Chapter 17

Diffuse Intrinsic Pontine Glioma: A Therapeutic Challenge

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Additional information is available at the end of the chapter

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Abstract

Diffuse intrinsic pontine glioma (DIPG) is a tumor of the brainstem, specifically in the pons, accounting for 10–20% of all of central nervous system (CNS) tumors in children. Unfortunately, DIPG is the leading cause of death in children with CNS cancers. Clinical interventions trying to effectively treat children with DIPG have failed despite 40 years of clinical trials. The critical location of these tumors eliminates extensive surgical resection as an option. Radiation therapy (RT) is the standard of care, and although it improves the clinical symptoms of most patients, the results are temporary, with tumor progression typically occurring months post radiation. Given the dismal prognosis associated with this disease and the challenge to find chemotherapeutic agents, especially molecularly targeted drugs that improve the survival of the patients, there is a strong incentive to move new treatments forward into clinical trials. The more effective treatment would potentially involve combinatory therapeutic regimens with new epigenetic drugs that can offer synergistic benefits and potentially increase therapeutic efficacy. The increasing knowledge of genomic, epigenomic, and proteomic characteristics of DIPG is opening doors to new therapeutic avenues and provides hope and promise for this devastating childhood cancer.

Keywords: diffuse intrinsic pontine glioma (DIPG), brainstem glioma, high-grade glioma (HGG), targeted therapy, combinatory mutations, precision medicine

1. Introduction

Diffuse intrinsic pontine glioma (DIPG) is a tumor that arises in the pons and diffusely infiltrates the brainstem. It is believed that DIPG originates from a dysregulation of a postnatal

neurodevelopmental process. It usually affects middle childhood, with a peak onset of 6–9 years of age. High-grade gliomas (HGGs) typically have a predilection for the ventral pons, a finding that would reflect the presence of a cell of origin as well as a signaling microenvironment favorable for tumor formation [1–3]. A study using early postmortem DIPG tumor tissue has shown that the Sonic Hedgehog (Shh) signaling pathway in DIPG tumor cells is involved in many developmental and oncogenic processes, such as neural embryogenesis and oligodendrogenesis. The dysregulation of this molecular system in DIPG leads to hypertrophy of the ventral pons and suggests a potential molecular origin for this poorly understood cancer [4]. According to the lessons learned from other pediatric brain tumors, such as medulloblastoma, neural stem or precursor cells would be the most likely cell type that could transform and give rise to DIPG [4–6].

In the United States, 200–300 children are diagnosed each year with DIPG [7]. Unfortunately, being the pediatric brain tumor with the highest mortality rate, DIPGs have poor prognosis with a less than 1-year survival, where less than 10% and 2% of patients survive after 2 and 5 years post-diagnosis, respectively [8]. The grim outcome first and foremost is due to the tumor's delicate anatomical location and significant infiltration. Extensive surgical resection is not a treatment option, leaving radiation therapy (RT) and chemotherapy as the only remaining therapies.

RT is the standard treatment for children with DIPG and results in improvement of symptoms in more than 80% of the patients; however, it rarely results in a cure. The conventional treatment consists of 1.8 Gy fractions delivered once daily, 5 days a week, for about 6 weeks to a total cumulative target dose of 54 Gy. Hyperfractionated doses up to 72 Gy have not shown improved efficacy in children and resulted in increased morbidity. On the other hand, hypofractionated RT may lead to similar outcomes as standard treatment. The median survival of patients treated with RT is only 10 months [9,10]. When RT is associated with standard chemotherapeutic agents, no survival benefit was shown, in neither the event-free survival (EFS) nor the overall survival (OS) of patients [11].

Another reason of poor prognosis is associated with the ineffective results using chemotherapeutic agents. Despite decades of research and use of different chemotherapeutic strategies, no survival advantage has been achieved. In the last 30 years, several clinical trials were done using various adjuvant chemotherapeutic drugs utilized prior to, during, or after radiotherapy in DIPG patients. The results were bleak: none of these clinical trials showed any improvement in survival of this pediatric cancer, leaving DIPG as the number one cause of brain tumor-related death in children [12]. In addition to the difficulty associated with finding effective therapeutics, it is also speculated that the tumor biology changes between the primary and recurrent tumors, leading to another problem—resistance to therapy. Furthermore, an additional challenge includes ways of overcoming the restrictive ability of the intact blood–brain barrier (BBB) in patients with DIPG.

