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Review

Diffuse Intrinsic Pontine Glioma: Translation of Genomic Knowledge to Clinical Practice

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Abstract

Pediatric brain tumors account for approximately 25% of all cancers in children and are currently the leading cause of cancer-related deaths in the pediatric population with an estimated incidence of 5.14 cases per 100,000 person years [1]. Up to 10-15% of all pediatric brain tumors arise in the brainstem, with the majority of these classified as the diffuse intrinsic pontine glioma (DIPG) subtype [2]. The outcome of children with DIPG remains dismal with a median survival of <1 year. Owing to the unique location of DIPGs, surgical intervention is unachievable due to difficulty of physical access and the diffuse infiltration of the tumor throughout the pons. Radiotherapy has thus far been the only method identified to provide transient benefit in the setting of a uniformly fatal outcome. Decades of laboratory studies and clinical trials have failed to identify any

chemotherapies delivering survival benefit, either alone or as an adjunct to radiotherapy. Advances in surgical biopsy techniques and next generation sequencing modalities have more recently provided a better understanding of the distinct pathophysiology of DIPGs and opened new windows of opportunity for the development of molecular-targeted therapies with hope of delivering more effective treatment for children suffering from DIPG whilst minimizing systemic toxicity. In this review, we will explore the recent advances in the genomic understanding and treatment of DIPG, and the resulting outcomes of the preclinical and clinical drug trials which are guiding the next wave of therapeutic discovery for this disease of unmet clinical need.

Keywords
Diffuse intrinsic pontine glioma; genetics; histone H3; epigenetic; targeted therapy; clinical trials

1. Introduction

DIPGs are aggressive high-grade gliomas (HGGs) derived from glial cells located in the pons of the brainstem with varying degrees of infiltration into neighboring structures. DIPG accounts for 80% of all brainstem gliomas and was traditionally classified based on anatomical location, radiographic location and where possible, by histopathology. The most recent edition of the World Health Organization (WHO) Classification of CNS Tumors published in 2016 uses molecular information in addition to histopathology and has redefined the majority of DIPGs as diffuse midline glioma, H3 K27M-mutant. Biopsied DIPGs have demonstrated the heterogenous histopathological spectrum of this tumor. They are usually high-grade astrocytoma's with increased microvascular proliferation, and are often associated with increased mitosis and necrosis, although up to a quarter of those biopsied lack these features and appear to be low grade on histological examination. Regardless of its histopathological grade at diagnosis, tumors under the DIPG/diffuse midline glioma, H3 K27M-mutant classification are aggressive in nature and have a poor overall prognosis [3].

DIPGs most commonly affect younger children with a peak incidence between the ages of 6 to 9, and a male to female ratio of 1:1. Patients typically present with a brief history of clinical symptoms and signs consistent with brainstem dysfunction, supported by typical radiologic findings on magnetic resonance imaging (MRI) (Table 1). Clinical signs in over 50% of presentations involve the classical triad of: 1) cranial nerve deficits (most commonly affecting the sixth and seventh cranial nerves resulting in facial asymmetry and diplopia); 2) cerebellar and bulbar signs (ataxia, dysmetria, drooling and dysarthria); and 3) long tract signs (hyperreflexia, upward going Babinski reflex and weakness) [4, 5]. Once the diagnosis is confirmed, treatment options are limited. Patients with histopathologically confirmed DIPG on current treatment protocols have a median survival of just 8-10 months with virtually all children succumbing within 2 years of diagnosis [6]. A small number of long-term survivors (LTSs) have been reported in various studies and case series and were systematically reviewed by Hoffman et al in 2018 [7, 8]. Although this review excluded LTSs reported with MRI findings

atypical for DIPG, many still lacked tissue biopsy to prove the diagnosis. Intriguingly, it does suggest that children presenting under the age of 3 or over the age of 10 had a better prognosis, with cranial nerve palsy, ring enhancement, extrapontine extension and necrosis at presentation being negative prognostic factors. Owing to its aggressive nature, DIPG remains the primary cause of death from brain tumors diagnosed in children. As such, it poses a significant challenge to all clinicians involved in the care of these children.

DIPG was first described by Dr Harris and Dr Newcomb in a pediatric case report in 1926. The 14-year-old boy presented following a minor head injury, and over the ensuing weeks was reported to have rapidly progressive cranial nerve palsies and pyramidal tract dysfunction, and he died within two months of presentation. The diagnosis of a pontine glioma was made clinically and the diffuse nature was confirmed histologically at post mortem [9]. Until recently, research initiatives into DIPG have been limited to therapies (radiation and chemotherapy) with limited benefit in other forms of HGG [10, 11]. Owing to the anatomical position of the tumor in the ventral pons, surgical interventions have had little role to play in its management [12]. There is no role for chemotherapy. Steroids may alleviate symptoms transiently but have the burden of severe side effects. Radiation is used as a palliative measure for symptom control, and has demonstrated a minimal survival benefit of a few months in some studies [13-15].

Table 1 Clinical and radiological features of DIPG

Common symptoms - Eye movement abnormality. - Diplopia. - Facial weakness and asymmetry. - Sudden onset hearing impairment. - Limb weakness, difficulty standing/walking and abnormal gaits. - Headache. - Nausea and vomiting.
Clinical findings Triad of 1. Cranial neuropathies (most frequently 6^{th} and 7^{th} cranial nerve palsies). 2. Cerebellar and Bulbar signs (ataxia, dysmetria, drooling, dysarthria). 3. Long tract signs (hyperreflexia, increased tone, clonus, weakness).
Radiological findings (on MRI) - Involvement of ventral pons, with or without an exophytic component into the prepontine cistern. - Basilar artery Encasement/encroachment. - Hypo/Isointense on T1- weighted imaging. - Hyperintense on T2-weighted imaging and FLAIR sequences.

2. Treatment

2.1 Medical Therapy

Traditional chemotherapies have proven to be ineffective in the treatment of DIPG, presumably due to poor blood brain barrier permeability and/or distribution throughout the

tumor mass. Regardless, conventional cytotoxics have continued to be trialed, alone and in combination, but without effect. Interestingly, a limited study investigating neoadjuvant high-dose methotrexate, BCNU, cisplatin and tamoxifen administered to DIPG patients until progressive disease, then followed by radiotherapy, reported an encouraging median overall survival of 17 months [16]. Since survival following radiotherapy is comparable to historical controls, the overall improved survival duration is likely attributed to upfront chemotherapy. However, long-term survival remained poor and the use of multi-agent medical therapies has not been adopted into routine clinical care.

