Clinicopathology & Molecular Analysis of Diffuse Intrinsic Pontine Glioma (DIPG) in Children - Insights from Past, Present, and Future Directions

Arupam Biswas

Student of Five Years Integrated M.Sc. in Life Sciences, Integrated Science Education and Research Centre, Visva-Bharati (A Central University & An Institution of National Importance), Santiniketan- 731235, INDIA.

Corresponding Author: arupambiswas007@gmail.com

ABSTRACT

Diffuse Intrinsic Pontine Glioma, or DIPG, is a rare, highly aggressive, heterogeneous group of brainstem tumors. Around 10-20% of primary brain tumors are considered pediatric brain tumors, of which 10-15% are diffuse brainstem tumors. It is considered untreatable and surgically unremovable due to its intrinsic position within the brain. Over the years, applying radiotherapy and chemotherapy has not shown a better outcome. However, gene-targeted therapy has proven successful, but it is still in the developing phase. This article covers the various aspects of DIPG, from clinical and molecular definitions to a vision for a universally accepted novel approach to beat this severe condition by joining fundamental science and translational research.

Keywords- Diffuse Intrinsic Pontine Glioma (DIPG), pediatric High-Grade Glioma (pHCG), H3K27M-Mutant.

I. INTRODUCTION

Brain tumors are the cause of death among most children and young people worldwide. As per statistics, the probability of a brain tumor occurrence in an individual throughout their lifetime is less than 1% (https://www.cancer.net/cancer-types/brain-tumor/statistics). In 2016 based on molecular characteristics and histological parameters, World Health Organization introduced a few specific but significant changes within the diffuse gliomas and other embryonal tumors(medulloblastoma), including diffuse midline glioma(DMG)/diffuse intrinsic pontine glioma(DIPG), H3K27M-mutant, and published an updated version of CNS Tumors classification (previously updated in 2007); and a couple of changes were also made in the tumor grading system(addition of "grade unknown") [1].

As one of the most lethal pediatric CNS cancers, DIPG or H3K27M-mutant appears to be a rare, highly aggressive, heterogeneous group of brainstem tumors that cause death among most brain tumor patients worldwide. About 10-20% of primary brain tumors are considered pediatric brain tumors[3], among which 10%-15% are diffuse brainstem tumors[4]. It is even more lethal than diffuse astrocytoma (Grade-II) and other tumor categories; that is why these tumors are considered the Grade-IV category as per the new classification[5]. These occur primarily in youngsters between 6 and 9 years (>80% are considered brainstem gliomas)[6,7]. In contrast, most of these brainstem gliomas (15%- 20%) are low-grade astrocytomas, and more than half (75%) are reported as diffuse pontine tumors, deemed aggressive, but the outcome is inferior[7]. However, it can occur in adults also; it is defined as glioblastoma (Grade-IV astrocytoma); therefore, pHCG and aHCG are both considered as similar as DIPG[8,9].

However, DIPG is a universally fatal disease and almost untreatable. The survival rate is meager. Every year, around 200-400 DIPG cases are accounted for in the USA[10]. More than 80% of children with DIPG died within the two-year time of diagnosis[11], and studies have shown that the patients with DIPG have a median survival of less than one year[12]. Unfortunately, over the past few decades, there has been a serious issue regarding the standard

treatment of DIPG; according to medical experts, through the surgical procedure, complete removal of the tumor is almost impossible due to its anatomical position and diffuse nature. Furthermore, the patients with DIPG did not show progressive improvement with chemotherapy and radiation therapy. Several scientists and researchers have identified new opportunities for novel treatments like targeted therapy, drug discovery through in-vivo and in-vitro procedures; in this manner, the comprehension of its systems will set out a phenomenal opportunity to concentrate further in the future.