The lack of reliable models along with poor knowledge of the biological basis of DIPG has been critical elements in failure to make progress in this disease. In the pre-CT and magnetic resonance imaging (MRI) eras, histological assessment of biopsies was routinely conducted to diagnose DIPGs. However, this standard of care was discontinued in the early 1990s, due to

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the high rate of morbidity and improvement of imaging techniques [13]. Recently, the increase in availability for biopsy and acquisition of autopsy specimen for experimental purposes, as well as the insight gleaned from recent studies, is beginning to unravel the genetic and epigenetic drivers of DIPG. Stereotactic biopsies in well-trained neurosurgical teams are a safe procedure [14] and are being incorporated for patients with DIPG. Improved methods of modeling DIPGs by mimicking genetic and epigenetic changes, preclinical testing, and translational studies will bring a strong incentive to move new treatments forward into clinical trials.

Given the different molecular subgroups within the disease and the combinatory mutations found through gene expression, mutational, and epigenetic analysis, the key for an effective treatment relies on combinatory therapy. Studies using deep sequencing analysis, comprehensive methylation, copy number, and mRNA expression profiling show that these subgroups are characterized by upregulation of MYCN (N-Myc), Shh signaling, and H3-K27M mutations [15–18]. The combinatory genomic aberrations have introduced one more challenge for designing therapeutic regiments—most of the combinatory mutations are novel and thus there is a deficit of preclinical data on combinatory drug regimens. However, the increase in knowledge of DIPG and development of novel *in vitro* and *in vivo* approaches is a promise for effectively targeting driver mutations with the use of combinatory drugs.

New promising approaches provide a glimpse of hope for patients who are battling this devastating tumor. Among the many devastating childhood cancers, DIPG patients desperately need access to new treatments. Increased availability of tumor tissue for preclinical investigation and the development of experimental model systems now provide important tools to guide future clinical trials. Advances in the knowledge of the molecular biology of DIPG are key to developing new therapeutic testing.

2. Diagnosis: clinical presentation, radiographic findings, and stereotactic biopsy

2.1. Clinical presentation

Cerebellar signs (e.g., ataxia, dysmetria, and dysarthria), pyramidal tract signs (e.g., hyperreflexia, clonus, increased tone, and presence of a Babinski reflex), and cranial nerve palsies (unilateral or bilateral) are the classic triad clinical presentation in DIPG. As the tumor grows, the pons become diffusely infiltrated and enlarged, the basilar artery is encased, and crucial nuclei of cranial nerve tracts V, VI, VII, and VIII within the pons are compressed. The symptom onset is acute, with a fast progression, where children typically experience 1 month or less of neurologic manifestations before they are diagnosed. Symptom duration greater than 6 months prior to presentation should prompt a search for an alternate diagnosis. The most common reported symptoms are abnormal or limited eye movements, diplopia, asymmetric smile, clumsiness, difficulty walking, loss of balance, and weakness [19,20]. Obstructive hydrocephalus presenting with headaches, nausea, and vomiting may be present due to increased intracranial pressure, resulting from expansion of the pons. Other less common

symptoms may occur, including behavioral changes, night terrors, and scholarly difficulties [8]. Among the rare symptoms are urinary retention and other voiding abnormalities without spinal cord lesions, which can be due to disruption of the pontine micturition center [21].

2.2. Radiographic findings

Advances in imaging technology over the last few decades and the development of MRI have significantly improved the accurate diagnosing of DIPG. MRI scan is the best noninvasive method to determine the size and the characteristics of the tumor. Thus, the comprehensive diagnosis of DIPG is based on MRI findings combined with the clinical presentation.

On MRI, the boundaries of a DIPG are hard to determine, as the tumor cells invade the surrounding tissue of the pons—the tumor appears as a large expansible brainstem mass. The epicenter of DIPG lies within the pons and the lesion involves the majority of its structure. Tumors typically show diffuse hyperintense bright signal on T2-weighted and are hypo- or isointense on T1 (**Figure 1**).