Corticosteroids are widely used as supportive treatment to manage symptoms but do not have any effect on the tumor tissue itself. Administration of dexamethasone at the time of diagnosis will decrease edema of the surrounding brainstem and pons, with often rapid improvement in symptoms and signs. However, the side-effects of prolonged administration of dexamethasone can be a clinical challenge resulting in a compromised quality of life made more difficult with the return of symptoms and signs once they are withdrawn [17].

2.2 Surgical Resection

DIPGs typically arise in the pons resulting in destruction of intrinsic nerve fiber tracts with spread to the adjacent midbrain, medulla and cerebellar peduncles. Cranial nerves VI and VII are most commonly affected, although cranial nerves V, X and XI can also be commonly affected as the disease progresses. Owing to the central brainstem being involved and the diffuse nature of the tumor, surgical resection is not recommended [12, 18-20].

2.2.1 Stereotactic Biopsy

It should be noted that surgical intervention may only be considered after referral to a highly specialized neurosurgical team, in conjunction with pediatric neuro-oncologists, before any treatment is initiated. In the past, the paucity of available surgical tissue has in part hindered the development of suitable preclinical models for research. Until recently, DIPG tissue acquisition was difficult due to the morbidity associated with the anatomical site of the tumor and tissue was only acquired at post mortem under consent and often following extensive treatment.

In 2007, Roujeau et al. published the first major report of upfront stereotactic biopsy of DIPG patients. The study found that of the twenty-four patients studied, only two suffered deficits from the procedure, with both being transient and lasting less than two months. Importantly, a histological diagnosis was made in all 24 patients, and that 22 had an infiltrative malignant glioma, with 1 having a pilocytic astrocytoma, and 1 having a low-grade glioma. This study concluded that tissue biopsies in DIPG have a high diagnostic yield associated with minimal morbidity and resulted in a consensus meeting in Paris in 2011 where it was agreed that biopsy of brain stem gliomas is technically safe with acceptable risks to inform on optimal therapeutic interventions [21]. Since then, similar clinical studies have continued to prove that there is minimal risk of persistent morbidity or mortality associated with the procedure whilst providing valuable pathologic and histologic insights [22-24].

This important innovation has led to increasing numbers of recent trials incorporating genomic analysis into their treatment protocols as a core indicator for potential targeted treatment options. With increased willingness to biopsy, the increased availability of tissue has led to an exponential increase in our understandings of the genomic, transcriptomic and epigenetic landscapes of DIPG, and an increase in pre-clinical models. Tumor biopbsy will also

enable direct analysis of treatment naïve and treatment resistant tissues from longitudinal samples of the same patient. Collectively, these resulting resources are leading to the rationalized introduction of new clinical trials for targeted therapies, which will be discussed in more detail below.

2.2.2 Liquid Biopsy

Detection of the Histone H3 mutations in CSF-derived tumor DNA from patients with diffuse midline glioma was first described in 2017 by Huang et al [25]. In a small cohort of 6 diffuse midline glioma patients, sufficient CSF-derived tumor DNA was isolated from 5 of the patients and Histone H3 mutations were detected in 4 of these using Sanger sequencing. More recently, using digital droplet PCR, Histone H3 and additional driver mutations were detected in both CSF and plasma-derived tumor DNA from 48 diffuse midline glioma patients, all with matching tumor tissue [26]. Furthermore, this approach was applied to the longitudinal surveillance of patients during their clinical management and showed that changes in circulating tumor DNA mutational allele frequencies accurately reflected tumor response to therapy. This highlights the potential use of patient-derived liquid biopsy as both a clinically relevant diagnostic and predictor of therapy response and disease progression in patients with DIPG.

2.3 Radiation Therapy

To date, radiotherapy is the only therapeutic option to have demonstrated transient benefits in length of survival from diagnosis. Whilst not curative, and merely palliative, radiation therapy remains the standard of care treatment for children with DIPG. It is the only treatment repeatedly and reproducibly proven to provide temporary symptomatic relief and short-term progression control. Conventional radiotherapy targets the tumor volume plus a clear margin of 1 to 2 cm of adjacent neural tissue, accumulating a total dose between 54 Gy and 59.4 Gy over a period of 6 weeks with five treatment days per week, at a dose of approximately 1.8 Gy per fraction. However, despite significant radiological tumor shrinkage achieved with radiotherapy, the response is transient [27].

Furthermore, clinical trials have endeavored to improve survival rates through administration of higher doses of radiation and the use of adjunct chemotherapy and radiosensitizers, however survival and symptomatic benefits of these radiotherapy variations have shown no additional benefit [28, 29]. Recent studies of radiotherapy in DIPG have instead focused on identifying the lowest radiation dose required to attain the same therapeutic effect in order to maximize quality of life by reducing the burden of treatment. Indeed, a recent Phase III trial investigating hypofractionated radiotherapy, cumulative dose of 39 Gy in daily fractions of 3 Gy in thirteen treatments over 2.6 weeks, resulted in nearly comparable outcomes to conventional fractionation but with less burden on patients [30]. The potential for re-irradiation following disease progression has also become a recent area of interest, with several limited cohort studies reporting neurological improvement and improved median survival [31]. Upcoming phase II clinical trials of a prospective nature with increased target populations are investigating the reproducibility and morbidity associated with this method.

3. Genomics of DIPG

Recent advances in next generation sequencing techniques and stereotactic biopsies to access tissues, has enabled the in-depth exploration of the potential genomic and molecular

alterations underpinning DIPG tumorigenesis. Importantly, these studies have revealed distinct biological driver aberrations that could be therapeutically exploited. This includes recurrent mutations or amplification in genes associated with epigenetic regulation, cell signaling pathways, cell cycle regulation, and DNA repair mechanisms, often co-occurring in mutually exclusive combinations. Here, we will discuss the key alterations and their potential to be therapeutically targeted.

3.1 Epigenetic Dysregulation

The most significant advance in our understanding of DIPG biology has come from the discovery of unique recurrent somatic heterozygous mutations in genes encoding for two different histone H3 variants, H3.1 (*HIST1H3B, HIST1H3C*) and H3.3 (*H3F3A*) that are essential for epigenetic control of gene expression [32]. These mutually exclusive mutations in the histone H3 genes similarly result in a distinct single nucleotide variant leading to an amino acid substitution in the highly conserved N-terminal histone tail: lysine-to-methionine at position 27 (K27M). Collectively, H3K27M mutations occur in 74-93% of DIPG tumors [32-37]. The *H3F3A* mutation (H3.3K27M) is found in approximately two-thirds (56%-80%) of DIPGs and is associated with a later age of disease onset (7-10 years) and poorer overall survival (9 months) [32, 33, 35, 38]. Interestingly, H3.3K27M mutations also exclusively occur in diffuse midline gliomas effecting the thalamus and spinal cord. In contrast, H3.1K27M mutations are restricted to approximately one-fifth (17-31%) of DIPGs, correlated with an earlier age of onset (4-6 years), distinct clinicopathological and radiological features, and slightly longer survival (15 months) [32, 38].

