in DIPG tumor vasculature, with MRP-1 being co-expressed in tumor cells. Similarly, studies conducted by Chaves et al (2020) and Deligne et al (2020) also examined the expression of BBB efflux transporters in DIPG murine xenograft and *in vitro* DIPG BBTB models, respectively, and confirmed P-gp, MRP-1, and BCRP-1 to be functionally expressed [197,199]. This suggests that DIPG treatment failure may also be attributed to the presence of drug efflux transporters located at the BBTB, excreting therapies prematurely from the tumor site [198]. The literature also reports, even under normal physiological conditions, that the brainstem possesses a lower capillary density and an even more impermeable BBB compared to other cerebral regions, further adding to the complexity of pontine drug delivery [185,200,201]. This is supported by a study conducted by McCully et al (2013), who employed *in vivo* microdialysis to compare the concentration of intravenously administered temozolomide across different brain regions in a primate model [185]. The results found significantly lower concentrations of temozolomide in pontine tissue compared with the CSF and cortex, suggesting a lack of drug penetration into the pons compared with other CNS regions [185]. Moreover, Subashi et al (2016) used genetically-engineered mouse models of cortical and brainstem pediatric HGGs to explore how tumor location may affect BBB permeability [202]. Using dynamic contrast-enhanced MRI, their results demonstrated tumor vasculature to be more permeable in the cortex than in brainstem. This suggests that the local biological environment may influence the way in which tumor cells interact with the BBB/BBTB, with the brainstem's BBB/BBTB again proving to be more robust than other cerebral regions [202].

In regard to the tumor immune microenvironment (TIME) of DIPG, it is suggested to be non-inflammatory and immunologically "cold", characterized by fewer immune and inflammatory cells, meaning DIPG tumors may be less responsive to immunotherapy in comparison to other brain tumors [16,203,204]. A study conducted by Lin et al (2018) compared the secretome of primary DIPG and adult glioblastoma cultures, which found DIPG to secrete significantly less chemokines and cytokines than adult glioblastoma [203]. Minimal T-lymphocytic infiltration was observed in the TIME of both biopsy and autopsy DIPG tissue samples, which supports the notion that DIPG cells reside in a dampened immune environment [16,203]. Additionally, it is documented in the literature that DIPG exhibits a low tumor mutational burden, which means that the surface of DIPG cells express limited neoantigens responsible for triggering T-cell mediated immune responses against tumor cells, which is linked to reduced responses to immune checkpoint inhibitors [38,205]. The low tumor mutation burden, in combination with the lack of T-cells present in the TIME, suggest therapies should instead focus towards inducing recruitment or introduction of immune cells, such as adoptive cellular therapies targeted to the tumor site, in order to be efficacious for the treatment of DIPG [16,203,205].

6. Novel technologies used to bypass or disrupt the blood-brain tumor barrier (BBTB) in DIPG over the last 5 years

Over the past five years, a range of technologies that bypass or disrupt the BBB/BBTB have been investigated in experimental studies and clinical trials to improve brain drug delivery for children diagnosed with DIPG. These have primarily included convection enhanced delivery (CED), nanoparticle-mediated (NP) delivery, and focused ultrasound (FUS) drug delivery technologies, and to a lesser extent, intranasal (IN) delivery and intracerebral osmotic pump delivery technologies, as seen in Tables 3 and 4. Treatment strategies have included single-technology and multi-technology regimens (e.g., NP delivery in combination with CED, FUS, or IN delivery), which have been used to deliver a range of pharmacological agents, either as monotherapies or combination therapies.

Like Table 1, Table 3 was similarly restricted to *in vivo* studies and orthotopic experimental models to better evaluate the translational potential of the technologies in human DIPG. The majority of the experimental studies reported statistically significant improvements in tumor regression and/or overall survival when compared with controls and/or free drug, with many of the experimental technologies (Table 3) progressing to clinical trials (Table 4), demonstrating promise in bypassing or disrupting the BBB/BBTB with CED, FUS, or NP delivery. However, the previous considerations discussed above (i.e., the tumor

location, type of cells, route of administration, treatment safety and tolerability, efficacy in combination with standard of care and other pharmacological therapies, and extent of survival benefit) similarly apply when evaluating the translatability of the experimental technologies summarized in Table 3. Some additional factors require consideration when evaluating the translatability of the type of technology used, which will be discussed below. Moreover, as the ALZET® intracerebral osmotic pump delivery system is specifically designed for experimental use in animals and does not feature in current DIPG clinical trials, the application of this technology will not be discussed further in this review paper, however, was included in Table 3 for completeness.