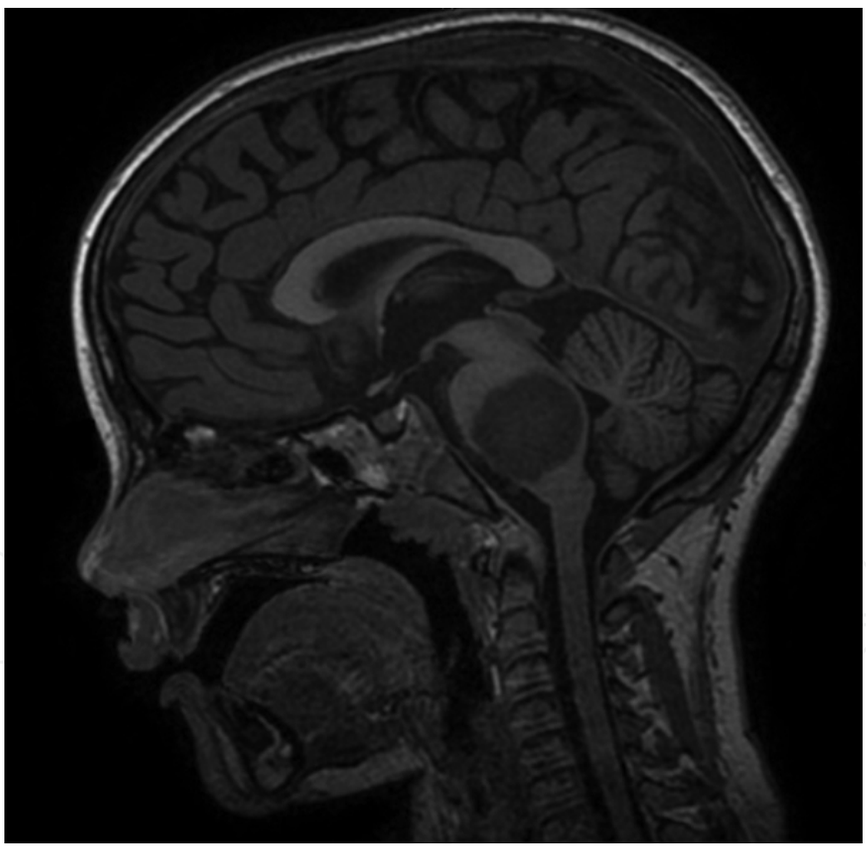

Figure 1. Precontrast sagittal volume T1 MRI of the brain of a DIPG patient showing diffusely infiltrating pontine mass.

On fluid-attenuated inversion recovery (FLAIR) imaging sequences, the tumor frequently appears homogeneous. MRI can also show pinpoint intratumoral hemorrhages, ventral

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involvement of the pons, encasement of the basilar artery, and possible sites of tumor extension [8,22,23].

One classification system developed by Choux et al. [24] classifies brainstem gliomas into diffuse, intrinsic focal, extrinsic focal, and cervicomedullary based on MRI. DIPGs are classified as type I tumors—those that diffuse throughout the brainstem. Type II, III, and IV tumors are characterized by more focal lesions and may have a more favorable outcome.

Compared to computed tomography (CT), MRI provides superior imaging of the posterior fossa of the brain and has a superior contrast resolution of soft tissue. Other advanced imaging techniques include magnetic resonance spectroscopy (MRS), perfusion imaging, and positron emission tomography (PET). These imaging methods show improved advantages such as tumor differentiation. However, MRI appearance is uncertain and stratification of patients based on the aggressiveness of their tumors could be helpful in deducing a more accurate diagnosis, leading to an improved understanding of these tumors.

2.3. Differential diagnosis in MRI

A universally diagnostic criterion has not yet been defined for DIPGs. Currently, the criteria that are typically accepted include symptom duration less than 6 months, at least two or three symptoms related to brainstem dysfunction, and pontine enlargement with evidence of diffusely infiltrative tumor centered in and involving greater than 50–66% of the pons [25].

In MRI, DIPGs present with distinct characteristics from pilocytic astrocytoma; for example, MRI shows the following aspects: (A) focal, well defined on T1 and T2 weighting, (B) minimum brainstem swelling, and (C) brainstem location without extension. The two most common types of pediatric brainstem tumors, DIPGs and pilocytic astrocytoma, can be accurately identified by MRI alone in most cases. Although MRI is not 100% specific, the vast majority of children diagnosed with DIPG by MRI do have a diffuse infiltrative glioma.

