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PERSPECTIVE

Improving long-term survival in diffuse intrinsic pontine glioma

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ABSTRACT

Introduction: Diffuse intrinsic pontine glioma (DIPG) is an almost universally fatal pediatric brain cancer. There has been no improvement in event-free survival (EFS) or overall survival (OS) despite immense effort through a multitude of clinical trials to find a cure. Recently, there has been a surge in the knowledge of DIPG biology, including the discovery of a recurrent *H3F3A* mutation in over 80% of these tumors.

Areas covered: The authors review the most recent approaches to diagnosis and treatment of DIPG including chemotherapy, biologics, surgical approaches, and immunotherapy.

Expert opinion: The authors propose four main opportunities to improve long-term survival. First, patients should be enrolled in scientifically sound clinical trials that include molecularly profiling either via stereotactic biopsy or liquid biopsy. Second, clinical trials should include more innovative endpoints other than traditional EFS and OS such as MRI/PET imaging findings combined with surrogates of activity (e.g. serial liquid biopsies) to better ascertain biologically active treatments. Third, innovative clinical trial approaches are needed to help allow for the rapid development of combination therapies to be tested. Finally, effort should be concentrated on reversing the effects of the histone mutation, as this malfunctioning development program seems to be key to DIPG relentlessness.

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KEYWORDS
Chemotherapy; DIPG; H3K27M; HDACS; immunotherapy; oncohistones; radiation therapy

1. Introduction

Diffuse intrinsic pontine glioma (DIPG), a brainstem high-grade glioma in childhood affecting about 250 children in the United States per year, is an almost universally fatal disease with less than 5% overall survival [1,2]. The median age at diagnosis is about 6 years old, although it has been described from infants to adults [3]. Standard diagnosis is made via clinical symptoms and imaging, with patients presenting with symptoms of cranial nerve palsies, cerebellar deficits, and pyramidal tract dysfunction. If a patient has obstruction of the fourth ventricle and resultant hydrocephalus, he/she may present with headaches, vomiting, or altered consciousness. These symptoms will progress over a short period of time (<3 months) [4]. The Standard of care remains the same as it did 30 years ago, palliative radiation therapy with a several month extension of life [3]. Overall survival (OS) is about 10–12 months after the diagnosis, with 7–9 month event-free survival (EFS) after radiation [2,3]. There remains a lot of variability in the treatment after radiation therapy, but despite an immense amount of clinical research using chemotherapy, biologics, or various radiation therapy techniques, there has been no meaningful improvement in EFS or OS [3]. In this perspective, we will review current research into the diagnosis and treatment of DIPG and discuss potential strategies for increasing the survival of affected patients.

2. Diagnosis

2.1. Imaging

Brain magnetic resonance imaging (MRI) has remained the standard for diagnosis of DIPG. Typical imaging findings are described as an expansile, infiltrative lesion comprising >50% of the pons that is hyperintense on T2-weighted MRI and fluid-attenuated inversion recovery (FLAIR) imaging, while hypointense on T1 imaging (Figure 1) [5]. There is frequently associated basilar artery encasement [3]. MRI became the standard for diagnosis because if classical imaging findings are present it correlated highly with the pathologic diagnosis [6]. Given the morbidity of biopsy was not insignificant, there was only a role for biopsy in so-called 'atypical' brainstem gliomas such as those with exophytic features [6]. Because of the lack of a histologic diagnosis, much work was done to define patients with shorter EFS as 'high-risk' based only on MRI findings. Risk factors for shorter EFS include the presence of enhancement with contrast [3,7,8]. Other findings predicting rapid progression after radiation therapy include the presence of a lactate peak on MR spectroscopy [9], and skewed apparent diffusion coefficient (ADC) histogram [10]. Interestingly, larger tumor volume at diagnosis is associated with longer PFS, possibly because for tumors to reach a larger size before symptoms appear they tend to be lower grade and grow slower [7,10,11]. One of the difficulties in following MRIs after radiation therapy is the incidence of increased FLAIR signal [pseudo-progression

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Article highlights

- DIPG is a common pediatric high-grade brain tumor with less than 5% overall survival.
- Previously a radiographic diagnosis, surgical biopsy has been proven safe in most patients.
- Recurrent mutations in histone (H3K27M) have been found in the majority of DIPG causing epigenetic dysregulation and aberrant transcription.
- Targeting the resultant epigenetic changes may be important in stopping DIPG growth.
- Newer techniques to bypass the blood–brain barrier via convection-enhanced delivery of therapies directly to the tumor show promise.
- DIPG is an immunologically 'cold' tumor, but multiple immunotherapies are trying to induce the immune destruction of DIPG.
- Ultimately, DIPG treatment will have to be multi-model if we have any hope of improving survival.