Table 1: Differences in classification of gliomas in 2007 and 2016 WHO Classification systems [2]

WHO 2007	WHO 2016
Diffuse astrocytoma	• Diffuse astrocytoma, IDH-mutant • Gemistocytic astrocytoma, IDH-mutant • Diffuse astrocytoma, IDH-wildtype • Diffuse astrocytoma, NOS
Anaplastic astrocytoma	• Anaplastic astrocytoma, IDH-mutant • Anaplastic astrocytoma, IDH-wildtype • Anaplastic astrocytoma, NOS
Glioblastoma	• Glioblastoma, IDH-mutant • Glioblastoma, IDH-wildtype • Glioblastoma, NOS
Oligodendroglioma	• Oligodendroglioma, IDH-mutant and 1p/19q-codeleted • Oligodendroglioma, NOS
Anaplastic Oligodendroglioma	• Anaplastic Oligodendroglioma, IDH-mutant and 1p/19q-codeleted • Anaplastic Oligodendroglioma, NOS
Oligoastrocytoma	• Oligoastrocytoma, NOS
Anaplastic Oligoastrocytoma	• Anaplastic Oligoastrocytoma, NOS
********	Diffuse Intrinsic Pontine Glioma, H3K27M-mutant (new addition)
Gliomatosis cerebri, Protoplasmic astrocytoma, Fibrillary astrocytoma	Removed

*Here, ******* = Did not exist; NOS = Not otherwise specified[2]*

II. CLINICAL FEATURES & IMAGING CHARACTERISTICS

Numerous classifications were proposed throughout the most recent couple of years; however, none of them could clarify them fittingly. Even though DIPG is primarily found in children, there is an equal proportion of occurring tumors in almost both genders compared to every age, i.e., 1:1, which is significantly more awful than other brainstem tumors[9]. The clinical features of patients with DIPG depend upon the tumor location, tumor nature, and tumor growth pattern and the patient's typical symptoms with brainstem tumors include cerebral signs like ataxia, dysmetria, dysarthria, Babinski reflex, motor impairment, cranial nerve (CN) palsies[4,13]. In DIPG, signs and indications of expanded intracranial pressing factor (because of obstructive hydrocephalus from the development of the pons) are seen in under 10% of youngsters[14]. Other vague side effects can be seen later at the diagnosis, including tactile anomalies, behavioral changes, urinary and respiratory issues, etc. DIPGs are generally identified by diffuse invasion and enlarging of the brainstem[9,15].

The earliest detection was based on CT and surgical observations; recently, MRI has been included in that list[16]. In DIPG, when limited surgical options and biopsy chances are available, there MRI plays a vital role in understanding the secret biological behavior of the tumor; thus, it has become an essential method to follow up the diagnosis[4]. In light of CT and MRI observations (gadolinium acts as the differentiation agent), these lesions seemed, by all accounts, to be hypo-intense (sometimes iso-intense) on T1-weighted and homogeneously (or heterogeneously) hyper-intense on T2-weighted images[4,5]. On primary MRI, these lesions appear in the pons (occupying more than half) and develop instead of uprooting them; this way, it is characterized as a large mass (more than 2 cm in size), often asymmetric, expansile, and infiltrative[4,17,18]. However, these may sometimes engulf the basilar artery. FLAIR images suggest a relatively homogeneous enhancement of the surrounding tissues[16].

Although the absence of scientific data immensely affects clinical grounds and molecular levels, scientists have brought their kind attention to a particular change in one of the histone three genes- H3.3(H3F3A) or H3.1(HIST1H3B), that is liable for DIPG[19]. A couple of reports proved that H3.3 [replacing lysine 27 on the amino-terminal tail for a methionine (H3K27M)] had achieved terrible perception than the changes in H3.1[18–21].