Patients with diffuse brainstem gliomas associated with neurofibromatosis type 1 (NF1) may mimic DIPG on imaging. However, they are usually low-grade gliomas (LGG—World Health Organization (WHO) grades I–II) which can be asymptomatic and present with a simple differential diagnosis based on family history and clinical examination [26].

In the context of an atypical presentation of DIPG (presentation with clinical features and circumscribed MRI characteristics), it is important to rule out potential differential diagnosis. These include embryonic tumors such as ATRT, PNET, and nonmalignant lesions such as infections, neurodegenerative conditions, and hemangioblastomas [27].

2.4. Stereotactic biopsy

Before the advent of effective imaging techniques, surgical biopsies played an important role in DIPG diagnosis. However, with improved radiologic capabilities—primarily MRI—until recently, stereotactic biopsies are only performed in the rare cases, where the diagnosis is uncertain based on MRI findings. Nonetheless, as neurosurgical experience with stereotactic biopsies of DIPGs grew and was proven to be safer, as well as neuropathologic expertise to

identify molecular subtypes increased, biopsy has been increasingly performed to not only identify the type of tumor present but to delineate potential molecular targets that could be therapeutically explored. In experienced hands, the permanent morbidity after stereotactic biopsy has been found to be less than 5%.

In histologic diagnosis, DIPGs are defined as a fibrillary astrocytoma, WHO grades II–IV, and in most of the cases resemble malignant gliomas in other locations [28]. However, the prognosis for DIPGs is not associated with the histological classification. DIPGs harboring the histone 3 mutation classified as WHO II and III have a poor OS, similar to WHO IV patients [29]. In addition, significant histopathological variability has been reported in DIPGs, where a single biopsy may not be representative of the histological classification of the entire tumor [23,29,30].

Important biological information obtained from biopsies may be used in future clinical trials, guiding new treatment regimens and allowing for advances in surgical and molecular analytical techniques [31]. The use of tissue obtained from pretreatment biopsies combined with antibodies to detect driver mutations gives the opportunity to identify the genomic mutational landscape of DIPG and provides opportunities to improve diagnosis, prognosis, and better understanding of the potential drug targets.

3. New advances: the future of genomics, epigenomics, and proteomics

Taking into consideration that DIPG may represent a biologically distinct subclass of glioma, there is a great need for the comprehensive investigation of tumor biology. Therefore, studies in this rare type of cancer cannot be performed without the knowledge of genomics and proteomics. The development of new technologies that can rapidly analyze DNA, RNA, and proteins and the progress in bioinformatics area are substantial advances that have largely been achieved in the past years. Analysis of mRNA, methylation, and proteomic profiling of DIPGs compared to healthy brain tissue identified two distinct subgroups characterized by upregulation of N-Myc and Hedgehog signaling pathways [15]. Combinatory analysis of whole-genome and whole-exome sequencing, copy number alterations, methylation, and gene expression profiling revealed three molecular subgroups in DIPG, highlighting novel therapeutic targets [18]. The three molecular subgroups consisted of upregulation of N-Myc (histone 3 wild-type DIPGs), silent genomes with fewer copy number alterations, and histone 3 K27M mutant DIPGs with *ACVR1* and *TP53* mutations. DIPGs of silent and H3-K27M molecular subtypes would benefit from therapies targeting altered histone modifications, while patients of the N-Myc subtype would benefit from therapy targeting N-Myc or ID2. Furthermore, DIPGs of the N-Myc and silent subgroups lacked amplification of receptor tyrosine kinases, indicating the inefficacy of inhibitors targeting these kinase pathways [18]. Therefore, numerous combinatory analyses of DIPG have identified the importance of the synergistic genetic and epigenetic basis of this fatal childhood cancer.

3.1. DIPG and tissue donation

A primary requirement for genomic analysis of cancer is tumor material. Much of the histological and prognostic information that we have about DIPG is from biopsies that were frequently performed until the early 1970s, before any of the current genomic techniques were available, and when CT/MRI were not widely accessible. After this period, the frequency of biopsies significantly decreased and histological information from pretreatment samples has not been available. Over the past 40 years, most DIPG patients participated in clinical trials without prior genomic profiling of their tumors. Therefore, the reason why these treatments failed is not clear.