(PsP)] shortly after radiation therapy. This has been particularly problematic in clinical trials, where patients may discontinue innovative therapy prematurely given the apparent progression, making EFS difficult to interpret in these studies [12]. There have been attempts at differentiating between PsP and true progression using median ADC values, but no difference between groups was found [13]. Measuring perfusion via arterial-spin labeling did show increased values in patients with PsP [14]. Interestingly, there is a trend toward survival benefit for those patients with PsP [15]. Further research needs to be done to differentiate PsP from true progression, especially to improve the clinical trial design.

PET is emerging as an alternative noninvasive way to monitor DIPG. The unique challenge in developing tracers for PET imaging is to ensure the tracers cross the blood-brain barrier and can differentiate the already high metabolism of the brain from tumor tissue [16]. Although PET ^{18} F-FDG is extensively used in a variety of pediatric cancers [16,17], this is not an ideal tracer for brain tumors as it has a lack of specificity given its high uptake in normal brain tissue [18]. A more promising tracer is ^{11} C-methionine which has shown increased uptake in DIPG, although its utility is yet to be determined [19]. Continued evaluation of different PET tracers is ongoing, and this may emerge as an important tool in differentiating treatment response (PsP) from true progression.

2.2. Biopsy

As previously stated, biopsy had not been a part of the diagnosis of DIPG given the morbidity of the procedure with no clear clinical benefit [6]. Unfortunately, because of the lack of tumor tissue, animal models were limited to genetically engineered mouse models (GEMMs). These models were useful in studying some aspects of DIPG, but likely did not accurately recapitulate

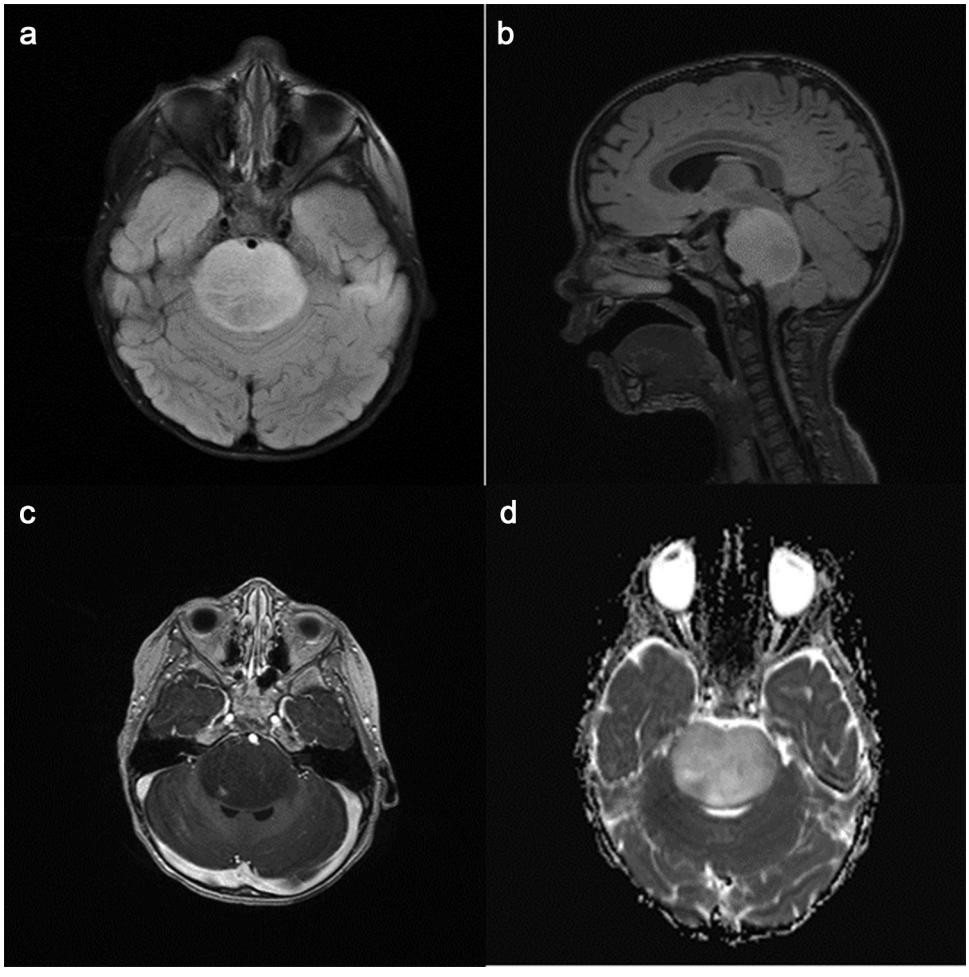

Figure 1. MRI images of a classic DIPG, including (a), (b) axial and sagittal T2 FLAIR, (c) T1 post-contrast images, and (d) Diffusion-Weighted Image.

all of the molecular characteristics of DIPG cells [20]. Autopsy studies utilizing postmortem tissue increased understanding of the biologic drivers of DIPG [21]. Autopsy tissue, however, does not possess the same molecular alterations as tissue obtained at diagnosis [22]; therefore, stereotactic biopsy of DIPG at diagnosis more accurately represents the molecular targets to be targeted with upfront therapy [23]. This tissue could be propagated via implantation into the mouse brainstem to produce patient-derived orthoptic xenografts (PDXs), allowing for new methods for testing novel therapeutics [24]. The usefulness of biopsy tissue in research and the increasing safety of stereotactic biopsy of DIPG, with permeant morbidity reported to be ~0.6% and diagnostic success of ~96% [25], have led to the increased use of upfront biopsy.