Moreover, other genomic changes have likewise been developed, such as ACVR1, p53, PDGFRA, and ATRX, in non-malignant tumors, including primitive neuroectodermal tumors (PNET) and cysts and different low-grade gliomas[4,5,7,8,13,16,17]

III. MOLECULAR MARKERS

As histone is liable for delivering the DNA bundles into the nucleosome; thus, it has become the essential and core unit regulating gene expression. Epigenetic modifiers can do histone modifications dynamically, such as methylation, acetylation, phosphorylation, and ubiquitination; thus, these histone mutations, so-called oncohistones, have been the reason for several cancer diseases. Several reports show that histone three somatic mutations happen due to the replacement of lysine with methionine, explicitly on position 27 (H3K27M) in the isoforms H3.1 and H3.3, encoded by genes HIST1H3B and H3F3A[22,23]; these can be found in over 30% of the pHCG cases and definitely in 60% of the DIPGs[24]. Besides, there are few differences observed between H3.1 and H3.3 the histone isoforms:

1. Earlier, CT and MRI showed that H3.1K27M tumors exist in the pons, whereas H3.3K27M tumors are found along the brain's midline[18,24].

2. The two tumor types have their arrangement of partner DNA transformations. Some changes like ACVR1 are related to H3.1K27M, while PDGFRA, MYC, CCND2, and TP53 transformations are related to H3.3K27M[14,16,25,26].

Table 2: A Chart showing genetic alterations in pediatric high grade glioma (pHCG) [29]

Entities	Mutations	Structural alterations
Anaplastic astrocytoma	**TP53**	PDGFRA amplification/rearrangment/indels
Glioblastoma	**TP53** H3F3A (H3.3) G34R/V PICK3CA/PIK3R1 BRAF V600E NF1 ATRX, DAXX PDGFRA IDH1 FGFR1	*CDKN2A* deletion *NTRK1-3* fusion genes *MET* or *EGFR* amplification *CDK4* or *CDK6* amplification *MYCN* amplification
Epithelioid glioblastoma	**BRAF V600E**	
Diffuse midline glioma/ Diffuse Intrinsic Pontine Glioma	**H3F3A (H3.3 K27M)** *HIST1H3A/B/C* (H3.1) ACVR1 G328	
Anaplastic pleomorphic xanthoastrocytoma	**BRAF V600E**	**9p21.3 deletion (CDKN2A/B)**
Anaplastic Ganglioglioma	TP53 **BRAF V600E** TP53	*CDK4* amplification Loss of *CDKN2A/B* and DMBT1

Here, Bold Script = present in >50%[29]

3. Transcriptome analysis unveiled that H3.1K27M tumors show a mesenchymal aggregate with angiogenesis and hypoxia being upregulated, but H3.3K27M tumors show an oligodendroglial aggregate[4,23,24].

4. The two kinds of tumors have distinctive clinical results. Metastatic backslides are habitually found in H3.3K27M patients, and they have a limited middle endurance of 11 months than 15 months in H3.1K27M patients. Besides, H3.3K27M tumors are more impervious to radiotherapy. Therefore, regardless of having various signs, the H3.1K27M and H3.3K27M statements lead to comparable epigenomic adjustments[4,24,27,28].

In most pediatric glioblastomas, TP53 mutations are pretty frequent and can be seen around 22-40% among patients with DIPG[16]. The loss of function of the TP53 gene is very much common in DIPG and often co-occurs with PGDFR amplification; the mutations of TP53 and H3.3K27M, and usually, PPM1D changes have been displayed to allow malignant growth cells to avoid cell passing and senescence by affecting the epigenetic regulations[4,14,16].

Similarly, ACVR1 is another frequent mutation in almost 30% of DIPG patients and co-segregate with H3.1. Earlier studies have revealed that ACVR1 mutation plays an essential role in early tumor progression and helps in therapeutic targeting. Generally, ACVR1 encodes a bone morphogenetic protein (BMP) type 1 receptor, and upon activating ACVR1 mutations, it increases BMP signaling. Furthermore, the BMP pathway is associated with a build-up of SMAD proteins in the

nucleus. These proteins have the ability to induce the EMT-related transcription factors (for example, SNAI1/2 and zinc finger E-box-binding homeobox 1/2 (ZEB1/2). Nevertheless, the activation of ACVR1 leads to phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), rat sarcoma/mitogen-activated protein kinase (RAS/MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling. These pathways induce mesenchymal transition and activate EMT-related transcription factors, including twist-related protein 1 (TWIST1)[14,16,25].