Whilst the precise functions of H3K27M are still to be determined, evidence suggests that the mutation imparts a gain-of-function activity inhibiting the Polycomb repressive complex 2 (PRC2) via direct sequestration of the histone methyltransferase Enhancer of zeste homolog 2 (EZH2) resulting in a global reduction in H3K27me3 [39, 40]. Despite a global hypomethylation phenotype in H3K27M DIPGs, selected genomic regions of histone hypermethylation (gain of H3K27me3) are also observed, particularly in intergenic regions. As expected, genes with aberrant gain or loss of H3K27me3 demonstrate decreased or increased expression, respectively, strongly implicating epigenetic control of gene expression as an underlying mechanism driving DIPG pathogenesis [40]. Since epigenetic modifications are reversible, many studies have focused on the therapeutic potential of epigenetic modifiers. These include the use of inhibitors of H2K27 demethylases (JMJD3 and GSKJ4), histone deacetylase inhibitors (panobinostat and sodium valproate), catalytic EZH2 inhibitors (EPZ6438) and BET inhibitors, all which have shown promise in preclinical models (Table 2, Table 3).

Interestingly, H3.1K27M and H3.3K27M mutations co-occur with exclusive genetic alterations, likely accounting for the distinct clinical features described above. *TP53* mutations, *PDGRFA* mutations/amplifications, amplification at 17p11.2 targeting *TOP3A*, amplification of *CCND2, ATRX, ATM* and *ASXL1* mutations largely co-segregate with H3.3K27M. In contrast, H3.1K27M exclusively co-occurs with *ACVR1* and *BCOR* mutations and an enrichment of downstream PI3K pathway mutations (*PIK3CA* and *PIK3R1*) [32, 35, 36, 41]. Interestingly, *MYCN/ID2* mutations are enriched in H3 wildtype DIPG [35]. The specific functional importance of these co-segregating genomic alterations is now an area of intense investigation and pre-clinical results strongly suggest that combination therapy targeting both H3K27M and specific partner mutations will be necessary in the clinic.

Table 2 Genomic abnormalities in DIPG and the targeted therapies tested						
Genomic abnormality	**Incidence (%)**	**Cellular changes**	**Clinical outcome**	**Targeted therapies tested**	**Therapeutic limitations**	**Ref**
MUTATIONS						
Histone H3	80	Hypomethylation of histone H3 proteins, initiated by the conversion of a lysine to methionine residue, resulting in aberrant cell-cycle function that initiates oncogenesis	Worse prognosis compared to non-histone mutated tumors	• HDAC inhibitors such as panobinostat have yielded promising results in preclinical studies, but clinical trials published so far have shown intolerable toxicity at the effective dose. Other potentially promising HDAC inhibitors with promising preclinical results include JMJD3 and GSKJ4. • EZH2 inhibitors currently investigated by preclinical trials. • BET protein inhibitors have shown promising results against histone dysregulation in preclinical and clinical studies of glioblastoma populations.	• Commonly occurs in combination with other mutations; further investigation into combination therapies is necessary.	[42-44]
1) H3.3 (H3F3A)	60-71		• Median OS = 9months • ↓ response to radiotherapy • ↑ metastases			
2) H3.1 (HIST1 H3B)	12-18		• Median OS = 15months • ↑ response to radiotherapy • ↓ metastases			
PDGFRA*	≈32	The most commonly mutated/amplified tyrosine kinase receptor in DIPG. Phosphorylation of these receptors triggers downstream activation of the PI3K and MAPK pathways.	• Co-segregate with histone H3.3 mutations • Enriched pro-neural expression • Clinically aggressive	PDFGRA inhibitors tested • Dasatinib/vandetanib/cabozanitinib (moderate potency) • Crenolanib (potent)	• Predominant cytostatic effect • Poor CNS penetration (limited by drug efflux transporters, e.g. P-glycoprotein)	[42, 45-49]
PPM1D/TP53	22-40	PPM1D and TP53 are cell cycle regulatory mutations which are mutually exclusive in DIPG. PPM1D mutation/amplification and its consequent overexpression leads to suppression of multiple targets, e.g. p53. Mutated TP53 with histone H3.3 mutations occur in 30% of DIPGs allowing for the evasion of cellular death.	• Co-segregate with histone H3.3 mutations • ↑ metastases	Small molecule PPM1D inhibitors currently investigated by preclinical trials.	NA	[50-52]
ACVR1	20-30	Gene encodes the activin A type tyrosine kinase receptor (ALK2) Mutation activates the BMP pathway causing phosphorylation of downstream proteins, upregulating the signaling targets ID1/2, subsequently promoting cell progression via interaction with the Rb1 and p21 pathways.	• Co-segregate with histone H3.1 mutations • ↑ median OS	No DIPG trials performed on ALK2 inhibitors • K02288 (highly selective ALK2 inhibitor) has been shown to inhibit the BMP-induced SMAD pathway; further preclinical models necessary.	NA	[33, 34, 53-55]