Even with the safety and efficacy of surgical biopsy, there remains a question of its clinical utility given there is yet to be a clear actionable target on molecular profiling. There have been several pilot studies to evaluate molecular stratification with biopsy. The INFORM study found it was safe and effective for biopsy, with only 1/20 patients with prolonged deficit and 20/20 with successful molecular profiling [26]. An additional pilot precision medicine trial found it was feasible to biopsy DIPG and develop a personalized treatment plan based on the results [27]. These trials illustrate biopsy will become an important part of clinical trials in stratifying patients based on the tumor's molecular characteristics.

To avoid surgical complications in evaluating the biology of DIPG, an alternative method of molecular testing could include liquid biopsy. Liquid biopsy attempts to detect circulating tumor DNA (ctDNA) that is shed into fluid compartments of the body [28]. This molecular biology technique detects the recurrent *H3F3A* mutation of these tumors to help confirm the diagnosis. Several studies using cerebrospinal fluid (CSF) and serum have shown high sensitivity of detection of *H3F3A* [27,29,30]. A pooled analysis of several liquid biopsy studies showed a sensitivity of 93% detection in the CSF and 77% in the plasma [31]. While CSF has a better sensitivity, there are limitations to obtaining CSF in patients with hydrocephalus, as a lumbar puncture in these patients could cause cerebral herniation. The serum approach would be ideal given its ease of obtaining and hopefully sensitivity should improve. Ideally, liquid biopsy could be used as another form of monitoring efficacy in clinical trial design.

2.3. Biology

Recently, a recurrent mutation in histone H3.3 (*H3F3A)* with a methionine amino acid substitution for lysine (H3 K27 M) has been found in most DIPGs. Additionally, mutations were found in the synonymous histone H3.1 (*HIST1H3B)* [32–34]. Overall, 80–90% of DIPGs have a mutation in either H3.3 (~60%) or H3.1 (~30%), while about 10% of tumors are considered wild type (WT) [35]. The outcome of these histone mutations is a dominant negative loss of histone methylation causing complex epigenetic changes and tumorigenesis [36]. Remarkably, these mutations follow a spatial relationship, as all of the mutations were found in the midline of the brain. In addition, the incidence of these mutations follows a temporal relationship, as H3.1 mutations are more commonly found in younger children (<5 years) and H3.3 mutations are more commonly found in older children [37]. Moreover, the discovery of the likely precursor of DIPG being an oligodendroglia-like cell, important in the myelination process of the pons, combined with evidence that peak incidence of DIPG corresponds with the peak age of myelination and growth in the pons, supports that these oncohistones function as a malfunctioning developmental program [35,38–42]. This dysfunctional program has numerous downstream effects, particularly the suppression of polycomb repressive complex 2(PRC2) through its interaction with zeste homologue-2 (EZH2) [32,36]. Interestingly, in murine models, expressing mutant histone proteins alone did not produce tumor growth, likely indicating it may create a favorable environment for tumor formation, but additional molecular alterations are needed [43,44]. Furthermore, in knocking down mutant histones in murine models decreases proliferation and growth, but does not ameliorate the eventual progression to death [45]. In reality, most DIPGs have additional molecular alterations in addition to histone mutations, including in *platelet-derived growth factor receptor α (PDGFRα*) and *TP53*, specifically in H3.3 mutant tumors, and *activin receptor 1* (*ACVR1*) in the H3.1 tumors [35]. Abnormalities in additional genes have been described in DIPG including *ATRX, VEGFR1, PIK3CA, PTEN, MET, AKT, NF1*, and *SMARCA4* [22,46]. In fact, even within tumors, there is tremendous heterogeneity with multiple subclones reported within a single tumor [47]. This illustrates the difficulty in finding a single targetable molecular driver for all of the DIPG.

3. Treatment

3.1. Radiation

Standard focal conformal fractionated radiation therapy (RT) remains the standard treatment of DIPG. The typical dose of RT is 30 fractions of 1.8 Gy for a total of 54 Gy [48]. Multiple attempts to hyperfractionate RT up to 66 to 75.6 Gy of radiation [49] failed to show an improvement in PFS or OS when compared to standard radiotherapy [50–52]. Conversely, attempts at hypofractionated RT to 5 Gy daily at a total of 25 Gy [53] narrowly failed to meet non-inferiority outcomes when compared to standard dose RT but did show improvement in the quality of life [54]. Several trials have tried to improve on the biologic effects of RT by adding radiation-sensitizer medications. A large review of 44 studies utilizing radiation-sensitizing agents showed a slight increase in median OS and PFS when compared to studies without radiosensitizers. It is difficult to directly compare these studies because of the heterogeneity of treatments and the lack of molecular information on most of the trials [49]. They also reported significant bone marrow toxicity in patients with concurrent treatments during RT [49]. Palliative re-irradiation after progression has been shown to be feasible, well tolerated in selected patients, as well as to improve symptoms and prolong survival by additional few months [55–57].