Over the years, the lack of tissue samples and biological information on DIPG caused many research groups to explore other ways to collect tissue samples. Among these, autopsy procurement of brain samples began to have a great meaning in the understanding of DIPG. Programs for postmortem specimen donations from research groups throughout the world in a variety of tumors had positive results. The contribution of autopsy tissue donation in DIPG is relatively new and yields promise to facilitate genome-wide studies in this disease.

A variety of research teams have been working in the recent years with postmortem tissue collection and several important publications show a number of potential targets for new treatments [18,32–35]. These studies also revealed that DIPG cannot be considered a single entity, and according to the underlying biology of the tumor, different types of treatment may be needed. Findings from preclinical drug testing conducted on accurate *in vitro* and *in vivo* models of DIPG will provide direction to future clinical trials.

3.2. Preclinical models

In vitro and *in vivo* models of pontine gliomas are helping to guide the understanding of DIPG and key genomic changes that help maintain the tumor's growth and resistance to therapy. Different approaches have been used to generate primary neurosphere cultures and allograft and xenograft mouse models to elucidate the biology of DIPG; however, they are unlikely to provide all the answers. Allograft models mimicking brainstem gliomas have been used to unravel expression signatures and to serve as a platform to test the efficacy of novel therapeutic agents, such as small molecule multi-kinase inhibitors [36,37]. Primary neurosphere cultures and xenograft models from DIPG tissue obtained at autopsy have provided remarkable advances in understanding tumor biology. Some of these include the identification of a cell of origin, methods of effective drug delivery, and identification of potential therapeutic targets [4,38–41]. A pitfall in these models is the exposure of autopsied tissue to chemotherapeutic agents. Therefore, research groups are increasingly focusing on deriving preclinical models from biopsied tissue [42]. Biopsy-derived preclinical models have been utilized for identification of genomic expression profiles and for testing potential therapeutic agents [40,43,44].

Effectively treating cancer in mouse models may not always yield similar results in humans. On the other hand, animal models can represent an alternative for screening of novel agents and combination of drugs, leading to the discovery of the most promising drugs for human

DIPG trials. A recent study identified a FDA-approved epigenetic drug, Panobinostat, to be a leading therapeutic candidate by testing a plethora of promising anticancer drugs in biopsy- and autopsy-derived preclinical models of DIPG [44].

A better knowledge of the genomic aberrations that are considered drivers in DIPG is essential to treat accurate animal models. Research is improving and several studies are focusing on the discovery of these important mutations and agreeing that novel combinations should be tested in genetically and histologically accurate preclinical models prior to their translation to the clinic [5,42]. This collaborative effort will elucidate many of the unanswered questions in DIPGs.

4. Molecular basis of DIPG: major driver mutations

4.1. Histone mutations

Recent studies and advances in DIPG and biopsy specimens available at the time of the diagnosis have permitted researchers to identify the mutations encoding for histones H3.1 (*HIST1H3B* and *HIST1H3C*), H3.2 (*HIST2H3C*), and H3.3 (*H3F3A*)—proteins known for packaging DNA into chromatin. Histone mutations are found in nearly 80% of children with DIPG, and its high frequency strongly suggests its potential as a driver mutation [45,46]. Clear evidence also indicates that the molecular pathogenesis of DIPG is distinct from non-brainstem HGGs [46].

The *K27M* (lysine replaced by methionine at amino acid 27) or *K27I* (lysine replaced by isoleucine at amino acid 27) mutations result from a gain of function and have the potential to lower overall amounts of wild-type H3 with trimethylated lysine 27 (*H3K27me3*). This results in a loss of methylation at this site. Also, sequestration of the polycomb repressive complex 2 (*PRC2*) further results in overall histone hypomethylation. Normally, the *PRC2* complex represses gene expression through histone methylation. In the absence of *PRC2* complex member *EZH2*, genes that should be silent by methylation are expressed and transcriptionally active, leading to the mechanism of *K27M*/*K27I* tumorigenicity [47].

Studies analyzing the differences between H3.3 and H3.1 subgroups are showing that they can have distinct cells of origin [48]. A distinct genomic expression pattern between these two subgroups, in addition to the higher frequency of H3.1 mutation in a younger age, could imply that H3.3 and H3.1 mutations target distinct progenitors. Another interesting finding is that *PDGFRA* amplification is seen mainly in combination with H3.3 mutation, while *ACVR1* is only seen mainly in combination with mutant H3.1 [18,48].