Accessory drivers						
PIK3R1/PIK3CA	15	Can also be resultant from upstream activating changes in receptor kinases. Oncogenes within the PI3K pathway are an obligate partner of histone H3.3 present in clonal populations	• ↑ angiogenesis • Stem cell formation	No DIPG trials performed on PI3K inhibitors • PI3K/mTOR inhibitor NVP-BEZ235 has demonstrated increased sensitization to RT and temozolomide with increased survival in non-brainstem glioblastoma murine models Agents tested • AKT inhibitors • Rapamycin	Agents tested were non-specific to DIPG	[42, 47, 55-57]
BCOR/BCORL1, NF1, PTEN	<1	NA	NA	NA	NA	[33]
COPY NUMBER ABNORMALITIES						
PDGFRA ampl	15-30	As above, see *				
CDK4/6 ampl	7-18	Amplifications involve the phosphorylation and inactivation of the Rb1 pathway, resulting in release of transcription factors required for cell cycle progression. Concomitant mutation/amplification of the tyrosine kinase receptor pathways occur in approx. 20% of DIPGs.	• ↑ cell proliferation	CDK4/6 inhibitor tested • Palbociclib	• Predominant cytostatic effect	[42, 55, 57]
CCND1/2/3 ampl	≈15			NA	NA	
1q gain, MET, IGF1R, MYCN/MYC	<1	Although commonly found in adult gliomas, are found only in a minority of DIPGs	NA	Agents tested • Monoclonal antibodies (e.g. nimotuzumab) • Small molecule inhibitors (e.g. erlotinib) • EGFRvIII-targeting peptide vaccines	• Therapies were based on effective agents used in adult regimes and weren't DIPG-specific.	[33, 42, 45, 58-65]
CELL CYCLE REGULATION						
PARP1/2 overexpression	N/A	Nuclear proteins involved in sensing DNA damage and initiating repair mechanisms to escape apoptosis.	• ↓ response to radiotherapy	PARP1/2 inhibitor tested • Veliparib/olaparib/niraparib (synergistic effect with RT and temozolomide) • Clinical trials currently underway (e.g. ABT-888)	NA	[45, 66-69]
WEE1 overexpression	N/A	A regulatory kinase at the G2 checkpoint which facilitates DNA repair and cell survival.	• ↓ cellular sensitivity to DNA-damaging agents	WEE1 inhibitor tested • MK-1775 (synergistic effect with RT in murine models) • Clinical trials currently underway	NA	[70-72]

Table 3 Selected preclinical DIPG targeted drug studies (published up to June 2018)

Drug/agent class	Agent/s investigated	Media	Effect observed (Yes/No)	Conclusion	Ref
CAR T-cells	Anti-GD2 CAR T-cells	In vitro and In vivo (murine models)	Y	• Anti-GD2 CAR T cells incorporating 4-1BBz costimulatory domains demonstrate potent cytotoxic effects towards H3K27M+ cells and engrafted tumors through antigen-dependent cytokine generation	[73]
EZH2 inhibitor and BET inhibitor	EPZ6438 and JQ-1	In vitro and In vivo (murine models)	Y	• Synergistic effects in inhibiting tumor growth by blocking cell proliferation and promoting cell apoptosis	[74]
HDAC inhibitor	Panobinostat	In vitro and In vivo (murine models)	Y	• Potent cytotoxic effects • High levels of cellular toxicity, low levels of normal cell viability • Synergistic effects with GSK-J4	[75, 76]
	Sodium valproate	In vitro	Y	• Cytotoxic effects as a monotherapy, with minimal toxicity to murine neural cells • Induces dose-dependent increases in in vitro DIPG cellular apoptosis • Its HDAC inhibition also sensitizes in vitro cells to DNA intercalating chemotherapeutics, such as carboplatin	[77]
Lytic infection agent	Parvovirus H-1	In vitro	Y	• Mild cytostatic effects observed after infection at sub-lethal doses	[78]
mTORC1/2 kinase inhibitor	TAK228	In vitro and In vivo (murine models)	Y	• Cytotoxic effects through inhibition of the mTOR/AKT pathway induced a significant survival benefit in some DIPG murine models • Inhibition of the AKT pathway by TAK228 enhances the cytotoxic effects of radiation, as demonstrated by in vitro cell cultures	[79]
	AZD2014 (mTORC1/2 inhibitor) *compared to* Everolimus (mTORC1 inhibitor)	In vitro	Y	• Combined mTORC1/2 inhibition has greater efficacy compared to MTORC1 inhibition alone, which has little to no effect • AZD2014 exhibits synergistic relationships with both radiotherapy and various chemotherapy agents	[80]
PARP1/2 inhibitors	Veliparib/Olaparib/Niraparib	In vitro and In vivo (murine models)	Y	• Cytostatic effects, most pronounced with niraparib • ↑ response to radiotherapy	[81]
PDFGRA inhibitors	Dasatinib and Cabozanitinib	In vitro	Y	• Cytostatic effects with synergistic effects between dasatinib and cabozanitinib	[82]
PI3K inhibitor and MEK inhibitor	Perifosine and trametinib	In vitro	Y	• Cytotoxic effects through synergistic inhibition of the PI3K/AKT and MEK/ERK pathways	[83]
PLK1 selective inhibitor (new generation)	BI 6727	In vitro	Y	• Significant cytostatic effects with mild cytotoxic effects • ↑ response to radiotherapy	[84]
VGEF inhibitor	Bevicizumab	In vivo (murine models)	N	• Inconclusive results due to insufficient drug biodistribution	[85]
Systematic drug screen	Screen of 83 agents	In vitro and In vivo (murine models)	Y	• 10 of the most potent agents were listed • BMS-754807, listed as the most potent agent, significantly inhibited cell proliferation in vivo regardless of the histone H3.3 mutation status	[86]

3.2 Cell Signaling Pathway Mutations

Recurrent mutations and gene amplification involved in key mitogenic and developmental signaling pathways have been described in a significant proportion of DIPGs and are commonly found to coexist with H3 mutations. The more commonly affected pathways include the PDGF, EGFR and BMP pathways.

The platelet-derived growth factor (PDGF) signaling pathway is involved in the regulation of cell growth and division, and angiogenesis. PDGF ligands bind to the cell membrane protein tyrosine kinase receptors PDGRFA and PDGFRB leading to activation of other proteins by phosphorylation. *PDGFRA* is one of the commonly mutated PDGF receptors found in malignancies and is the most commonly mutated and/or amplified tyrosine kinase receptors in DIPG, occurring in 5% and 30% of tumors respectively [42, 45-47]. *PDGFRA* mutations/amplification most commonly co-occur with H3.3K27M. The PDGFRA inhibitors, dasatinib and cabozanitinib, have been investigated in preclinical DIPG drug studies. These compounds have demonstrated cytostatic modes of action and are synergistic when administered together. However, PDGFRA inhibitors are known to have poor CNS penetration, limited primarily by drug efflux transporters. Further animal studies are necessary to investigate whether the co-administration of an efflux transporter inhibitor may improve CNS drug concentrations and subsequent drug efficacy. Inhibition of vascular endothelial growth factor (VEGF) is known to reduce downstream activation of PDGRA. VEGF inhibitors have been investigated in DIPG cell lines, so far with inconclusive results. Despite the preclinical data to date, the development of a genetic mouse model driven by PDGF-B overexpression, strongly supports a role for this pathway in DIPG tumorigenesis [87]. Recent effort has also focused on targeting its downstream effector pathway, PI3K/AKT/mTOR, that is also strongly implicated in DIPG.