6.1. Convection enhanced delivery (CED)

Convection enhanced delivery (CED) is a novel technique where one or more small catheters are stereotactically inserted either in or near the tumor site to directly deliver therapeutic agents, which can be done at the time of biopsy [8,206]. Although invasive, local delivery with CED provides numerous advantages when compared to systemic drug delivery, the most significant being the ability to bypass the BBB/BBTB. This enables higher drug concentrations to accumulate at the target site, enhancing on-target efficacy and reducing off-target toxicity [207]. Unlike simple diffusion (i.e., the mechanism by which systemically administered therapeutics permeate tissues), CED utilizes convection to generate a local hydrostatic pressure gradient, which enables more uniform drug distribution over a greater surface area [207]. Imaging markers can also be co-administered with the infusate during CED, which is advantageous for examining drug biodistribution in real-time [208,209]. Additionally, CED is especially useful for tumors that have an intact BBB/BBTB to prevent 'leakage' of the infusate into the periphery, which is argued to be characteristic of DIPG [210].

Despite the advantages of CED, successful drug delivery depends on a range of factors, all of which can impair the efficacy and translatability of the technology. Firstly, a range of pharmacological agents delivered by CED have been investigated in both experimental studies and clinical trials for DIPG (Tables 3 and 4). Although these drugs were selected for CED based on their anti-tumor efficacy, their compatibility with CED must also be considered. Drug physicochemical properties (e.g., lipophilicity, susceptibility to enzymatic degradation or efflux transporters, surface characteristics, receptor binding, and size) influence the efficacy of CED, as they affect drug flow, clearance, and volume of distribution at the tumor site [211]. In particular, large hydrophobic molecules that are positively charged exhibit a reduced volume of distribution when administered by CED, and such characteristics should be avoided when designing CED therapeutics [206,212,213].

The tissue characteristics and location of the delivery site also influences effective drug delivery by CED, as pathogenic changes induced by tumor infiltration (e.g., increased interstitial pressure, oedema, and heterogenous vasculature changes) can result in enhanced systemic infusate loss and alteration of drug distribution [211,214,215]. Moreover, drug delivery within or in close proximity to certain brain structures, such as white matter tracts, ventricles, and ependymal surfaces, has been associated with CED failure, as these structures may direct infusate flow away from the tumor site, act as a sink for infusate collection, or cause leakage into the ventricular or cisternal CSF [206,216,217]. This is particularly relevant for pontine drug delivery in DIPG, given that the pons houses both transverse and longitudinal white matter fibres and lies in close proximity to the fourth ventricle [213,218,219].

Tumor size is another important parameter influencing effective drug delivery by CED, as the CED treatment field must encompass the entire tumor area to be effective, given that the drug will revert to passive diffusion in the absence of the pressure gradient [213,220]. In order to cover a greater drug distribution area, the infusion flow rate can be increased, however at the cost of increasing catheter backflow (i.e., the retrograde movement of infusate along the catheters insertion tract), which can result in inadvertent drug loss and toxicity in off-target brain regions [211,213]. The presence of leptomeningeal disease dissemination, which has been identified in up to one third of post-mortem DIPG patients, may therefore be a significant limitation to therapy, as CED's infusion parameters are unlikely to accommodate for disease spread beyond the pontine region [12,24,221].

In addition to the flow rate, further variables affecting the efficacy of CED include the infusate viscosity, the number of CED infusions (e.g., single or continuous) and the catheter material, diameter, and placement, factors in which vary or are unknown across the experimental studies and clinical trials shown in Table 3 and Table 4 [207,211,213,217]. The lack of standardized infusion techniques may be a significant hindrance in the clinical translatability of the technology to the clinic, where many variables are yet to be optimized for the effective implementation of CED for DIPG [60,220]. Additionally, side-effects commonly occur as a result of CED, ranging from headache, ataxia, dysarthria and transient facial weakness to more serious complications, such as infection, seizures, and hemorrhage [222,223].

