

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Diffuse Intrinsic Pontine Glioma

Santosh Valvi and Nicholas G. Gottardo

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.78578>

Abstract

Diffuse intrinsic pontine glioma (DIPG) is a leading cause of brain cancer-related death in children. These aggressive high-grade gliomas cannot be effectively treated and are associated with dismal prognosis. Whilst radiation therapy (RT) prolongs survival, it is a palliative therapy, as half of children with DIPG die within 1 year of diagnosis and almost all are dead by 2 years. These statistics have not changed for decades, despite a multitude of clinical trials. No chemotherapeutic regimen has been shown to improve survival, emphasizing the need to find novel and effective treatments. One of the principal reasons for this poor outcome was our limited knowledge of the biology of DIPG's. Due to their location in brainstem, surgical resection is not feasible and up until recently, even performing a limited biopsy was considered too dangerous. In the last decade, DIPG tumor tissue has become available through autopsies and biopsies. This combined with the genome revolution has resulted in a transformation in our understanding of the underlying biology of this disease. Moreover, viable DIPG cells can now be grown in the laboratory which have allowed development of *in-vitro* (neurospheres) and *in-vivo* models (allograft and xenograft). This chapter summarizes recent advances in DIPG and potential novel therapies.

Keywords: diffuse intrinsic pontine glioma (DIPG), advances in biology, novel therapies

1. Introduction

Diffuse intrinsic pontine glioma (DIPG) is a high-grade glioma originating in the pons and occurs predominantly in children. It is one of the most dreaded pediatric cancers as they are essentially incurable. At the same time, it has become one of the most intense areas of research in the past few years. Due to the lack of tumor samples, there was limited information available about the genetic and molecular abnormalities in DIPG. These tumors were considered

to mimic other pediatric and adult high-grade gliomas and therapies were based on these inaccurate assumptions. Radiation therapy (RT) is the only treatment modality available, that has been demonstrated to prolong survival and improve quality of life (ref) but is not curative. Almost all children die with a median survival of 1 year. Indeed, 50% of children with DIPG die within 1 year of diagnosis and almost all are dead by 2 years (ref). Despite a myriad of clinical trials, no effective treatment has been identified so far. But recently, there has been an exponential increase in the pre-clinical research involving DIPG and many, previously unknown, abnormalities contributing to DIPG pathogenesis have been identified. These may provide treatable targets and help improve the outcome of patients with DIPG.

2. Epidemiology

Tumors of the central nervous system (CNS) constitute the largest group of solid tumors and the second most common pediatric cancer [1, 2]. Around 20% of CNS tumors are brainstem gliomas [3] of which 80% arise within the pons as DIPG [4] with approximately 200–300 children in the United States [5] being diagnosed each year. Survival rates for children with cancer have improved dramatically since the 1960s; from an estimated 5-year survival of 28% to approximately 80–85% at present [6]. The outcome for patients diagnosed with brain tumors has also improved with more than 70% expected to survive at least 5 years from the diagnosis [7]. Pediatric CNS tumors are a very heterogeneous group of diseases with over 100 distinct histological types and survival differs markedly amongst the different histological types. Unlike other childhood cancers, survival for DIPG has not changed [5]. The median survival for children with DIPG is less than 1 year from the diagnosis [8] with more than 90% dying within 2 years of diagnosis [4, 9]. Although DIPG constitute only 10–15% of all pediatric brain tumors, they are the leading cause of death in this group [10]. More than three decades of research and different treatment modalities have not yielded any survival improvement.

3. Clinical features

The symptoms and signs of patients with DIPG occur secondary to the involvement of different parts of the brainstem, which include the midbrain, pons and medulla. The brainstem serves as a conduit through which axonal tracts pass to the spinal cord, cerebrum or exit as cranial nerves. Due to the diffuse nature of DIPG, the tumor infiltrates the white matter affecting the adjacent cranial nerves and white matter tracts [11]. As the pons contains important nuclei critical for life-sustaining function, any damage caused by the tumor or its treatment has devastating effects. DIPG predominantly occurs in the middle childhood. Median age at diagnosis is 6–7 years, with males and females affected equally [11–13]. Interestingly, adults with DIPG tend to have a longer survival which may indicate a less aggressive and biologically different tumor from that in children [14]. Typically, the presentation is with neurological symptoms of less than 3 months duration [15] with the “classic” triad of cranial