It is known that the type of histone H3 mutation can predict the prognosis and OS of DIPG patients in a more accurate way than clinical, histological, or radiological characteristics of the tumor [29]. The discovery of the histone mutations and its importance are an incentive to the reintroduction of biopsy at the time of diagnosis, permitting to identify the genomic landscape of the patient and determination of a better treatment plan.

4.2. Partner mutations

Studies have shown that although about 80% DIPGs harbor histone mutation as expected, nearly all H3 mutant DIPGs also harbor partner mutations that vary across patients [23,34,46, 49]. Histone 3 mutations can be seen in combination with a variety of genomic alterations, such as *ACVR1*, *TP53*, and *PDGFRA* (**Table 1**).

Gene	Alteration	Possible targeted therapy
H3F3A/HIST1H3B and *HIST1H3C*	Mutations	Panobinostat
TP53	Mutations	Sertraline Thioridazine Wee1 inhibitors
ACVR1	Mutations	ALK2 inhibitors
ATRX	Mutations	Cisplatin Fluorouracil Carboplatin Oxaliplatin
PIK3CA/PIK3R1	Mutations	Temsirolimus Metformin everolimus Chlorpromazine Sirolimus
PDGFRA	Amplification	Axitinib Dasatinib Pazopanib Ponatinib Sorafenib Mebendazole Sunitinib
PPM1D	Mutations	Arsenic trioxide

Table 1. Specific mutations and copy number abnormalities and possible target treatments in DIPG.

Recent whole-genome sequencing studies reveal that 20–30% of DIPGs—usually patients less than 5 years old—contain mutations in the *ACVR1* gene, which encodes the type 1 bone morphogenetic protein (BMP) receptor, *ALK2* [18,50,51]. The high percentage of *ACVR1* mutation in DIPG provides strong evidence that it is an oncogenic driver in this cancer. Almost always *ACVR1* mutation occurs in combination with *HIST1H3B K27M*, encoding mutant histone H3.1, which is also associated with a younger age. When a HGG arises early in development and affect infants, usually the prognosis is better and the mutation burden is lower, suggesting that the tumor would be generated with fewer mutations [46,52].

Mutations in *ACVR1* gene activate the *ALK2* receptor, increase phosphorylation of SMAD proteins, and up-regulate genes in BMP developmental signaling pathway. These mutations are also described in patients with fibrodysplasia ossificans progressiva (FOP), although amino acid substitutions that occur in DIPGs have not been found in FOP patients [51]. Because FOP is not associated with cancer predisposition, it is likely that *ACVR1* mutations provide a selective advantage in the presence of other critical partner mutations, rather than driving tumor initiation [52].

Pathways common in a variety of tumor types occur also in DIPG. One example is *TP53* checkpoint, harmed by *TP53* mutations, which occurs in approximately 55% of patients with HGGs and is associated with *H3F3A*, *ATRX*, and *DAXX* mutations [45]. Mutant p53 proteins have an extended half-life and can be detected by immunohistochemistry (IHC) due to their protein accumulation [53]. In 9–23% of DIPGs, there are also mutually exclusive mutations in the *TP53* target gene *PPM1D*, which plays a role downstream of p53 in the DNA damage response [51,54].

Also, the association of p53 abnormalities in the context of *PDGFRA* amplification or *PI3K* mutations raises the possibility that the *PI3K* signaling pathway constitutes a major component of the pathogenesis of DIPG [49]. *PDGFRA* is known to be expressed in malignant gliomas and plays a role during embryonic development, suggesting an embryonic origin for DIPG, given the incidence of this disease in middle childhood [55].

Other important genomic alterations include ATRX, TERT, MYCN, and PTEN. The identification of driver mutations in DIPG helps more than to confirm the diagnosis at a molecular level: it provides relevant clinical and prognostic information, leading to the improvement in the genomic and epigenetic knowledge of DIPG. The combination of different mutations in a singular patient elucidates the fact that DIPG is a complex and varied pathology comprised of different molecular subgroups that share the same clinical features as well as a grim prognosis.