6.2. Focused ultrasound (FUS)

Focused ultrasound (FUS) is another novel drug delivery technology which employs the use of intravenously administered microbubbles and targeted ultrasound to temporarily disrupt focal regions of the BBB and enhance brain drug delivery [210]. Ultrasound energy causes circulating microbubbles to oscillate and induce mechanical stress against the BBB/BBTB endothelium, resulting in TJ disruption, decreased P-gp expression, and increased caveolae-mediated transcytosis, which transiently increases the paracellular and transcellular permeability of the BBB/BBTB to systemically administered pharmacological agents [17,224–226]. In the tumor interstitial space, FUS and microbubble-mediated BBB/BBTB opening has also been documented to result in an increase in convective transport, which favors the transport of larger therapeutics to their site of action [17,227]. Aside from BBB opening, another interesting application of FUS is its ability to locally activate pharmacological agents at the tumor site, avoiding off-target treatment effects. This is currently being investigated in a clinical trial utilizing an intravenous formulation of aminolevulinic acid (5-ALA), which reacts with energy delivered by FUS to induce necrosis and apoptosis following uptake by DIPG cells (NCT05123534).

FUS is minimally invasive, considered safe, and can achieve BBB opening in various brain regions, including the brainstem, which can be monitored by MRI [201,226,228–230]. Radiation has been documented to be safe in combination with FUS and also has a synergistic effect on BBB opening, which may be of use in enhancing FUS drug delivery in DIPG patients [231]. Additionally, FUS-mediated BBB opening enables the systemic extravasation of CNS immune cells and immunomodulation of the TIME, which has been suggested to enhance the anti-tumor effects of immunotherapy agents [226,231,232]. FUS-mediated BBB opening could also be used to improve the application of liquid biopsy in DIPG patients, as it allows for increased levels of cell-free DNA (cfDNA) to accumulate peripherally in serum or CSF, which could be used to detect tumor biomarkers, identify tumor mutations, and inform individualized treatment strategies [231].

However, like CED, there are many variables affecting the effectiveness and clinical application of FUS-mediated brain drug delivery. The majority of experimental FUS studies shown in Table 3 failed to demonstrate a survival benefit in murine models of DIPG, which may have firstly been due to the type of therapeutic agent selected for FUS delivery [193,233,234]. The physicochemical properties of the pharmacological agent again influences the efficacy of FUS, whereby smaller agents with positively charged surface modifications (i.e., that promote adsorption to the negative charge of the microvascular endothelial surface) or ligands designed to initiate caveolae-medicated transcytosis are more likely to penetrate across the opened BBB and accumulate at the tumor site [225]. Differing drug pharmacokinetic profiles in the

murine population, along with pathogenic changes induced by tumor growth (e.g., high intra-tumoral pressure, alterations in vasculature, and endothelial changes) may have also contributed to FUS treatment failure in the experimental studies, which are important considerations for clinical translation [193,233]. The presence of disseminated disease extending beyond the opened BBB foci may have also resulted in the lack of survival benefit, which in practice, may require repeated sonication across multiple brain regions to ensure adequate disease coverage [233–235]. However, the long-term safety profile of chronic and repeated BBB opening, especially in pediatric patients, is still being investigated [236].

Moreover, the intrinsic acoustic properties of the cranium and other skeletal structures also influences the efficacy of FUS [235,237]. These factors may be challenging to mitigate when treating pediatric patients with FUS, given that the skull characteristics of children are mostly unknown and that the pons lies in close proximity to other spinal bones, which may reflect ultrasound and cause heterogenous tissue exposure [231]. Additionally, the ultrasound devices currently available for clinical FUS applications have primarily been developed and investigated for the management of adult patients. As a result of this, FUS-mediated BBB opening has required ongoing technical evaluation in the pediatric population to ensure the safety of the technology, especially for children with DIPG (e.g., NCT04804709, NCT05630209, NCT05615623 and NCT05762419) [231,238]. Sonication parameters that require both optimization and standardization for the effective implementation of FUS for DIPG include the: (i) timing between opening the BBB and drug administration; (ii) microbubble composition, size, and dose; (iii) ultrasound intensity; (iv) transducer frequency; and, (v) treatment frequency and duration [225,226]. Optimization of these sonication parameters is essential to ensure safe BBB/BBTB opening, as vascular injury, hemorrhage, and neuronal damage can occur if parameters are exceeded [239].