nerve deficits (diplopia and facial asymmetry), long tract signs (hyperreflexia, clonus, upward Babinski, increased tone and decreased strength) and cerebellar signs (ataxia, dysmetria and dysarthria), which is seen in about 50% patients [16, 17]. In most cases, abducens nerve palsy is the earliest sign and is a sensitive predictor for DIPG [17]. Obstructive hydrocephalus with signs of raised intracranial pressure are seen in <10% of patients [18]. Other symptoms for example behavioral changes, night terrors, and school difficulties may also occur.

4. Imaging characteristics

4.1. Magnetic resonance imaging (MRI)

DIPG is diagnosed clinically on the basis of history, clinical signs and MRI findings [18]. The classic MRI appearance is of an expansile lesion centred in the pons that frequently extends laterally into the cerebellar peduncles and hemispheres and often extends vertically into the midbrain and medulla (**Figure 1A**). It is poorly marginated, occupying more than 50% of the axial diameter of the pons [11]. Necrosis can be seen but cysts are rare [14]. The tumors are hypointense with indistinct margins on T1-weighted images (**Figure 1B**) and hyperintense on T2-weighted/fluid-attenuated inversion recovery (FLAIR) images (**Figure 1C and D**) [4]. Post-gadolinium enhancement as commonly seen in pilocytic astrocytomas is often minimal or absent in DIPG (**Figure 1E**) [19, 20]. With an average molecular weight of 545 kDa, gadolinium largely exceeds the penetration cut-off of the blood brain barrier (BBB) (400–600 Da) with limited contrast enhancement in DIPG suggesting a largely intact BBB [14, 21]. Other MRI features typical of DIPG include ventral involvement of the pons and encasement of the basilar artery (**Figure 1F**) [5].

4.2. New imaging techniques

4.2.1. MR spectroscopy (MRS)

MRS provides a measure of brain chemistry. The most prominent peaks in the brain spectrum on Proton MRS are N-acetyl aspartate (NAA), creatine, and choline. NAA is a neuronal marker which is usually decreased in tumors. Choline is associated with the metabolism of membrane turnover and is generally increased in tumors. In DIPG, MRS shows a modest increase in choline levels and a decrease in NAA levels [14]. Additionally, peaks from lactate and mobile lipids are often elevated [11]. The abnormalities in these normally occurring brain metabolites may provide insight into the biology of DIPG and become invaluable tools in DIPG radiodiagnosis.

4.2.2. Perfusion and diffusion techniques

Some of the newer perfusion and diffusion MRI techniques are being tested in prospective trials and although not a standard for DIPG diagnosis currently, may prove beneficial in the future.