EGFR mutations and amplification are among the most common genetic aberrations found in adult glioblastomas but are scarcely found in DIPGs. It is therefore not surprising that despite the large number of clinical trials investigating EGFR-targeting agents, none have shown efficacy so far. Similarly, there are varying reports on the prevalence of other mutations in kinase pathways, including *MET* mutations, which are seen in a minority of DIPGs. Trials conducted so far have failed to prove their potential as a DIPG therapeutic target [45, 59-61].

The bone morphogenetic protein (BMP) signaling pathway is affected in 20-30% of DIPGs, predominantly in the form of activating mutations in *ACVR1*, a gene encoding type I BMP receptor (ALK2), that exclusively co-occur with H3.1K27M. To date, 7 different *ACVR1* mutations have been described in DIPG, resulting in ligand-independent constitutive activation of the pathway, upregulation of phosphorylated SMAD1/5 transcriptional effectors and upregulation of downstream BMP targets, leading to promotion of cell cycle progression via the RB and p21 pathways [33, 37, 88, 89]. Similar gain-of-function mutations have been described in the rare inherited bone disease, fibro-dysplasia ossificans progressiva (FOP) [90]. Despite significant biological differences between FOP and DIPG, including the inherited nature of FOP, the study of ACVR1 functions in FOP may be useful in the overall DIPG analysis. Limited ALK2 inhibitor drug studies have been performed on either tumor types and interpretation of results has been inconclusive. The role of ACVR1 in DIPG is yet to be determined [91]. With the availability of highly-specific ALK2 inhibitors, further preclinical studies are necessary to investigate their effects on potentially susceptible DIPG cell lines.

3.3 Cell Cycle Dysregulation

Genetic abnormalities involving essential cellular pathways are also found in a third of DIPGs, commonly coexisting with oncogenic mutations in the histone genes or kinase pathways as accessory drivers. The more commonly affected pathways include the RB and PI3K-Akt pathways.

Genetic abnormalities in the RB pathway that are described in DIPG include focal deletion of *RB* itself, amplifications of *CCND1/2/3* (cyclin D1/D2/D3), and downstream *CDK4/6* (cyclin-dependent kinase 4/6), collectively occurring in approximately 30% of tumors [42, 55, 57]. These cellular proteins contribute to phosphorylation of RB and the coordination of G1/S cell cycle progression. The increased activity of these proteins contributes to tumor growth. The CDK4/6 inhibitor, palbociclib, has been studied in DIPG murine models demonstrating a cytostatic mechanism of action but with limited efficacy [57]. As these abnormalities are often coexistent with other genetic aberrations, combination therapy may potentially prove to have greater effectiveness.

The PI3K/AKT/mTOR pathway is commonly implicated in glioblastoma as well as in other tumor types. Mutations in this pathway affect cellular signaling associated with control of the cell cycle and include alterations in tyrosine kinase receptors, growth factors, and interacting pathways including Ras/Raf/MEK/ERK. These mutations affect approximately 15% of DIPGs and include recurrent mutation/amplification of *PIK3CA*, mutations of *PIK3R1*, and mutation/loss of *PTEN*. Recently, there has been substantial preclinical activity in determining the efficacy of PI3K/mTOR inhibitors in DIPG, often in combination with other targeted agents. Inhibition of PI3K/AKT pathway with perifisone in combination with the MEK inhibitor, trametinib, synergistically reduced DIPG cell viability, while use of the dual HDAC/PI3K inhibitor, CUDC-907, as a radiosensitizer looks promising, acting by inhibiting radiation-induced DNA repair pathways [83, 92]. However, more preclinical work is needed to further investigate the potential role of inhibiting these pathways, with particular consideration directed to ensuring adequate agent tumor penetration.

The Hedgehog pathway has been investigated as another potential therapeutic target owing to earlier studies demonstrating its upregulation in DIPG [93]. However, these molecular abnormalities are uncommon, and found to be insufficient to cause DIPG formation in murine models. Although the role of the Hedgehog pathway in tumor formation remains unclear, studies into the efficacy of Hedgehog pathway inhibitors, including Smoothened inhibitors, are still currently being undertaken in clinical trials [2, 93-95].

3.4 Inactivation of DNA Repair Mechanisms

Mutations in the TP53 pathway are the second most common genetic aberration in DIPG with mutations or copy number alterations observed in 42-71% of tumors and can co-occur in the presence or absence of H3 mutations. Interestingly, *PPM1D*, encoding a phosphatase (WIP1) that negatively regulates p38 MAPK and is induced by TP53, is mutated in approximately 10% of DIPG. Functionally, *PPM1D* mutations are equivalent to *TP53* mutations, and they are largely mutually exclusive with PPM1D mutations observed in 50% of DIPGs that are wildtype for *TP53* [50-52, 96]. These mutations appear to function primarily as accessory drivers in the oncogenic process and research into their therapeutic potential is scarce. However, they may have a role in therapeutic synergy with other agents in combination therapy.

Other DNA repair proteins found to be affected in DIPG include the *PARP1/2* and *WEE1* proteins. Their prevalence in DIPG cohorts has not yet been formally studied. Nonetheless, their

potential as molecular targets have been studied in preclinical models with both PARP1 inhibitors and WEE1 inhibitors demonstrating radio-sensitization effects. Further studies are needed to investigate their clinical efficacy [66-68, 71, 72, 97, 98].

4. Translation of DIPG Genomics

With the recent surge in our understanding of DIPG genomics, attention has now turned to translating this knowledge into meaningful therapeutic strategies using preclinical models. Several drug classes are currently being trialed in preclinical studies both *in vitro*, and *in vivo* using murine models (Table 3). Studies published to date have mainly tested agents targeting epigenetic dysregulation and aberrant kinase pathway activity, using targeted therapies either FDA-approved or in late-stage clinical trials for other tumor types. Many studies have shown therapeutic potential of targeted therapies *in vitro*. However, the cytostatic or cytotoxic effects observed *in vitro* only partially translate into *in vivo* murine models due to inability to efficiently cross the blood brain barrier, high levels of systemic toxicity owing to their wide-ranging effects on different pathways and cell types, and only modest improvements in survival. These findings, in part, highlight the critical importance of relevant and reliable *in vitro* and *in vivo* models that has until recently been lacking for DIPG.