6.3. Nanoparticle-mediated (NP) drug delivery

The application of nanoparticles for targeted drug delivery is becoming increasingly attractive for the treatment of brain cancers, including DIPG [157,193,240–245]. Owing to their nanometer size (usually $\leq$100 nm), high drug-loading capability, and enhanced tissue selectivity, nanoparticles provide several advantages over conventional formulations, aiming to improve therapeutic efficacy and reduce the potential of systemic adverse effects [246,247]. Their physicochemical properties (i.e., size, charge, shape, composition and surface properties) can be modified to augment drug release profiles (e.g., controlled drug release or triggered drug release in response to certain stimuli), promote better cellular uptake and, most importantly, bypass biological barriers, including the BBB/BBTB [177,246,247].

Drug loaded nanoparticles may traverse the BBB/BBTB either transcellularly (e.g., by surface ligands that can bind to and trigger internalization by endothelial receptors or transporters) or paracellularly (e.g., by passive diffusion), and can further improve drug accumulation by avoiding clearance by the reticuloendothelial system (e.g., by surface polyethylene glycol coatings) and evading BBB/BBTB efflux pumps and drug metabolizing enzymes [177,246,248]. Another significant advantage of NP drug delivery, especially for DIPG, is the ability to administer drug-loaded nanocarriers in combination with other drug delivery technologies, such as CED, FUS and IN delivery, as shown in Table 3 and Table 4 [97,193,241,244].

Although the majority of the nanoparticle-mediated drug delivery strategies significantly prolonged survival in the DIPG murine models (see Table 3), the translation of the technology to the clinic has not been successful so far, which may be due to a variety of factors [14,249]. Firstly, the nanoformulations employed across the studies, including the functionalized macrophage exosomes [242], passionfruit-like gold nanoarchitectures [243], sequential targeting in crosslinking (STICK) nanoparticles [157], and the peptide nanofiber precursor platform [244], are highly complex in their design and synthesis. For nanoformulations to be clinically translated, the complexity of their design and synthesis needs to be minimized to enable large-scale pharmaceutical manufacture and quality control [246].

To facilitate BBB/BBTB transport, the physicochemical properties of the nanoformulation have to be optimized. In general, properties that enhance BBB/BBTB permeability include a positive surface charge, spherical or rod shape, and a size of 20–50 nm – whereby 20 nm is large enough to evade renal clearance and small enough facilitate passage across the BBB/BBTB, and 50 nm is an appropriate size to trigger receptor-mediated transcytosis [240,250]. The size of the nanoparticle is also important in determining its drug loading capacity, with larger nanocarriers being able to transport more drug molecules [251]. However, this requires careful consideration, given nanoparticles traverse the BBB/BBTB in a size-dependent fashion, favoring those with smaller particle sizes [240]. Although positively charged nanoparticles exhibit improved adsorption to the negatively charged endothelial surface, cationic surface charges can trigger the production of reactive oxygen species and disrupt the integrity of the BBB/BBTB, which may cause adverse effects in the patient [252]. However, the magnitude of these effects depends on the degree of cationic charge exhibited by the nanoparticle. Another consideration for ensuring the appropriate cellular uptake and intracellular trafficking of the nanoparticle at the BBB/BBTB is the surface ligand density. Although ligands are advantageous for a range of applications, including improved targeting and cellular uptake, higher densities can increase the nanoparticle size, induce steric hindrance, diminish stealth activity, and hinder carrier uptake or release from the basolateral membrane [252]. Different nanoparticle types, such as polymer nanoparticles (e.g., poly-D,L-lactic-*co*-glycolic acid [PLGA] formulations), inorganic nanoparticles (e.g., gold, silver, iron, and silica formulations), or organic nanoparticles (e.g., lipid-based nanoparticles such as liposomes and micelles), also influence the stability, biocompatibility, and toxicology profile of the nanomedicine [240,246,253]. Additionally, the route of administration requires consideration, as each differs in the physiological barriers that the nanocarriers have to overcome to reach the site of action, which inevitably results in heterogeneous patterns of nanoparticle biodistribution throughout the body [254].