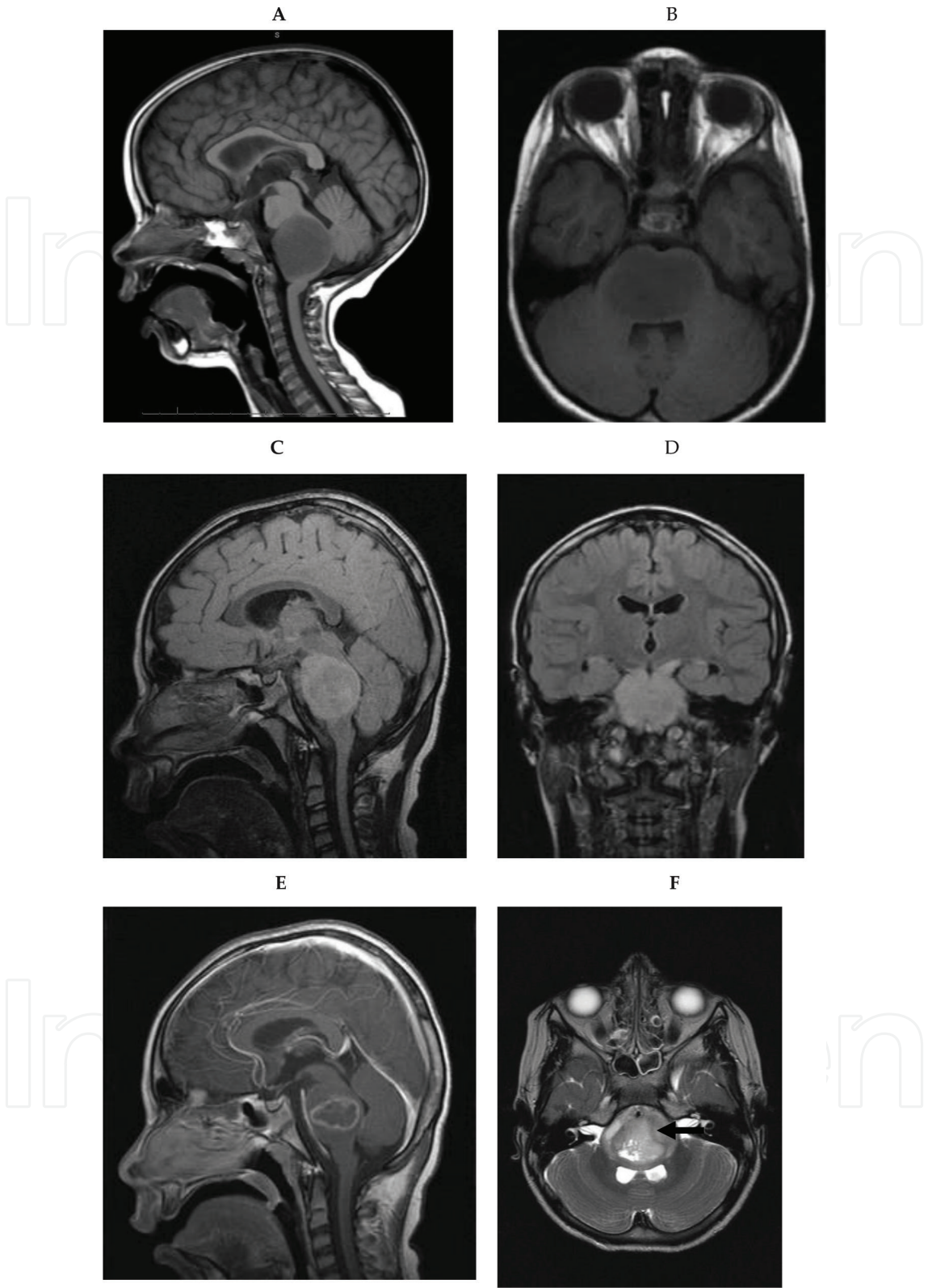


Figure 1. T1-weighted pre-contrast sagittal (A) and axial (B) MRI pictures showing poorly margined, expansile and hypointense pontine mass. It is hyperintense on T2-weighted/FLAIR sagittal (C) and coronal (D) images. (E) There is minimal post-gadolinium enhancement. (F) Basilar artery encasement by DIPG (black arrow).

MR perfusion measures regional blood volume and flow reflecting the vascular nature of neoplasms [22]. Serial changes in tumor vascularity may be useful to monitor the effectiveness of therapy. Diffusion tensor imaging may provide visualization and quantitative characterization of the major white matter pathways in DIPG [23, 24]. This superior delineation between tumor and normal brain compared to the conventional MRI techniques may prove useful, especially to perform biopsies and obtain DIPG samples. Specific MRI sequences, including single-voxel spectroscopy (SVS), multi-voxel MRS and dynamic susceptibility contrast (DSC) MRI may help in predicting short or long survival interval from diagnosis in patients with DIPG [25].

4.2.3. Molecular drug imaging

Imaging of radiolabeled drugs like monoclonal antibodies and tyrosine kinase inhibitors can be achieved by using PET technology [26]. A recently introduced PET imaging technique of zirconium-89 (⁸⁹Zr)-labeled bevacizumab in children with DIPG demonstrated considerable inter- and intra-tumoral heterogeneity in drug delivery [27]. Therapeutic potential and toxicity both can be quantified by such non-invasive, in patient techniques of tumor imaging and drug distribution [28]. Thus, these newer imaging modalities provide quantitative physiologic and functional information to complement the anatomic visualization provided by conventional imaging. However, these techniques need further validation and have yet to impact treatment decisions [11].