4.1 Patient-Derived Cell Models

Increased frequency of early post-mortem, stereotactic biopsies and refinement of DIPG culture conditions and supplementation has enabled the development of cohorts of patient-derived DIPG cell models. Slowly but surely these models are now becoming sufficient in number to accurately represent the heterogeneity of the disease and are often extensively characterized at a genomic level with accompanying clinical annotations [99, 100]. These models have provided an invaluable resource to the research community with which to explore underlying biology and identify new therapeutic avenues. The accompanying genomic and molecular annotations of these models is now enabling the next wave of phenotype-genotype analyses to uncover primary genetic drivers of disease, relevance of co-occurring alterations and functional importance of targetable pathways, in a cohort and even patient-specific context [99, 100]. However, the normal limitations of *in vitro* cell models also apply to DIPG. These include potential of the cells to genomically or molecularly "drift", and responses to be different in the absence of supporting tissues and other signals, including immunomodulation. As a result, *in vitro* studies are often subsequently validated using *in vivo* disease models.

4.2 Orthotopic Xenograft Models

The availability of increased patient-derived cell models has also permitted the development of orthotopic cell-derived DIPG xenograft models that replicate the invasive nature of the disease and maintain key genomic and molecular characteristics. Direct injection of human DIPG cells into the fourth ventricle/pons of immune-deficient mice has not only enabled the interrogation of gene function and tumor biology but enabled evaluation of potential therapies on DIPG *in situ*, including an intact blood brain barrier, and potential systemic toxicities. Moreover, the labelling of tumor cells prior to injection, most commonly using luciferase constructs, to permit imaging,

allows researchers to monitor tumor growth and response to therapy using longitudinal imaging techniques [93]. Patient-derived orthotopic xenograft models, whereby patient tumor and stromal cells are injected without culture, also represents a valuable *in vivo* preclinical system. Typically, these patient-derived xenografts are maintained by serial passaging in immune-deficient mice to avoid any potential culture artefact. The limitation is related to absence of expression of reporters for imaging. Whilst these models remain the gold standard for *in vivo* investigation for DIPG preclinical studies, the use of immune-deficient mouse strains largely precludes the investigation of immunotherapeutic agents.

4.3 Genetic Mouse Models

The first genetic model to closely resemble DIPG preceded more recent genomic and molecular tumor characterization and was driven by germline Ink4a-ARF deletion and PDGFB overexpression in the Nestin-expressing neural stem cells of the fourth ventricle and aqueduct in neonatal mice [87]. This method utilized the replication-competent avian sarcoma-leucosis virus long-terminal repeat with splice acceptor/tumor virus A (RCAS/Tva) system whereby RCAS-PDGFB-expressing DF1 chicken fibroblast cells were injected into *Nestin tv-a;Ink4a-ARF*$^{-/-}$ mouse pups at postnatal day 3. Despite very closely representing human DIPG histopathology, tumors do not exclusively arise in the pons. Subsequent variations of this model have since been developed to more closely replicate the genomics of human DIPG. This includes RCAS-PDGFB, RCAS-Cre, RCAS-H3.3K27M expressing DF1 cells intracranially injected into *Neastin tv-a;p53*$^{lox/lox}$ neonatal mice and RCAS-PDGFB, RCAS-Cre, RCAS-H3.3K27M expressing DF1 cells intracranially injected into *Pax3 tv-a;p53*$^{lox/lox}$ neonatal mice [39, 101]. Most recently, in utero electroporation of the pontine nuclei-containing lower rhombic lip of embryonic day 12.5 mouse embryos with a piggyBac transposon vector to overexpress H3.3K27M in combination with a non-transposable gRNA/Cas9 targeting *Trp53* (H3.3K27M;p53 KD) resulted in neoplastic transformation with 100% penetrance 6 months following surgery [102]. Moreover, electroporation of a transposable shRNA targeting ATRX into this same model (H3.3K27M;ATRX/p53 KD) resulted in focally restricted lesions and reduced tumor latency to 4 months whilst the additional overexpression of wildtype *PDGFRA* cDNA using the piggyBac transposon (H3.3K27M;PDGFRA;ATRX/p53 KD) led to increased invasion and further reduced tumor latency to 21 days [102]. The different histopathological features of these models provide some insight into the importance of co-occurring genomic alterations and aberrant protein interactions on tumorigenesis. Whilst genetic mouse models of DIPG provide the benefit of studying tumors that arise *in situ*, in immunocompetent animals, they are currently restricted to narrow or broad cells of origin harboring limited and specific genetic alterations and are therefore only representative of a small cohort of the disease. As our understanding of tumor biology and the genetic and molecular interactions underlying DIPG continue to increase, so too will our ability to generate additional models that accurately represent the known heterogeneity of disease.

5. Clinical Application

Comprehensive reviews of DIPG-specific clinical trials have previously been published, analyzing a total of 55 DIPG-specific trials covering the period from 1984 to March 2011 [10, 11]. Despite the large number of clinical trials, no clear improvement in either the quality of life or length of survival has been demonstrated, and most studies are, in hindsight, complicated by the wide

variance in the selection criteria and absence of accompanying genomic and molecular data. Additionally, a systematic clinical review from the Netherlands has emphasized the necessity of cross-national cooperation to prevent potential epidemiological bias [5]. The pharmacologic agents investigated to date are largely based on therapies proven to convey survival benefit in the treatment of adult gliomas. The only agents with activity against DIPG genomic targets which have been investigated are anti-EGFR tyrosine kinase inhibitors, and results to date are inconclusive, possibly owing to varied selection criteria. One study requiring tissue biopsy on diagnosis, investigated the effects of erlotinib, and achieved an improved median survival of 12months. However, the outcome was reported as inconclusive, and further Phase III studies were deemed necessary to validate the results [58].

In this review, we have also tabulated clinical trials published between March 2011 to June 2018 (Table 4). The agents investigated are still primarily common chemotherapeutic agents with anti-EGFR agents continuing to be one of the few drug classes being tested targeting known genetic abnormalities in DIPG tumors. Short-term stable disease was reported in an 8-patient clinical trial using a PI3K pathway-specific AKT inhibitor and an mTOR inhibitor, but it failed to improve overall survival. Generic alkylating agents such as temozolomide have been extensively studied in several trials, with no proven survival benefit. Other studies included agents such as VEGF inhibitors and mTOR inhibitors also resulted in negative or inconclusive results (Table 4).