Although nanotechnology provides many advantages over conventional pharmacological agents, a balance must be maintained between successful BBB/BBTB penetration, on-target efficacy, safety, and feasibility of manufacture, which are challenges that must be addressed for the effective implementation of NP drug delivery for DIPG.

6.4. Intranasal (IN) drug delivery

Intranasal (IN) delivery is another novel drug delivery technology being investigated for the treatment of DIPG [97,241]. IN delivery exploits the anatomical link between the nasal cavity and the brain, where drugs gain access to the CNS along the olfactory and trigeminal nerves by intracellular (e.g., pinocytosis or endocytosis) or extracellular (e.g., convection or paracellular diffusion) transport mechanisms across the nasal mucosa [255]. Drugs administered via the nasal cavity can bypass the BBB/BBTB, facilitating drug delivery to rostral brain regions (i.e., via olfactory nerves and olfactory bulbs) and/or caudal brain regions including the brainstem and pons (i.e., via trigeminal nerves) [256]. Given that the respiratory epithelium is rich in trigeminal nerve endings, IN drug delivery targeting this region of the nasal cavity is likely beneficial for the treatment of DIPG, as opposed to drug deposition in the upper nasal passage which houses the olfactory nerves [241,257]. Moreover, IN delivery is well-tolerated, convenient for self-administration, and is a potential alternative to other drug delivery technologies that are invasive, possess high surgical risks, or require specific skills and expertise to implement [255,256]. Nasal administration provides a large surface area for drug absorption and also avoids first-pass metabolism in the liver, meaning lower doses of medications

can be administered to mitigate potential side effects [258].

However, the efficacy of IN drug delivery is limited by a range of factors, including active mucociliary clearance as well as the presence of P-gp efflux pumps and drug metabolizing enzymes (e.g., CYP enzymes, peptidases, and proteases) in the nasal cavity [256,259]. Pharmacological agents may also be absorbed by local blood vessels or lymphatics at the lamina propria or removed via CSF clearance mechanisms in the subarachnoid compartment after traversing the perineural space [126,256]. Low drug retention time, mucus composition, and inadvertent nasopharynx drainage also compound effective nose-to-brain delivery, along with the inability to deliver large volumes of drug given the small size of the nasal cavity (~200 μL) [259,260]. As with other novel drug delivery technologies, the physicochemical properties of the therapeutic agent (e.g., size and lipophilic-hydrophilic balance) again influence the efficacy of IN delivery, determining the drug's ability to traverse across mucous, epithelial, and/or paracellular junctional barriers in the nasal cavity [261]. For example, lipophilic molecules with small molecular weights <1 kDa are well absorbed across the nasal mucosa, whereas large hydrophilic agents >1 kDa exhibit poor permeability and tend to become trapped in the mucus [256]. Moreover, the type of device (e.g., spray, dropper, or nebulizer) and formulation (e.g., liquid, particulate, or semisolid) also influences IN delivery, along with the ability of patients or carers to administer the dose correctly and consistently [259].

These IN delivery challenges may become less problematic when therapeutic agents are delivered via nanotechnology. For example, both experimental studies conducted by Sasaki et al (2022) and Louis et al (2018) investigated the efficacy of a nanoliposomal formulation of irinotecan (or its metabolite) administered via IN delivery in rodent DIPG models in order to better protect the drug from degradation, enhance retention at the tumor site, and improve drug release and solubility [97,241]. These studies both reported the irinotecan nanoliposomal formulations to inhibit tumor growth and significantly prolong survival in the xenograft models. However, the nasal cavity and upper airway anatomy of humans differs from animals, especially rodents, which may impact the clinical translation of experimental results [258]. Further studies optimizing the balance between nasal penetration, brain delivery via trigeminal pathways, and feasibility of administration, are required to determine whether IN drug delivery provides a viable alternative to other technologies that bypass the BBB/BBTB for DIPG.