5. Pathology

Grossly, DIPG tumors tend to spread contiguously, extending to involve the midbrain, medulla, and cerebellar peduncles [29, 30]. Up to 20% of patients are reported to have leptomeningeal disease at diagnosis [31] and almost 56% have spinal metastases or leptomeningeal dissemination at the time of recurrence or autopsy [32, 33]. Microscopically, the majority of tumors resemble malignant gliomas in other regions. Tumor cells appear relatively small, with prominent cytoplasmic intermediate filaments and cell processes [34]. Tumor cells pervade normal cells (**Figure 2A and B**), diffusely expanding the pons and distorting, displacing and destroying nerve fiber tracts that normally course through it [34]. Anaplasia, increased mitotic activity (**Figure 2C**), tumor necrosis (**Figure 2D**) and vascular proliferation (**Figure 2E**) are often present [5]. A histopathological hallmark is perineuronal satellitosis in which collection of tumor cells are found around pontine neurons [35]. DIPG is histologically classified as fibrillary astrocytoma, World Health Organization (WHO) Grades II–IV [36] but the prognosis is not associated with histological grade [37, 38]. There can be marked intratumoral heterogeneity with a high proportion of samples showing focal areas of WHO grade I phenotype [39].

5.1. Diffuse midline glioma, H3K27M-mutant

In the latest WHO classification, DIPG have been grouped with other midline gliomas (thalamus, spinal cord) forming a new diagnostic entity. These tumors are characterized by a