3.2. Chemotherapy

Despite tremendous research and effort, there has been no improvement in the OS of patients with any chemotherapy. Myelosuppressive chemotherapy was trialed extensively without any improvement in OS, including high-dose chemotherapy with stem-cell rescue [58,59]. In adults with glioblastoma, the use of temozolomide showed improved survival in patients with methylated MGMT promoter; however, temozolomide was not successful in patients with DIPG [60,61]. Interestingly, despite the negative results, there still is wide variation in providers use of chemotherapy as one survey found 44% of physicians recommended adjuvant chemotherapy after RT [62]. Part of the reason for the poor response to chemotherapy or other biologics is the tight blood–brain barrier (BBB) in DIPGs. There are several techniques that attempt to circumvent this barrier, including the use of focus ultrasound [63], nanoparticles [64], and convection-enhanced delivery (CED) [65]. Of these, CED has been the most widely used and utilizes a surgically implanted catheter to deliver the drug of interest directly to the region of the pons, thus bypassing the BBB which allows for higher local drug levels. Studies using CED have successfully been conducted in patients with DIPG, including radiolabeled antibody [^{124}I]-8H9 [66]. There are ongoing trials with the use of conventional chemotherapy (irinotecan) and the histone deacetylase (HDAC) inhibitor panobinostat via CED [67–69].

4. Emerging therapies

4.1. Epigenetic modifiers

There are multiple new therapies (Table 1) that are capitalizing on the recent advance in the knowledge of DIPG biology, either trying to reverse the epigenetic changes caused by oncohistones or directly targeting molecular pathways altered in DIPG. Increased acetylation of histone proteins in histone mutated tumors increases transcriptional activation via changes in chromatin structure [70,71]. The previously mentioned HDAC inhibitor panobinostat was shown to reverse some of the epigenetic changes related to the histone mutation and was active in patient-derived xenograft (PDX) models (Figure 2) [21,72]. The major limitation of panobinostat is its limited CNS penetration and significant off-target toxicity [72]. There is an ongoing phase 1 clinical trial (NCT02717455) to define the maximal tolerated dose and toxicity profile of this agent. There are also attempts to bypass these issues by delivering panobinostat via CED [73]. An additional HDAC inhibitor in a clinical trial is the dual PI3 K/HDAC fimepinostat (NCT03893487), which demonstrated significant pre-clinical cytotoxic effect, particularly when combined with radiotherapy in pediatric DIPG cell lines [74]. Another approach to treatment is to target the oncohistone-induced hypomethylation. The CNS-penetrant inhibitor of Jumonji domain-containing protein 3 (JMJD3) GSKJ4 restores methylation and inhibits proliferation leading to increased survival in PDXs [75,76]. Furthermore, combining both epigenetic approaches in pre-clinical studies by using a dual HDAC and lysine-specific demethylase 1 (LSD1) inhibitor Corin appear to be effective by decreasing xenograft growth *in vivo* [77]. Outside of attempting to restore methylation or acetylation states in DIPG directly, several inhibitors of EZH2 and PRC2, important components of the oncohistone pathogenesis, have been developed and have shown pre-clinical efficacy [78,79]. Analogous to PRC2, polycomb repressor complex 1 (PRC1) causes epigenetic changes via chromatin remodeling promoting tumorigenesis [80]. Targeting PRC1 via its BMI1 subunit by PTC-209 decreases the viability of DIPG cells *in vitro* and *in vivo* in PDX models [81,82]. Additionally, targeting transcriptional machinery by inhibiting RNA polymerase II (RNAPII) via bromodomain and extra-terminal (BET) has shown *in vivo* efficacy against DIPG PDXs alone or in combination with EZH2 inhibitors [83]. Inhibition of cyclin-dependent kinase 7 (CDK7) a transcriptional protein involved in activation of RNAPII by THZ1 was effective but did not have significant CNS penetration in pre-clinical models [84]. More recently a multi-kinase inhibitor which inhibits CDK7 and has CNS penetration, TGO2, showed efficacy in PDXs [85]. Overall, despite the promising pre-clinical data for epigenetic modifiers, safety, and efficacy profiles are still yet to be determined, and human trials are either ongoing or forthcoming.