Low drug bioavailability around the tumor due to its anatomical position within the blood brain barrier and its encapsulated morphology has also been proposed as a reason for lack of conclusive and positive outcomes in DIPG clinical trials. As a result, studies are now being undertaken to maximize drug delivery to the site. Besides the conventional chemotherapy delivery through intravenous therapy, other novel routes of drug delivery are being investigated. Of these methods, convection enhanced delivery (CED) has been the most extensively studied with *in vivo* murine and primate studies proving efficacy of drug delivery [103, 104]. CED utilizes a site-directed catheter which delivers a high concentration of the therapeutic agent directly to the tumor bed. This method was developed with the purpose of achieving reliable drug levels of agents with low bioavailability across the blood brain barrier. Although a number of trials have been conducted investigating the application of CED with conventional anticancer treatments, only recently has the delivery of targeted or radioimmunotherapy agents been described for DIPG patients suggesting that it is a safe and rational therapeutic option for these patients [105-108]. Despite potential barriers to the successful application of CED, including unequal distribution of the drug to variegated tumor tissue and necessity of neurosurgery expertise for its insertion, this is an avenue with promise that warrants further attention.

In addition to CED, several other innovative methods are also on the horizon. These include intranasal chemotherapy administration, drug delivery via continuous interstitial infusions, and through the medium of human neural and mesenchymal stem cells [109]. These ideas are still in the preclinical stages of investigation but have already showed promise in murine experimental models [110-112].

In addition to the most recent clinical trials outlined in Table 4, there are also a number of ongoing trials yet to be published (Table 5). Many of these ongoing trials are testing more promising agents targeting a range of different genomic abnormalities specific to DIPG tumors. Their results are much anticipated and will provide a platform on which directed therapeutic regimes can be formed through more comprehensive genomic understanding on a biopsy-oriented, case-specific level.

Table 4 Selected recent DIPG clinical trials with ≥ 5 patients (published from March 2011 – June 2018)

Drug class	Agent/s investigated	Study type	Study phase	Study population (DIPG)	Inclusion criteria	Effect observed	Conclusion	Ref
AKT inhibitor and mTOR inhibitor	Perifosine and Temsirolimus	Prospective	I	8	MRI	Y/INC	Stable disease achieved for a median of 3 months in 6 children, survival benefit no clarified, no dose-limiting toxicity	[113]
Alkylating agent	Temozolomide	Prospective	I	15	MRI	N	No clinically significant benefit observed, reported median survival of 15.6months	[114]
		Prospective	N/A	21	MRI + Biopsy	N	No significant survival benefit, reported median survival of 11.7months observed with greater levels of toxicity	[115]
- with radiosensitizers	- Vincristine or - Cis-platinum	Retrospective	N/A	50	MRI/unclear criteria	Y/INC	Improved survival with median survival of 13months	[116]
Anti-angiogenic protocol (Angiocomb protocol)	Thalidomide and Etoposide and Celecoxib	Prospective	N/A	41	unclear criteria	Y/INC	Significantly improved overall survival with median survival of 12 months, with 1-year and 2-year survival rates of 61% and 17% respectively	[117]
Autologous dendritic cell vaccine	AEMPS PEI 15-215	Prospective	I	9	MRI	INC	The vaccine generated non-specific and specific antitumor immunological response in treated patients This therapeutic approach has demonstrated reasonable safety Survival outcomes were not measured in this study	[118]
Kinase inhibitor (anti-EGFR)	Nimotuzumab	Prospective	II	44	MRI	INC	Median survival 3.2months, a few demonstrated prolonged survival	[119]
Kinase inhibitors	Vandetanib **vs** Vandetanib and Dasatinib	Prospective	I	55 (32 vs 23)	MRI	INC	Difference in radiation field was another variable studied between the two trial groups. No significant survival benefit was identified as a difference between the two trial groups. A few reported patients appeared to have demonstrated prolonged survival, but this was not the primary outcome and was not thoroughly explored.	[120]
Monoclonal antibody (anti-VEGF)	Bevacizumab	Prospective	I	15	MRI	N	No survival benefit proven (median survival 10.4months), several discontinued therapies due to substantial toxicity (grade 3 hepatotoxicity and grade 4 thrombocytopenia)	[121]
		Retrospective	N/A	14	MRI	N	No significant increase in progression-free survival, treatment may have potentially caused a higher incidence of distant diffuse disease	[122]
Recombinant interferon	Pegylated Interferon α-2b (PEG-Intron)	Prospective	II	32	MRI	INC	No significant survival benefit, with 14% survival rate at 2years	[123]
Telomerase inhibitor	Imetelstat (GRN163L)	Prospective	II	9	MRI	N	Premature study closure due to toxicity and CNS bleeding from resultant intracranial bleeding	[114]

N/A = not applicable, Y = benefit observed, N = no benefit observed, INC = inconclusive results (e.g. not statistically significant)

Table 5 WHO registry-listed clinical DIPG drug trials up to June 2018[#] [124]