5. Challenges in the treatment: BBB and combinatory mutations

5.1. The blood–brain barrier

The infiltrative nature of DIPG makes effective therapy extremely difficult and characterizes this tumor as one of the most, if not the most challenging childhood cancer. The successful and efficient delivery of effective therapy to the DIPG tumor is the major challenge that contributes to the poor treatment options in children with this disease. For the effectiveness of a therapy, the agent needs to have certain important characteristics: (A) it has to be active and reach its molecular target in the tumor cell in adequate concentrations, (B) it has to reach the target for an adequate amount of time, and (C) the tumor cells need to be sensitive to the compound. Many factors can affect the level of a drug in the brain tumor site, including the concentration of this drug in the bloodstream, amount of protein to tissue binding, and the degree of central nervous system (CNS) penetration [8]. In DIPG, the BBB is often intact and has the ability to restrict the delivery of chemotherapeutic agents. Even for drugs that are

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capable of crossing the cerebral capillary bed, it is difficult to achieve optimal concentrations due to systemic toxicity.

Strategies have been used to overcome the BBB and to direct those agents to the specific anatomic region or tumor mass, reducing the disturbance of normal neurological functions. Among these strategies are the temporary disruption of the BBB, modification of drugs to enhance their ability to permeate the BBB, and local delivery methods such as the convection-enhanced delivery (CED) to deliver drugs directly into the extracellular space [38,56].

Also known as interstitial infusion, CED is a technique designed to deliver high concentrations of drugs directly into the tumor, allowing intratumoral injection of novel therapeutic agents. The use of hydrostatic pressure allows the distribution of a homogeneous concentration of molecules over large distances by displacing extracellular fluid with the infusate. Several clinical trials in patients with neurodegenerative disorders and malignant gliomas have been done with the use of CED, including ongoing phase I/II clinical trials in DIPG, and published studies have shown CED in DIPG to be feasible and safe [57–60]. The use of CED into murine brainstem had been well tolerated by mice with and without brainstem tumors, increasing median survival in preclinical models [38]. However, it is necessary to develop novel therapeutic agents for delivery via CED and also to improve the technique of CED in order to provide a better outcome and a new hope of treatment for children with DIPG.

5.2. Combinatory mutations

Novel genome-wide studies and increasing availability of tumor tissue, from autopsy and surgical biopsy samples, show that each individual tumor harbors multiple mutations, as well as copy number abnormalities, gene expression, and methylation patterns. While there are several ongoing clinical trials using target therapies, targeting only specific mutations in a patient has rarely been effective. Studies are showing that using chemotherapy alone or in combination with RT does not lead to any additional survival benefit [8,10,11].

In this context, multi-targeting combinatory regimens are the new promise for DIPG. Considering that DIPG is not a single disease and that HGGs harbor distinct genomic aberrations compared to adult glioblastomas, the heterogeneity of DIPG can be correlated with age of onset and the range of genomic mutations particular to each subtype. It is also believed that DIPG heterogeneity partially accounts for its resistance to current targeted therapies.

The main challenge is to combine different molecularly targeted chemotherapeutics that in a mutual mechanism of action would target the distinct driver mutations of each patient. For this purpose, it is essential to deeply investigate the mechanism of action of these drugs, as well as the pathway of each mutation found in DIPG. Preclinical studies conducted *in vitro* and *in vivo* are crucial to gain a perspective of what can be done in the clinic. Also, it is necessary to discover proper drug concentrations, study the ability of the agent to overcome the BBB, and minimize the possible adverse effects.

Hopefully, a better understanding of the molecular landscape of DIPG patients will lead to the use of combinatory therapy not only in preclinical models but also in clinical trials, aiming for an optimal personalized drug combination that can be used in children with DIPG.

5.3. Clinical trials

Clinical trials are the best way to evaluate treatment for DIPG and to test if the therapeutic agents are effective or not. While determining the origin of DIPG is important, it is also essential to evaluate new drug targets, biological agents, and immunotherapeutic strategies in clinical trials to determine if they can be used in the clinic. Numerous molecularly targeted chemotherapeutic agents have been tested in the past year with and without RT (**Table 2**).