4.2. Other targeted inhibitors

Like many other cancers, receptor tyrosine kinases (RTKs) are important in the pathogenesis of DIPG. Examples of abnormal signaling pathways include PDGF and PDGFRα which leads to aberrant PI3 K/AKT/mTOR and RAS/RAF/MEK/ERK signaling and increased cell growth and survival [86,87]. Additional molecular alterations in *PTEN*, which negatively regulate PI3 K, as well in PIK3CA and PIK3R1, have been reported in a subset of DIPG causing activation of this pathway [88]. Targeted therapies attempting to ameliorate the consequence of these molecular alterations in DIPG are in development. One such target, PDGFRα, by the multi-kinase inhibitor dasatinib did not show any clinical response, albeit this was in phase 1 trial [89]. Dasatinib does not have significant CNS penetration, which may be a cause of its lack of efficacy *in vivo* [90]. Targeting mammalian target of rapamycin (mTOR) by TAK228 demonstrated significant *in vitro* and *in vivo* inhibition of DIPG [91]. Inhibiting mTOR by temsirolimus combined with the AKT inhibitor perifosine showed no responses in relapsed DIPG, but again this was a limited phase 1 study therefore further investigation is needed [92]. Combing inhibition of both RAS/MAPK and PI3 K pathways by targeting AKT and MEK by perifosine and trametinib, respectively, showed significant *in vitro* effect [87]. Recently an additional RAS/MAPK protein, ERK5, was found to be important in DIPG growth, and inhibition by TG02 in DIPG PXAs led to prolonged survival [85]. Recurrent mutations in an *ACVR1*, which encodes for the ALK2 receptor, occur in over 80% of H3.1K27 M tumors and cause increased BMP/SMAD4 signaling with increased cell growth [93]. Treatment with the ALK2 inhibitor LDN212854 was recently shown to inhibit both *in vitro* and *in vivo* growth of *ACVR1* mutant DIPG [94].

Dysregulation of cell-cycle checkpoints is common in cancer and is a possible target in DIPG treatment. As a review, the cyclin-dependent kinases (CDKs) 4/6 combine with cyclins (D1,

Table 1. Published and ongoing trials using NIBS to ameliorate motor symptoms in schizophrenia.

Treatment Type	NCT Number	Title	Medication	Phase
Biologic	NCT02233049	Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication	Erlotinib Everolimus Dasatinib	Phase 2
	NCT03605550	A Phase 1b Study of PTC596 in Children with Newly Diagnosed Diffuse Intrinsic Pontine Glioma and High Grade Glioma	PTC596	Phase 1
	NCT03355794	A Study of Ribociclib and Everolimus Following Radiation Therapy in Children with Newly Diagnosed Non-biopsied Diffuse Pontine Gliomas (DIPG) and RB+ Biopsied DIPG and High Grade Gliomas (HGG)	Ribociclib Everolimus	Phase 1
	NCT02644460	Abemaciclib in Children With DIPG or Recurrent/Refractory Solid Tumors	Abemaciclib	Phase 2
	NCT03416530	ONC201 in Pediatric H3 K27M Gliomas	ONC201	Phase 1
	NCT02717455	Trial of Panobinostat in Children with Diffuse Intrinsic Pontine Glioma	Panobinostat	Phase 1
	NCT03893487	Fimepinostat in Treating Brain Tumors in Children and Young Adults	Fimepinostat	Phase 1
	NCT03696355	Study of GDC-0084 in Pediatric Patients with Newly Diagnosed Diffuse Intrinsic Pontine Glioma or Diffuse Midline Gliomas	GDC-0084	Phase 1
	NCT01837862	A Phase I Study of Mebendazole for the Treatment of Pediatric Gliomas	Mebendazole	Phase 1/2
	NCT03598244	Volitinib in Treating Participants with Recurrent or Refractory Primary CNS Tumors	Savolitinib	Phase 1
	NCT03387020	Ribociclib and Everolimus in Treating Children with Recurrent or Refractory Malignant Brain Tumors	Everolimus/Ribociclib	Phase 1
	NCT03709680	Study of Palbociclib Combined with Chemotherapy in Pediatric Patients With Recurrent/ Refractory Solid Tumors	Palbociclib Temozolomide Irinotecan	Phase 1
	NCT01884740	Intraarterial Infusion of Erbitux and Bevacizumab For Relapsed/ Refractory Intracranial Glioma In Patients Under 22	SIACI of Erbitux and Bevacizumab	Phase 1/2
Chemotherapy	NCT02992015	Gemcitabine in Newly Diagnosed Diffuse Intrinsic Pontine Glioma	Gemcitabine	Phase 1
	NCT02758366	Prolonged Exposure to Doxorubicin in Patients With Glioblastoma Multiforme and Diffuse Intrinsic Pontine Glioma	Doxorubicin	Phase 2
	NCT03243461	International Cooperative Phase III Trial of the HIT-HGG Study Group (HIT-HGG-2013)	Temozolomide + Valproic Acid Vs. Temozolomide + Chloroquine	Phase 3
Convection Enhanced Delivery	NCT03566199	MTX110 by Convection Enhanced Delivery in Treating Participants with Newly Diagnosed Diffuse Intrinsic Pontine Glioma	Panobinostat Nanoparticle Formulation MTX110 via CED	Phase 1/2
	NCT03086616	CED With Irinotecan Liposome Injection Using Real Time Imaging in Children with Diffuse Intrinsic Pontine Glioma (DIPG)	CED of Nanoliposomal irinotecan (nal-IRI)	Phase 1
	NCT04264143	CED of MTX110 Newly Diagnosed Diffuse Midline Gliomas	MTX110 and gadolinium	Phase 1
Immunotherapy	NCT04049669	Pediatric Trial of Indoximod With Chemotherapy and Radiation for Relapsed Brain Tumors or Newly Diagnosed DIPG	Indoximod	Phase 2
	NCT04185038	Study of B7-H3-Specific CAR T Cell Locoregional Immunotherapy for Diffuse Intrinsic Pontine Glioma/Diffuse Midline Glioma and Recurrent or Refractory Pediatric Central Nervous System Tumors	SCRICARB7H3(s); B7H3-specific chimeric antigen receptor (C	Phase 1
	NCT02359565	Pembrolizumab in Treating Younger Patients with Recurrent, Progressive, or Refractory High-Grade Gliomas, Diffuse Intrinsic Pontine Gliomas, Hypermutated Brain Tumors, Ependymoma or Medulloblastoma	Pembrolizumab	Phase 1
	NCT03396575	Brain Stem Gliomas Treated with Adoptive Cellular Therapy During Focal Radiotherapy Recovery Alone or With Doseintensified Temozolomide (Phase I)	Tumor RNA-Dendritic Cell Vaccine with GM-CSF w/wo Dose Intensive Temozolomide	Phase 1
	NCT03690869	REGN2810 in Pediatric Patients with Relapsed, Refractory Solid, or Central Nervous System (CNS) Tumors and Safety and Efficacy of REGN2810 in Combination with Radiotherapy in Pediatric Patients With Newly Diagnosed or Recurrent Glioma	REGN2810	Phase 1/2
Other	NCT03739372	Clinical Benefit of Using Molecular Profiling to Determine an Individualized Treatment Plan for Patients with High Grade Glioma	Multiple Medications	Other
	NCT03478462	Dose Escalation Study of CLR 131 in Children and Adolescents with Relapsed or Refractory Malignant Tumors Including but Not Limited to Neuroblastoma, Rhabdomyosarcoma, Ewings Sarcoma, and Osteosarcoma	CLR 131	Phase 1