PDGFRA is also frequently observed amplification which can be seen in most (1/3rd) of pediatric gliomas; it is also involved in the RTK-RAS-PI3K-Akt flagging pathway. PDGFRA triggers the enactment of PI3K and MAPK pathways through phosphorylation at different phosphotyrosine spaces. Furthermore, PIK3R1, PIK3CA and others are also the primary drivers of PI3K pathways and have played a crucial role in the progression of DIPG. Besides, studies have shown that some transcription regulators such as MYC and MYCN can be present in DIPG, which generally enhances the gene expression genome-wide. Thus, in the future, thorough investigation and understanding of these various biological pathways will create more opportunities to find a better cure, such as a therapeutic target for DIPG[4,5,8,16,30].

Table 3: A chart showing different molecular markers and their significance in pHCG [31]

Marker	Relevance to Pediatric HGGs
ADAM3A	DNA analysis of 38 predominantly pretreatment pediatric HGG samples (including 13 DIPGs) revealed homozygous loss at 8p12 in 16% of cases. This novel deletion includes the ADAM3A gene.
Akt	Ras/Akt activation is observed in pediatric HGG and may be associated with poor prognosis.
BRAF	The *BRAFV600E* mutation has been found in pediatric high-grade astrocytomas.
CDKN2A/B	Approximately 50% of supratentorial tumors expressed CDKN2B and ~75% of infratentorial tumors were positive for CDKN2B expression.
	Pediatric DIPG samples showed high-frequency loss of 17p and 14q and lack of CDKN2A/CDKN2B deletion.
EGFR	A lesser degree of *EGFR* amplification is observed in children compared with adult HGG. Marked overexpression of *EGFR* is observed in pediatric HGG relative to pediatric low-grade glioma.
EGFRvIII	*EGFRvIII* deletion mutations have been observed in pediatric HGG, but no *EGFRvIII* activating mutations.
IDH1	Until recently, most data had shown that *IDH1* mutations were rare or absent in pediatric HGG. However, a recent study found *IDH1* mutations in 7 AA/GBM tumors from children ≥14 years old.
MGMT	*MGMT* overexpression is rare in pediatric HGG but is associated with poor prognosis.
	Methylation of the *MGMT* promoter may be prognostic for survival in pediatric HGG.
PDGFRA	*PDGFRA* is overexpressed in the majority of pediatric HGG cases and amplified in ~ 1/3 of pediatric HGG, including DIPG.
P53	p53 overexpression and mutation correlate independently with adverse outcome and appears to vary with age.
VEGF 121 isoform	The VEGF 121 isoform, which promotes mitogenesis and vascular permeability, has been linked with anapaestic astrocytoma and glioblastoma

IV. TREATMENT PROCESS

It is always have been a challenging task to give appropriate treatment to patients with DIPG. The very first method that is applied to patients is to remove the tumor **surgically**. Historically, in 1880, Dr. Edward H. Henoch of Berlin reported a young eleven-year-old girl suffering from various neurological disorders, including

ataxia, nausea, dysmetria, and specifically CN VI palsies. Later, the autopsy showed a segregated pontine mass with no sharp limits that appeared to broaden diffusely into the region of the pons with various minor foci; that was the first reported case of DIPG. Again in 1887, going through the clinical assessment of a six-year-old patient, Dr. Henry Hun presented a detailed explanation of DIPG to the American Neurological Association, which was later cited in an article and published in 1889 by Dr. Mary Putnam Jacobi. However, in DIPG, the intricate nature of the pons has posed a significant limitation to surgical intervention. Although in the past, surgery and biopsies have both been attempted in patients, it does not come without significant risks. However, *"Stereotactic biopsy of DIPG can be considered as a safe procedure in well-trained neurosurgical teams and could be incorporated in protocols."* [4]

Recently, a large-scale study was conducted in which 130 DIPG patient biopsies were screened. The research team reported transient morbidity of 3.9% with no mortality[32] . Further, they displayed the possibility of operating more than one biopsy upon a single patient, providing adequate tissue for cellular and biomolecular analysis. Alongside gliomas (grade II-IV), PNET & germ cell tumors (including germinomas and teratomas) can occur in the brainstem as diffusive in nature; the strategies of detection and treatments have no common link with gliomas [16,32].