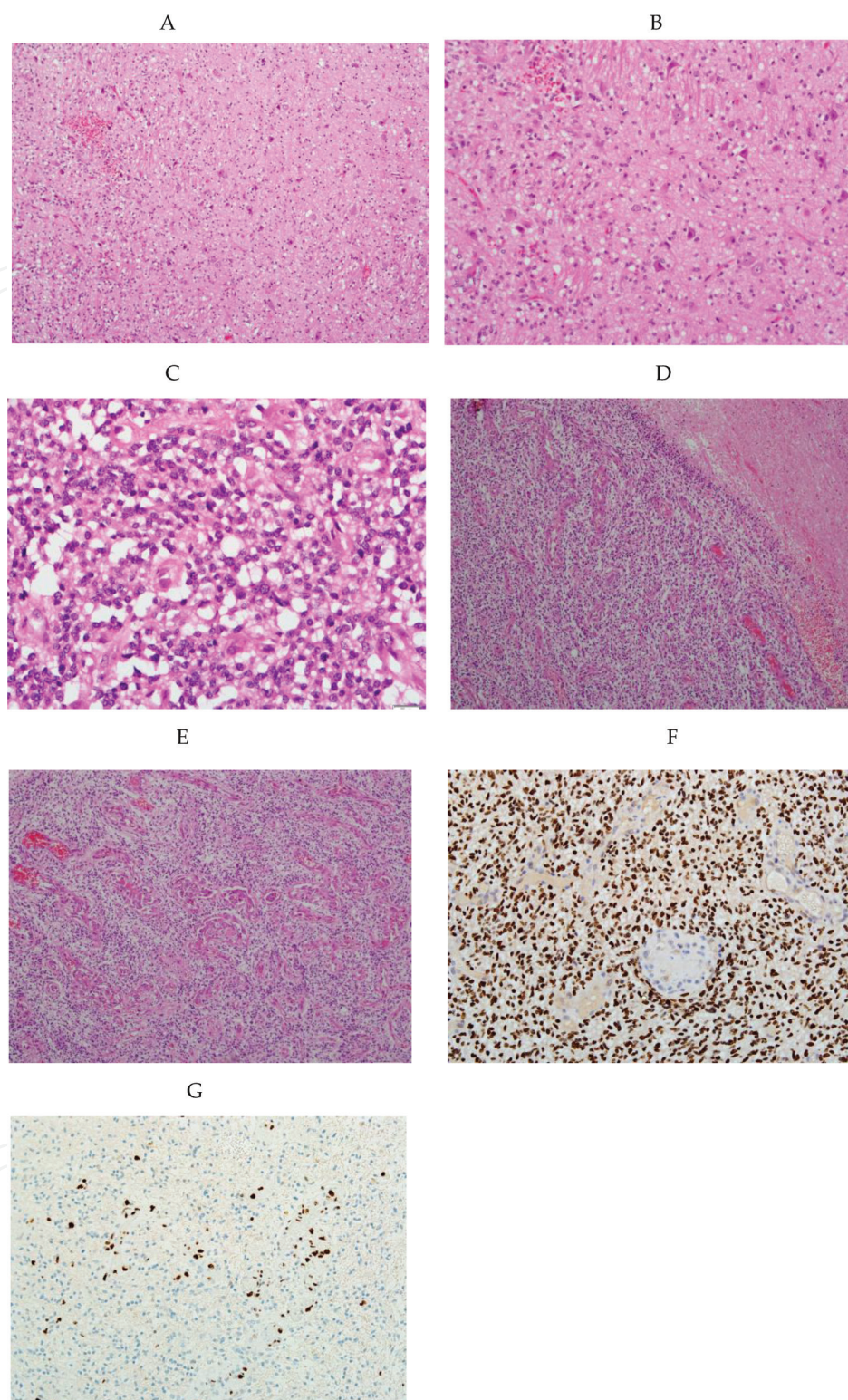


Figure 2. Histologic features of DIPG. Tumor infiltrating medullary neurons, H & E 100× (A) and 200× (B). (C) Tumor showing mitotic figures, H & E 400×. (D) Tumor/necrosis interface showing pseudo-palisading tumor necrosis. (E) Exuberant tumor-associated microvascular proliferation. (F) Tumor cells showing positive nuclear H3K27 M staining with sparing of the endothelial nuclei of admixed blood vessels H3K27 M 200×. (G) Loss of staining of tumor cell nuclei with the trimethylated antibody with retained staining in the endothelial cells, H3K27me3 200×. Images courtesy of Dr. Jason Dyke (Royal Perth Hospital, Australia).

specific histone mutation (H3K27 M) and are called diffuse midline glioma, H3K27M-mutant [40]. A mutation-specific antibody can be used to detect this mutation on immunohistochemistry (**Figure 2F**).

6. The role of biopsy in diagnosis

Prior to the routine MRI use, it was estimated that up to 15% of patients diagnosed with DIPG had a non-glial tumor or non-tumor process [41, 42]. The role of surgical intervention was controversial and, in some centres, biopsy procedures were frequently undertaken for histological confirmation. Although it is not 100% specific, with the wide availability in the early 1990s MRI became the modality of choice for DIPG diagnosis. Biopsy confirmation was thought to be an unnecessary risk as it did not alter the management [43]. The lack of clear biopsy benefit combined with improved diagnostic imaging capabilities led to MRI scans becoming the diagnostic standard of care for DIPG in the United States [18, 44]. More recently, there has been a renewed interest in performing stereotactic biopsies in patients with DIPG [45]. In France, biopsies were routinely performed with minimal morbidity and high diagnostic yield [46], nonetheless, it was not a common practice. The increase in the available DIPG tumor samples has yielded valuable data toward improving our understanding of the biology of DIPG [45, 47, 48]. Some of the identified biologic markers have been shown to correlate with progression-free survival (PFS) and may be useful to stratify patients in future clinical trials [49]. Considering the potential for new diagnostic and therapeutic methods combined with low morbidity associated with surgical procedures [50], the option of biopsy is being reconsidered and may eventually be included as part of the routine diagnostic evaluation for DIPG [51]. The second Consensus Conference on Pediatric Neurosurgery recommended biopsy in DIPG to ascertain biological characteristics to enhance understanding and targeting of treatment, especially in the setting of clinical trials [52]. Biopsy from a single area may not be representative of the entire tumor [53] but it may still provide important molecular information.