7. Future advances for the management of DIPG

There are numerous factors affecting the development and progression of effective therapies in DIPG. However, a significant challenge is the inability of pharmacological agents to penetrate across the BBB/BBTB and gain access to the CNS. A range of novel technologies have been investigated to bypass or disrupt the BBB/BBTB and improve brain drug delivery in DIPG, including CED, FUS, NP delivery, and IN delivery. Although these technologies have advantages when compared to conventional drug delivery strategies, each possess unique challenges that are yet to be optimized from bench to bedside, especially for the treatment of DIPG. However, NP drug delivery is emerging as a promising option for DIPG treatment, especially as it can be combined with additional delivery technologies, which may mitigate certain limitations posed by CED, FUS and IN delivery [97,193,241,244].

Another emerging pathway that could be exploited for brain drug delivery in DIPG is the glymphatic system, which describes an additional CSF flow pathway through the brain. Prior to reabsorbing into the systemic circulation or lymphatics, part of the CSF is thought to flow from the subarachnoid compartment into the brain tissue via the periarterial spaces of penetrating arteries (i.e., Virchow-Robin spaces), enabling the exchange of CSF with the interstitial fluid prior to draining into perivenous spaces [128,262]. Modulating properties that influence this novel flow pathway, such as body position, aquaporin-4 activity, and sleep, may provide new opportunities to improve drug delivery from the CSF (i.e., by direct intrathecal injection or via surgically implanted Ommaya reservoirs) into the brain tissue, overcoming the current limitations of rapid CSF clearance and poor CSF-CNS penetration [128,263–269]. Ommaya reservoirs are dome-shaped silicone reservoirs located under the scalp that connect to an indwelling catheter positioned in the ventricles of the brain [270]. Although Ommaya reservoirs have been used in clinical practice for decades, they are emerging as a means for administering immunotherapy agents directly into the ventricular CSF spaces for DIPG patients (e.g., NCT04185038, NCT04196413 and NCT04099797), which could also prove useful for glymphatic-modulated drug delivery in the future [103,271,272]. However, the concept of the glymphatic system remains contentious, especially as most studies have utilized rodent models, requiring further investigation into the mechanisms governing CSF-interstitial fluid exchange and how it affects human brain drug delivery [128,268,269].

Moreover, given the significant variability in the routes of administration across both experimental studies and clinical trials, further research is required to determine which routes best achieve drug accumulation in the pons, including for conventional administration strategies (e.g., intravenous, intrathecal, intracerebroventricular, or oral) and novel techniques (e.g., CED, IN delivery, or FUS). Improvements in preclinical experimental studies, such as establishing orthotopic tumors, generating tumors from human derived DIPG cells, optimizing the species used for DIPG modelling, and evaluating treatment efficacy in combination with standard of care radiation therapy and other pharmacological therapies, are further required to better recapitulate the human disease state and enhance the translatability of experimental results.

Finally, the biological characteristics of the brainstem's BBB/BBTB, both under normal physiological conditions and in response to DIPG, are poorly understood – and it is these characteristics that determine drug penetration, exposure, and retention at the tumor site. Although the accepted theory among the majority of the literature is that the BBB/BBTB remains intact in DIPG, findings in human DIPG samples have challenged this, suggesting therapeutic failure may be due to reduced tumoral perfusion, rather than an intact BBB/BBTB [18]. However, further characterization of the changes that occur in the vasculature and in the expression of receptors, transporters, efflux pumps, and drug metabolizing enzymes at the BBB/BBTB in DIPG is still required, as this inevitably informs the development of novel targeting strategies. Although the ultimate aim is to cure DIPG, goals such as prolonging survival, establishing disease "remission", and improving quality of life, may be the most attainable outcomes for this deadly disease in the medium term. However, a biology-driven approach to rational formulation design coupled with early tumor biopsy to identify patient-specific molecular targets, will ideally improve brain drug delivery and progress treatment towards precision medicine approaches, improving the way we manage and treat DIPG [38,273].

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Lauren M. Arms: Writing – original draft, Funding acquisition. **Ryan J. Duchatel:** Writing – review & editing, Supervision, Funding

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Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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