On the other hand, lessions that occur in the brainstem which seems to be non-malignant, have various neurodegenerative symptoms such as alexander disease, para-coccidioidomycosis, acute demyelinating encephalomyelitis etc)[9].

Patients exposed to **radiation therapy** have a better chance of surviving in their lifetime on an average of three to six months; however, it is still considered an aggressive palliative process[16]. As a standard diagnosis procedure, intensity-modulation radiation therapy (IMRT) has recently been analyzed in DIPG patients, given in single dose consecutively for 6 weeks (5 days in a week) for quite a long time[33]. As steady consideration as corticosteroids is utilized to treat the peri-tumoral edema, most patients exposed to this therapy have gained some short-term improvement in their body system[34].

Nevertheless, 85% of patients showed steroid-prompted incidental effects because ideal dosages were lacking[35]. Various studies showed that both the lower doses (<50 Gy) and higher doses of radiation therapy (66-78 Gy) given to DIPG patients are not increasing the level of survival possibility; therefore, it was proposed that as the furiously and vigorously growing tumor cells are not responding to the given standard treatments, so they might respond if the concentrations of doses are increased[4,15,16]. Conversely, several hypo-fractionation studies have reported similar results with standard fractionation without giving definite survival benefits. Young children were subjected to hyper-fractionated doses (twice a daytime), following which there are few symptoms of

illness such as Leptomeningeal dissemination/disease (LMD) in all DIPG cases within 3-6 months[36].

Radiation therapy seems to be advantageous; even though radio-sensitizers has not indicated any progress to this date. Thus far, the application of radiotherapy alongside radio-sensitizers have not been highly productive in the long term. Recent studies showed, in the addition of 5-aminolevulinic acid (5-ALA) to the current irradiation of DIPG or DMG, resulting in selective malignant cell cytotoxicity, termed as photodynamic treatment (PDT)[37].

The functional importance of these conventional **chemotherapeutic agents** (such as temozolomide, TMZ) has been investigated thoroughly at various phases of the DIPG (prior to, concurrent with, and adjunctive to radiotherapy[38]. It is used alone in multiple combinations or at a variable dose with different intensities; however, none of these agents/combinations at different dose intensities with or without radiotherapy showed better survival outcomes than radiotherapy alone[36].

Immunotherapy given either in isolation or in combination with known chemotherapeutic agents is another potential treatment option[39]. For example, a recent case report demonstrates the utilization of nimotuzumab combined with temozolomide and radiation therapy in a patient with LMD of DIPG [36]. Among of many, the critical impediments of this disease's treatment are effective due to targeted therapy.

Several factors can influence the given drug bioavailability to a DIPG tumor, including the specificity of the drug or agent, the level of blood flow to the tumor & brain diffusion, and drug metabolism [40]. In targeted therapy, the probable dynamic compound or drug should have the following qualities:

It should,
a. reach its target, be specific.
b. have adequate concentration.
c. stay active long enough
d. be effective.

Researchers are also trying to make epigenetic changes within the genes via using different drugs. HDAC inhibitor such as vorinostat, panobinostat are showing improvements among patients with DIPG, however their effects are monitored thoroughly. Besides, effects of combined vorinostat and temsirolimus(an mTOR inhibitor) in experiment arms with and without radiotherapy (NCT02420613) are ongoing. Another clinical trial is trying to test BMI1 inhibitor, PTC596 (NCT03605550), even though these are promising, but recently a report by Balakrishnan et al., 2020 showed antitumor effect with other BMI1 inhibitors, PTC209 or PTC028. A Phase I/II clinical trial (ongoing) is validating the safety of a synthetic peptide vaccine specific for the H3.3K27M epitope in combination with poly-ICLC and Nivolumab (the PD-1 inhibitor), among newly diagnosed DIPG patients and other gliomas showing positive for H3.3K27M (NCT02960230) [41].