Title	Clinical trial ID	Chemotherapy	Radiotherapy
Study of suberoylanilide hydroxamic acid (SAHA) with temsirolimus in children with DIPG	NCT0242061	Vorinostat; temsirolimus	Single daily fractions of 1.8 Gy for 30 treatments over 6–7 weeks. Total dose of radiation 54 Gy
Intra-arterial chemotherapy for the treatment of progressive DIPGs	NCT01688401	Melphalan hydrochloride intra-arterially	No
Study of the combination of crizotinib and dasatinib in pediatric research participants with DIPG and HGG	NCT01644773	Crizotinib; dasatinib	No
Biological medicine for DIPG eradication (BIOMEDE)	NCT02233049	Erlotinib; dasatinib; everolimus	No
Lenalidomide and radiation therapy in HGGs or DIPGs	NCT01222754	Lenalidomide	Five days per week to a prescription dose of 54–59.4 Gy
Molecular profiling for individualized treatment plan for DIPG	NCT02274987	Individualized treatment plan for each patient and different approaches depending on the molecular profile of the patient's tumor (specialized tumor board recommendations)	Standard radiation therapy followed by molecular based therapy with FDA-approved drugs
Anti PD1 antibody in DIPG	NCT01952769	MDV9300 (pidilizumab); cyclophosphamide	Yes
A phase I study of mebendazole for the treatment of pediatric gliomas	NCT01837862	Mebendazole; bevacizumab; irinotecan	No

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Title	Clinical trial ID	Chemotherapy	Radiotherapy
DIPG reirradiation (ReRT)	NCT01469247	No	Starting dose 24 Gy in 2 Gy fractions
Safety and efficacy of cabazitaxel in pediatric patients with refractory solid tumors including central nervous system tumors	NCT01751308	Cabazitaxel (XRP6258)	No
WEE1 inhibitor MK-1775 and local radiation therapy in treating younger patients with newly diagnosed DIPGs	NCT01922076	WEE1 inhibitor AZD1775	Yes. Five days a week for 6 weeks (up to 30 fractions)
Convection-enhanced delivery of 124I-8H9 for patients with non-progressive diffuse pontine gliomas previously treated with external beam radiation therapy	NCT01502917	No	Radioactive iodine-labeled monoclonal antibody 8H9
Valproic acid and radiation followed by maintenance valproic acid and bevacizumab in children with HGGs or DIPG	NCT00879437	Valproic acid; bevacizumab	Total dose of between 54.0 and 59.4 Gy in 30–33 fractions over 6–7 weeks
Erivedge (vismodegib) in the treatment of pediatric patients with refractory pontine glioma	NCT01774253	Erivedge (vismodegib)	No

Table 2. Clinical trials conducted for patients with DIPG in 2015.

Although clinical trials are a big hope in finding a treatment that could lead to a better prognosis, it also has its own challenges—not all the patients qualify for participation; the families have a crucial responsibility in the decision to participate or not; the length of time to complete a trial can be long, given the rarity of this cancer; and finally, there are strict guidelines that need to be followed in order to guarantee the patient safety and minimize the risk.

Every time that a clinical trial is formulated, it has great potential, but not a guarantee of benefit. However, it is important to recall the example of so many other pediatric cancers, such as leukemia, lymphoma, and Wilms' tumor, which obtained their success in treatment because of the persistence of the researchers and physicians in clinical trials.

The biggest hope is that in the future, patients can be divided into subgroups according to their genetic, epigenetic, and proteomic molecular particularities through a biopsy of their tumor. This way, targeted therapy could be individualized—however, the only way to achieve this goal is through improvement of research and investment in more clinical trials.

5.4. What to expect?

Although DIPG still remains a lethal cancer with an abysmal prognosis, scientists and physicians have for the first time the appropriate tools and knowledge to change the outcome of this disease in children affected with DIPG. The new generation of DIPG clinical trials will focus on new studies of the molecular landscape diversity of children affected with this cancer and will aim to assess the tumor, identify tumor targets, select appropriate agents, and determine the adequate dosing for a treatment selection.

Finally, it is also crucial that the collaborative efforts in this disease continue, given the small number of patients and the difficulty to obtain tumor tissue. The historical tragedy of DIPG should not discourage researchers and physicians from continuing forward. The recent data show improved knowledge that we never previously had about this devastating childhood cancer—and this improvement is a substantial step in opening novel avenues for promising approaches.

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