rTMS: repetitive transcranial magnetic stimulation; DLFPC: dorsolateral prefrontal cortex, SHRS: St. Hans Rating Scale, EPS: extrapyramidal side effects, iTBS: intermittent theta burst stimulation, IFG: inferior frontal gyrus cTBS: continuous theta burst stimulation, IPL: inferior parietal lobe, TULIA: test of upper limb apraxia, CR: coin rotation, SMA: supplementary motor area, SRRS: Salpêtrière Retardation Rating Scale, RETONIC: Personalized noninvasive Neuromodulation by rTMS for Chronic and Treatment-Resistant Catatonia, OCoPS-P: Overcoming Psychomotor Slowing in Psychosis trial, BrAGG-SoS: Brain Stimulation And Group Therapy to Improve Gesture and Social Skills in Psychosis trial.

D2, or D3) to phosphorylate retinoblastoma-associated protein (RB1), sequestering it from E2 F and allowing E2 F transcription factor to induced entry into the cell cycle [95]. Direct inhibition of CDK4/6 by palbociclib showed *in vitro* effects, but ultimately had poor BBB penetration for an *in vivo* effect [96,97]. An additional CDK4/6 inhibitor abemaciclib has significantly increased CNS penetration [98] and is currently in clinical trials for DIPG (NCT02644460). G2 checkpoint, mediated by WEE1 kinase, is important in pausing mitosis of cells which allows DNA damage repair and continued dividing. This is an