Table 4 : A List of past and present clinical trials among pHCG (mostly DIPG patients) [41]

ID	Clinical Trial	Epigenetic Target	Compound
NCT02899715	Panobinostat in treating younger patients with progressive diffuse intrinsic pontine glioma	HDAC	Panobinostat
NCT02717455	Trial of panobinostat in children with diffuse intrinsic pontine glioma	HDAC	Panobinostat
NCT03566199	MTX110 by convection-enhanced delivery in treating participants with newly diagnosed diffuse intrinsic pontine glioma	HDAC	MTX110 (panobinostat nanoparticle formulation MTX110)
NCT01189266	Vorinostat and radiation therapy followed by maintenance therapy with vorinostat in treating younger patients with newly diagnosed diffuse intrinsic pontine glioma	HDAC	Vorinostat
NCT02420613	Vorinostat and temsirolimus with or without radiation therapy in treating younger patients with newly diagnosed or progressive diffuse intrinsic pontine glioma	HDAC and mTOR	Vorinostat, Temsirolimus
NCT00879437	Valproic acid and radiation followed by maintenance valproic acid and bevacizumab in children with high-grade gliomas or diffuse intrinsic pontine glioma	HDAC	Valproic acid
NCT03893487	Fimepinostat in treating brain tumors in children and young adults	HDAC and PI3K	Fimepinostat
NCT03605550	A Phase 1b study of PTC596 in children with newly diagnosed diffuse intrinsic pontine glioma and high-grade glioma	BM1	PTC596
NCT02960230	H3.3K27M peptide vaccine with nivolumab for children with newly diagnosed DIPG and other gliomas	H3K27M-epitope and PD-1	H3.3K27M Peptide, Nivolumab

Table 5: Targetable mutations in DIPG and their effects[16]

Gene	Alteration	Impact	Result
ACVR1	Missense	Loss of function	Arrest glial cell differentiation and drives tumourigenesis
PDGFRA	Amplification	Gain of Function	Upregulation of PI3K/AKT/mTOR pathway, increased proliferation
CDK4/6	Amplification	Gain of Function	Upregulation of cell cycle, increased proliferation
PTEN	Deletion	Loss of function	Loss of inhibition of PI3K/AKT/mTOR signalling pathway, increased proliferation
PPM1D-p53	Truncation	Loss of function	Impairs DNA repair mechanism, evasion of apoptosis

A recent article showed that a natural library of 756 compounds was screened, and among them, interestingly, distinguished six natural compounds that showed inhibitory impacts on DIPG multiplication and harbor autonomous development through initiating growth cell apoptosis and cell cycle capture. Besides, RNA-sequencing and functional validation uncovered the underlying molecular mechanisms of these mixtures with anti-DIPG functions and distinguished new cellular factors like FN1 and EIF3CL, required for DIPG survival as potential therapeutic targets. The functional role of the EIF3CL in cancer cells has never been reported[6].

However, it has been highly expressed in human glioma tissues through the analysis of TCGA datasets compared to other types of cancer.

Taking everything into account, researchers have given enough evidence that diffuse midline glioma, H3K27M-mutant, or DIPG, is one of the most challenging tumors to beat. Nevertheless, recent molecular breakthroughs may have opened up new possibilities for a better outcome. Targeted therapies, some are still in clinical trials, along with optimization and combination of the newly invented drugs and other therapies may give patients the required answer that they are waiting for. We hope the future goals will ultimately be directed at deciphering DIPG's highly invasive nature, which might lead to new therapeutic strategies that could finally satisfy the urgent need for effective therapy.

ACKNOWLEDGEMENTS

FUNDING & CONFLICT OF INTEREST

REFERENCES

[1] Louis, D. N. *et al.* The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathologica* vol. 131 803–820 (2016).

[2] Iyengar, P. & Tsao, M. S. Clinical relevance of molecular markers in lung cancer. *Surgical Oncology* **11**, 167–179 (2002).