Drug class	Agent/s investigated	Trial ref no.	Current status	Registration date
Single agents				
Alkylating agent	Melphalan hydrochloride	NCT01688401	Suspended	10/09/2012
	Temozolomide	EUCTR2007-000128-42-DE	C/NP	02/06/2008
		NCT03396575	R	04/01/2018
Anthracycline	Doxorubicin	NCT02758366	R	27/04/2016
Antimetabolite (pyrimidine analogue)	Gemcitabine	NCT02992015	R	29/11/2016
Anti-PD1 Antibody	Pidilizumab	NCT01952769	NR/NP	15/09/2013
	Pembrolizumab	NCT02359565	R	09/02/2015
Anti-VEGF agents	Bevacizumab	NTR3518	R	09/07/2012
		NCT00890786	C/P	29/04/2009
CDK4/6 inhibitor	Abemaciclib	NCT02644460	R	15/12/2015
	Ribociclib	NCT02607124	R	16/11/2015
HDAC inhibitor	Panobinostat	NCT02899715	R	13/09/2016
		NCT02717455	R	22/02/2016
	MTX110 (panobinostat nanoparticle preparation)	NCT03566199	R	12/06/2018
	Valproic acid	RBR-7ygspd	Withdrawn	30/05/2016
	Vorinostat	NCT01189266	NR/NP	25/08/2010
Immunomodifier	Lenalidomide	NCT01222754	C/P	15/10/2010
Integrin inhibitor	Cilengitide	NCT01165333/ EUCTR2009-016870-33-FR	C/NP	16/03/2010
Kinase inhibitor	AZD1775	NCT01922076	R	12/08/2013
	ONC201	NCT03416530	R	23/01/2018
Monoclonal antibody (anti-EGFR)	Nimotuzumab	NCT00600054	C/P	11/01/2008
Oncolytic adenovirus	DNX-2401	NCT03178032/ EUCTR2016-001577-33-ES	R	18/08/2016
PDGFR Inhibitor	Crenolanib	NCT01393912	NR/NP	11/07/2011
Peptide vaccine	H3.3K27M Peptide Vaccine	NCT02960230	R	02/11/2016
	WT1 peptide-based vaccine	JPRN-UMIN000013257	C/P	26/02/2014
Recombinant interferon	Pegylated interferon α-2b (PEG-Intron)	NCT00036569	C/P	10/05/2002
Topoisomerase I inhibitor	Irinotecan	NCT03086616	R	16/03/2017
Multiple agent regime				
Adenoviral vector encoding cytokine IL-12 and Small-molecule activator ligand	Ad-RTS-hIL-12 and Veledimex	NCT03330197	R	16/10/2017
ALK inhibitor and kinase inhibitor	Crizotinib and Dasatinib	NCT01644773	NR/NP	17/07/2012
Antineoplastons	Atengenal and Astugenal	NCT02742883	NR/NP	12/04/2016
Antimetabolite (pyrimidine analogue), Topoisomerase I inhibitor and Monoclonal antibody (anti-VEGF) and Kinase inhibitors (anti-EGFR)	Gemcitabine, Irinotecan and Bevacizumab and Erlotinib and Everolimus	NTR2391	R	24/06/2010
Cellular preparation	Autologous dendritic cells pulsed with lysated allegenic tumor lines	NCT02840123/ EUCTR2015-003362-84-ES	C/P	31/05/2016
Cyclin D1/CDK4/CDK6 inhibitor and mTOR inhibitor	Ribociclib and Everolimus	NCT03355794	R	22/11/2017
HDAC inhibitor and Monoclonal antibody (anti-VEGF)	Valproic acid and Bevacizumab	NCT00879437	C/P	09/04/2009
HDAC inhibitor and Kinase inhibitor	Vorinostat and Temsirolimus	NCT02420613	R	15/04/2015
Kinase inhibitors	Dasatinib and Erlotinib and Everolimus	NCT02233049	R	29/08/2014
Kinase inhibitors	Vandetanib and Dasatinib	NCT00996723	C/P	15/10/2009
Monoclonal antibody (anti-VEGF) and Kinase inhibitor (anti-EGFR) and Alkylating agent	Bevacizumab and Erlotinib and Temozolomide	NCT01182350	C/P	12/08/2010

# All clinical trials up to June 2018 listed in the WHO trial registry involving a chemotherapeutic intervention for DIPG patients n≥5 have been included
R = recruiting; NR = not recruiting
C = completed; P = published; NP = no publications found

It should be noted that the rarity of DIPG has a significant impact on the ability to accelerate clinical research. The difficulty in recruiting sufficient patient numbers to demonstrate a potential survival advantage necessitates multi-institutional international trials and therefore can restrict the number of simultaneous trials. Accordingly, the weight of preclinical data will play an important role in prioritizing the next wave of clinical investigation.

6. The Future

Given the lack of success in achieving improved patient outcomes for DIPG to date, future focus is on tumor genomic and molecularly-informed targeted therapies and improving drug accessibility to the site of disease. To achieve this, better understanding of the genomic landscape of DIPGs can yet be achieved through improving the protocolization of tissue and liquid biopsies for tumor subtyping, and cross-national collaboration of DIPG research with coordination of DIPG databases, such as the International DIPG Registry and SIOPE DIPG network, to achieve a comprehensive epidemiologic profile for the malignancy.

A greater number of promising preclinical studies focused on DIPG targeted therapies is anticipated in the coming years. This will lay the groundwork on which further translational clinical trials should be conducted. Owing to the complex facets of DIPG, recent preclinical research has expanded to cover a broader range of treatment options as well as adjunct supportive therapies, utilizing both intrinsic and extrinsic pathways to target this tumor.

Extrinsically, recent identification of potential DIPG targeted therapies have put a greater emphasis on targeting the tumor's unique genomic makeup (Table 2). Historically, DIPG clinical trials have investigated chosen agents based on their effectiveness against anatomically and histologically similar adult counterparts, which have unfortunately failed to demonstrate any clinical success (Table 4). In recent years, a number of promising DIPG genomic-targeting therapeutic agents have been reported with positive outcomes in both *in vitro* and *in vivo* murine models (Table 3). Improvements in the numbers and heterogenous representation of preclinical *in vitro* and *in vivo* models will significantly enhance the transferability of preclinical results to the clinical setting. Several clinical trials extending from these studies are currently in the recruitment phase. A rationalized approach matching therapies to patient-specific tumor gene and pathway dependencies provides clinical potential for improving quality of life and/or survival outcomes for children with DIPG.

Intrinsically, recent developments in immunotherapy have demonstrated promising results in the utilization and augmentation of the children's own immune system to target and destroy tumor cells. Recent preclinical investigations of DIPG genomically targeted CAR-T cells have achieved promising *in vitro* and *in vivo* results, but are yet to be translated to clinical practice [73]. This may be challenged by the microenvironment of DIPG tumors, which are neither highly immunosuppressive nor inflammatory [125]. Therefore, concurrent research into methods of the delivery, activation, and retention of these cells in the tumor environment will be necessary. However, this area remains one of enormous potential and intense interest.

Owing to the inaccessibility of DIPG's anatomical location, further development in CNS drug delivery methods and cellular engineering is necessary to improve the potential clinical efficacy of both molecular and cellular agents. In addition to further research into invasive approaches such as CED and infusions, recent developments in non-invasive ultrasound-guided delivery of

therapeutic agents through microbubbles could also provide means of accurately targeting tumor cells to maximize therapeutic effect whilst minimizing systemic toxicity. It is also likely that the modification of already established targeted therapies with proven preclinical activity, to increase blood brain barrier permeability, could significantly strengthen the therapeutic arsenal required to treat this disease.

Further research into concurrent treatments such as radiotherapy can also be anticipated in the coming years. Some positive results have been seen in the re-irradiation trials published, and trials of larger numbers will be necessary to investigate the efficacy, dosage and toxicity of additional radiotherapy.

7. Conclusion

To improve the morbidity and mortality of children with DIPG, it is imperative to develop cross-national/international collaboration in protocoled biopsying and genomic analysis, and the consequent identification and development of genomically informed targeted therapies. Improvements in *in vivo* models and greater insight into effective methods of drug and cellular delivery will aid in improving efficacy in clinical translation. Together with concurrent radiotherapy and supportive treatments, increasing clinical trials of preclinically tested DIPG targeted therapies has the potential to improve the quality of life and survival outcomes in children with DIPG.

Author Contributions

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Competing Interests

The authors have declared that no competing interests exist.

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