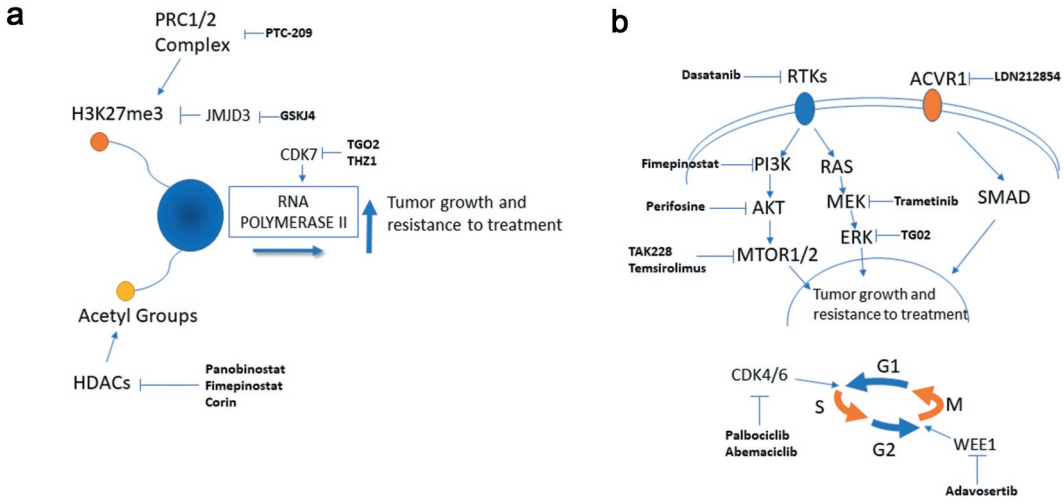

Figure 2. Schematic diagram of drug targeting. (a) Epigenetic processes, (b) Growth factor pathways and cell-cycle checkpoints in DIPG. RTKs: Receptor tyrosine kinase, HDACs: Histone deacetylase, PRC1/2: Polycomb repressive complex 1/2.

important mechanism used by DIPG cells to resist radiation therapy [99]. Inhibition of WEE1 increased cell death in conjunction with radiation in DIPG cell lines [99]. The WEE1 inhibitor adavosertib has recently finished a phase 1 clinical trial with results pending (NCT01922076). An additional inhibitor with activity against DIPG is ONC201. ONC201 was thought to have anti-tumor activity via binding of the dopamine receptor 2 (DRD2/3) and resultant activation of tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL) [100]. More recently, it was discovered that the mechanism of action was related to the binding of human mitochondrial caseinolytic protease P (CLpP) causing inhibition of protein synthesis/growth [101]. Clinical interest in ONC201 in histone mutated tumors increased after a single adult patient with an H3 K27 M tumor had a profound and sustained response to ONC201 [102]. Further responses were found in other diffuse midline gliomas and DIPGs [103,104]. There are ongoing phase 1 trials to further investigate ONC201 in pediatric patients with DIPG (NCT03416530). Given the previously stated heterogeneity of these tumors, it is highly unlikely that any one of these medications will be effective alone.

5. Immunotherapy

Immunotherapy revolutionized treatment for many cancers, including turning metastatic melanoma into a curable disease [105]. Developing immunotherapy for a heterogeneous solid tumor such as DIPG with a microenvironment that, unlike melanoma, is devoid or 'cold' of immune infiltration is more challenging [106,107]. There still is great enthusiasm for immune therapy, and there are several different approaches being tried in clinical trials.

5.1. Checkpoint inhibition

Checkpoint inhibition involves blocking the inhibitory signal of T-cells when interacting with tumor cells. This is accomplished via antibodies targeting programmed death 1 (PD-1) and/or cytotoxic T-lymphocyte associated protein 4 (CTLA-4), amongst others, which elicits cytotoxic T-cells to attack tumors [108]. Unfortunately, PD-1 expression is low and there is a lack of T-cell infiltration in DIPG which makes it less likely that checkpoint inhibition will be effective [106,107]. Checkpoint inhibition utilizing the PD-1 inhibitor pembrolizumab was tested in children with DIPG with dismal results (NCT02359565) [109]. PD-1 inhibition in patients with hypermutated tumors is more effective, as first described in hypermutated colon cancer [110,111]. There are ongoing trials evaluating this approach in pediatric brain tumors (NCT02359565). However, since DIPG has a low mutational burden, this approach is less likely to be effective [112]. Recently, there is a interest in modulating the immune response by inhibition of indolamine 2,3-dioxygenase (IDO), an enzyme involved in creating an immune inhibiting microenvironment [113]. Inhibition of IDO when combined with temozolomide was synergistic in glioma models [114], and trials are ongoing in pediatric DIPG with the IDO inhibitor indoximod (NCT04049669).

5.2. Vaccine therapy

A separate approach to augment the anti-tumor immune response is the use of vaccine therapy. The rationale for this therapy is to induce a cytotoxic T-cell response by introducing an antigen (protein or DNA/RNA) to induce an immune response, thereby tipping the scales of the immune microenvironment to immunostimulatory. Peptide vaccines containing the antigens of EphA2, IL-13Ra2, and survivin demonstrated some clinical response, including PsP in pilot studies [115,116]. Additionally, a peptide vaccine taking advantage of the recurrent mutant histone protein H3 K27 M by targeting the mutant histone directly has been successful in pre-clinical studies [117]. Again, this approach will be limited by the cold immune environment in DIPG as well as the fact that these vaccines will only induce an immune response in patients with specific HLA types (in this case A2). Another vaccine approach is the use of autologous dendritic cell

vaccine (ADCV) [118]. ADCVs are made by leukapheresis of monocytic cells from patients, exposing them with tumor-cell antigens, and then giving them back as a vaccine [119]. A trial evaluating this is ongoing (NCT03396575).