[3] Leach, J. L. *et al.* MR Imaging features of Diffuse Intrinsic Pontine Glioma (DIPG) and Relationship to Overall Survival: Report from the International DIPG Registry. doi:10.1093/neuonc/noaa140/5854320.

[4] Jallo, G. I., Noureldine, M. H. A. & Shimony, N. *Brainstem Tumors Diagnosis and Management*. (2020).

[5] Gajjar, A., Reaman, G. H., Racadio, J. M. & Smith, F. O. *Brain Tumors in Children*.

[6] Chen, J., Lin, Z., Barrett, L., Dai, L. & Qin, Z. Identification of new therapeutic targets and natural compounds against diffuse intrinsic pontine glioma (DIPG). *Bioorganic Chemistry* **99**, (2020).

[7] Hu, J., Western, S. & Kesari, S. Brainstem glioma in adults. *Frontiers in Oncology* vol. 6 (2016).

[8] Walter, A. W. & Hilden, J. M. *Brain tumors in children*. *Current Oncology Reports* vol. 6 (2004).

[9] Vanan, M. I. & Eisenstat, D. D. DIPG in children - What can we learn from the past? *Frontiers in Oncology* **5**, (2015).

[10] Ostrom, Q. T. *et al.* CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro-Oncology* **15**, (2013).

[11] Veldhuijzen Van Zanten, S. E. M. *et al.* Palliative and end-of-life care for children with diffuse intrinsic pontine glioma: Results from a London cohort study and international survey. *Neuro-Oncology* **18**, 582–588 (2016).

[12] Johung, T. & Monje, M. Diffuse Intrinsic Pontine Glioma: New Pathophysiological Insights and Emerging Therapeutic Targets. *Current Neuropharmacology* (2016) doi:10.2174/1570159x14666160509123229.

[13] Vanan, M. I. & Eisenstat, D. D. DIPG in children - What can we learn from the past? *Frontiers in Oncology* vol. 5 (2015).

[14] Kluiver, T. A., Alieva, M., van Vuurden, D. G., Wehrens, E. J. & Rios, A. C. Invaders Exposed: Understanding and Targeting Tumor Cell Invasion in Diffuse Intrinsic Pontine Glioma. *Frontiers in Oncology* **10**, 1–13 (2020).

[15] Warren, K. E. Diffuse intrinsic pontine glioma: poised for progress. *Frontiers in Oncology* (2012) doi:10.3389/fonc.2012.00205.

[16] Srikanthan, D. *et al.* Diffuse intrinsic pontine glioma: current insights and future directions. *Chinese Neurosurgical Journal* **7**, 1–11 (2021).

[17] Grimm, S. A. & Chamberlain, M. C. Brainstem glioma: A review. *Current Neurology and Neuroscience Reports* (2013) doi:10.1007/s11910-013-0346-3.

[18] Chiang, J. *et al.* Clinical, imaging, and molecular analysis of pediatric pontine tumors lacking characteristic imaging features of DIPG. *Acta Neuropathologica Communications* **8**, 1–12 (2020).

[19] Castel, D. *et al.* Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathologica* (2015) doi:10.1007/s00401-015-1478-0.

[20] Khuong-Quang, D. A. *et al.* K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathologica* (2012) doi:10.1007/s00401-012-0998-0.

[21] Mathew, R. K. & Rutka, J. T. Diffuse intrinsic pontine glioma: Clinical features, molecular genetics, and novel targeted therapeutics. *Journal of Korean Neurosurgical Society* (2018) doi:10.3340/jkns.2018.0008.

[22] Lowe, B. R., Maxham, L. A., Hamey, J. J., Wilkins, M. R. & Partridge, J. F. Histone H3 mutations: An updated view of their role in chromatin deregulation and cancer. *Cancers* vol. 11 1–24 (2019).

[23] Kallappagoudar, S., Yadav, R. K., Lowe, B. R. & Partridge, J. F. Histone H3 mutations—a special role for

H3.3 in tumorigenesis? *Chromosoma* **124**, 177–189 (2015).