5.3. CARs

Finally, immunotherapy using chimeric antigen receptors (CARs), which has revolutionized the treatment of acute lymphoblastic leukemia (ALL) [120], has begun to be developed as a therapy against DIPG. CARs utilize patient-derived T-cells transfected via a viral vector to respond to a specific antigen, and infuse them back into the patient [121]. Unfortunately, unlike ALL, which has a common antigen such as CD-19, DIPG lacks a single antigen to target because of the molecular heterogeneity. There have been a few attempts to develop CARs for DIPG including some that are becoming available in clinical trials now (NCT04185038) [121,122]. One of the more promising recent approaches has been to target disialoganglioside GD2, which is expressed widely in DIPG tumor cells [123]. The anti-GD2 CARs showed promising efficacy in PDXs, with mice having profound and durable responses [123]. Unfortunately, a robust immune response in the brainstem could cause devastating symptoms or death and the treatment for this (i.e. corticosteroids) would limit the benefit of the immunotherapy. In fact, when using the anti-GD2 CAR in mice there were many toxic deaths, likely secondary from brain swelling and herniation [123]. If these treatments were to go forward, they very likely would have to be done with discussion of the need for a temporary or permeant diversion of CSF.

6. Expert opinion

A decade ago, little was known about the biology of DIPG, but recently there has been an explosion in knowledge of the pathogenesis of this deadly tumor. With that, we are still in the infancy in understanding how DIPG develops, resists treatment, recurs, and evades the immune system. While we hope that in 5 years the survival of affected children will improve, realistically we feel this process will take longer and will have to involve multimodal treatments. We propose that to improve the survival of DIPG we should take the following steps.

First, all tumors should be molecularly profiled through either stereotactic biopsy or liquid biopsy in the context of a scientifically sound, well-designed clinical trial. Even though the majority of DIPGs will have histone mutations, there is enough molecular variability to no longer treat this as one single disease, and therapies should be targeted toward specific subtypes of DIPG. Without the knowledge of the molecular driver, it is nearly impossible to accurately assess clinical trial data. Ideally, liquid biopsies would be preferred to limit the morbidity to patients, but surgical biopsy has been shown to be very safe and would yield more information. We should continue to increase knowledge of the molecular drivers of DIPG, allowing a precision medicine approach. The hope would be that when a patient is diagnosed, she will have her tumor molecularly profiled and an individual plan is developed for her based on the targets and drugs available.

Second, we propose novel clinical trial designs and endpoints. One of the limitations with determining the efficacy of novel therapies given in combination or shortly after RT is the profound biologic effect RT can have, making it difficult to detect small biologic effects on the tumor. This may lead to the abandonment of biologically active compounds erroneously. There are also many variables as to why OS and EFS could be shorter, such as PsP or tumor hemorrhage that may or may not be related to the innovative treatment. Ideally, combining new imaging techniques, either PET or MRI, with clinical outcomes, would help differentiate from PsP and true progression and allow for a more accurate EFS outcome. These outcome measures should also be coupled with biologic outcome measures, such as serial liquid biopsy, much like the monitoring done for BCR-ABL in chronic myeloid leukemia.

Third, we do not think a single treatment or therapy has the potential to cure DIPG; therefore, we need to develop strategies to rapidly evaluate combinations of treatments. One of the issues with the current trial design is one target/one drug/one trial could take several years to publish, and if the results are negative or equivocal, the drug is abandoned, even if it might be reasonable to combine it with another agent or approach. We need rational combinations of treatments and the ability to rapidly test them together. Increasing pre-clinical research funding and improving biologic databases will help define novel targets in DIPG. To help incentivize drug companies to develop novel therapeutics in the United States, the Research to Accelerate Cures and Equity for Children Act (RACE for Children Act) was signed into law and is to go into effect in 2020. This law mandates that pharmaceutical companies develop pediatric studies on all novel cancer drugs where there is a possible pediatric cancer indication. Hopefully, this will improve access to novel agents, although how these studies will be done is still to be determined. While this is a good beginning, we propose that DIPG represents a unique form of pediatric cancer that requires a unique set of rules. Pharmaceutical companies should be incentivized/compelled to allow the use of proprietary drugs, if a rational drug/target (determined by a consortium/peer-reviewed panel) exists in DIPG, even if this target is discovered after the drug is approved for another cancer. There would also be incentives to companies to allow combinations of treatments such as multiple small-molecule inhibitors, immunotherapy, BBB disrupting therapies, etc.

Finally, we believe that improving survival in DIPG will be linked to developing a therapy that can ameliorate or eliminate the effects of the oncohistones. While we do not understand the entire role of the oncohistones in the pathogenesis of DIPG, it is likely these epigenetic changes allow the tumor DNA to be more susceptible to further mutations and the subsequent development of subclones of treatment resistance disease. HDAC inhibitors are an attempt to reverse this phenomenon, but ultimately, they are only treating one symptom of this malfunctioning developmental program. If we are able to reverse the oncohistone's epigenetic changes completely, we have hope that improvement in survival may be possible in the next 5–10 years.

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