[24] Wan, Y. C. E., Liu, J. & Chan, K. M. Histone H3 Mutations in Cancer. *Current Pharmacology Reports* vol. 4 292–300 (2018).

[25] Taylor, K. R., Vinci, M., Bullock, A. N. & Jones, C. ACVR1 mutations in DIPG: Lessons learned from FOP. *Cancer Research* vol. 74 4565–4570 (2014).

[26] Carvalho, D. *et al.* ALK2 inhibitors display beneficial effects in preclinical models of ACVR1 mutant diffuse intrinsic pontine glioma. *Communications Biology* (2019) doi:10.1038/s42003-019-0420-8.

[27] Daoud, E. v. *et al.* Adult brainstem gliomas with H3K27M mutation: Radiology, pathology, and prognosis. *Journal of Neuropathology and Experimental Neurology* **77**, 302–311 (2018).

[28] Nakata, S. *et al.* Histone H3 K27M mutations in adult cerebellar high-grade gliomas. *Brain Tumor Pathology* **34**, 113–119 (2017).

[29] Chiang, J. C. H. & Ellison, D. W. Molecular pathology of paediatric central nervous system tumours. *Journal of Pathology* vol. 241 159–172 (2017).

[30] Kluiver, T. A., Alieva, M., van Vuurden, D. G., Wehrens, E. J. & Rios, A. C. Invaders Exposed: Understanding and Targeting Tumor Cell Invasion in Diffuse Intrinsic Pontine Glioma. *Frontiers in Oncology* vol. 10 (2020).

[31] T. J. MacDonald, D. Aguilera & C. M. Kramm. Treatment of high-grade glioma in children and adolescents. *Pediatrics* **76**, 853–854 (1985).

[32] Wang, Z. J. *et al.* Diffuse intrinsic pontine glioma biopsy: A single institution experience. *Pediatric Blood and Cancer* **62**, 163–165 (2015).

[33] Aktan, M., Koc, M. & Kanyilmaz, G. Survival following reirradiation using intensity-modulated radiation therapy with temozolomide in selected patients with recurrent high-grade gliomas. *Annals of Translational Medicine* **3**, (2015).

[34] Dietrich, J., Rao, K., Pastorino, S. & Kesari, S. Corticosteroids in brain cancer patients: Benefits and pitfalls. *Expert Review of Clinical Pharmacology* vol. 4 233–242 (2011).

[35] Veldhuijzen van Zanten, S. E. M., Cruz, O., Kaspers, G. J. L., Hargrave, D. R. & van Vuurden, D. G. State of affairs in use of steroids in diffuse intrinsic pontine glioma: an international survey and a review of the literature. *Journal of Neuro-Oncology* **128**, 387–394 (2016).

[36] Vanan, M. I. & Eisenstat, D. D. DIPG in children - What can we learn from the past? *Frontiers in Oncology* (2015) doi:10.3389/fonc.2015.00237.

[37] Kast, R. E. *et al.* A new treatment opportunity for DIPG and diffuse midline gliomas: 5-ALA augmented irradiation, the 5aai regimen. *Brain Sciences* **10**, 1–8 (2020).

[38] Chassot, A. *et al.* Radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *Journal of Neuro-Oncology* (2012) doi:10.1007/s11060-011-0681-7.

[39] Vanneman, M. & Dranoff, G. Combining immunotherapy and targeted therapies in cancer treatment. *Nature Reviews Cancer* vol. 12 237–251 (2012).

[40] Warren, K. E. Beyond the blood: Brain barrier: The importance of central nervous system (CNS) pharmacokinetics for the treatment of CNS tumors, including diffuse intrinsic pontine glioma. *Frontiers in Oncology* vol. 8 (2018).

[41] Leszczynska, K. B., Jayaprakash, C., Kaminska, B. & Mieczkowski, J. Emerging Advances in Combinatorial Treatments of Epigenetically Altered Pediatric High-Grade H3K27M Gliomas. *Frontiers in Genetics* vol. 12 (2021).