7. Treatment

More than 250 clinical trials in the last three decades have failed to improve the poor outcome of DIPG [20]. Due to the location it is not amenable to the surgery and chemotherapy agents are yet to show any response. Radiation therapy remains the standard of care at present. The barriers to achieve a cure for DIPG may include the inability of the surgical resection, drug delivery difficulties secondary to the blood-brain barrier (BBB) and intra- and inter-tumoral heterogeneity in the identified molecular aberrations [54].

7.1. Surgery

Surgical resection of DIPG is not feasible due to the presence of vital structures where the tumor is located. Rarely, surgical intervention like ventriculoperitoneal shunt or endoscopic

third ventriculostomy may be necessary to relieve raised intracranial pressure secondary to hydrocephalus. Many patients benefit from steroids which help by reducing peritumoral edema.

7.2. Radiation therapy (RT)

Conventional RT in a dose of 54–60Gy over a 6 week period is commonly utilized in the treatment of DIPG [9]. Temporary improvement or stabilization of symptoms is seen in 70% of patients, but almost all patients have progressive disease. The mean progression-free survival (PFS) is 5.8 months with radiotherapy and 5 months without it [4]. As RT is effective temporarily, non-conventional doses and delivery schedules were investigated. In hyperfractionated RT, the total dose is divided into smaller doses and given more than once a day. Hyperfractionated RT in the form of 1Gy twice a day, to the total dose of 72Gy failed to improve the outcome [37]. Hypofractionated RT is given over a smaller period of time than standard RT by dividing the total RT dose into larger doses and treatments given once a day or less often. Studies investigating the role of hypofractionated RT with 39Gy [55] or 45Gy [56] delivered over 3 weeks have revealed similar survival outcomes, but may be more acceptable to families due to the shorter delivery times.

7.3. Chemotherapy

Various chemotherapy and targeted agents have been used to potentiate the beneficial effects of RT. These agents were combined before, with, and after RT without much success [9, 10].

7.3.1. Intensive chemotherapy

Chemotherapy combinations used with RT in the setting of phase I–III clinical trials. These include lomustine, vincristine and prednisone [41], cisplatin, etoposide, vincristine, ifosfamide and oral valproic acid [57], myeloablative thiotepa, isotretinoin and vinorelbine [58] and multiple other agents at relapse [59]. One trial evaluated the role of preradiation chemotherapy [60]. The outcome was uniformly poor.

7.3.2. Radiosensitizing agents

- i. Temozolomide (TMZ): TMZ is an alkylating prodrug which is converted into its active metabolite monomethyl 5-triazeno imidazole carboxamide. TMZ causes DNA damage by alkylating O⁶-guanine, N⁷-guanine and N³-adenine residues [61]. Due to its proven efficacy in high grade gliomas, low toxicity and radiosensitization potential, temozolomide was trialed to potentiate RT efficacy without much success [8, 62, 63]. Addition of lomustine to adjuvant temozolomide [64] was not beneficial. O⁶-methylguanine DNA methyltransferase (MGMT) contributes to TMZ resistance by repairing alkylated O⁶-guanine nucleotides. But this does not appear to be the cause of TMZ resistance as MGMT is not expressed in DIPG [47]. However, 3-methylpurine-DNA glycosylase (MPG), enzyme

responsible for repair of N⁷-gaunine and N³-adenine nucleotides, and its ATM-dependent regulation may play a role in TMZ resistance in DIPG [65]. A recently closed phase II study stratified patients to receive TMZ and erlotinib based on MGMT methylation and EGFR expression status [66].

- ii. Topotecan: topotecan acts as a radiosensitizing agent by stabilizing the DNA topoisomerase I complex, interfering with DNA replication and DNA repair. Concurrent administration of topotecan with RT was found to be ineffective [67, 68].
- iii. Other radiosensitizing agents like motexafin-gadolinium [69] and carbogen [70] also showed similar poor results.

8. Biology

More than 250 therapeutic clinical trials including several targeted agents have not improved the dismal prognosis of DIPG [10]. The reason for this, at least in part, has been attributed to our lack of understanding of the biology of this disease. More has been published on the biology and pathophysiology of DIPG in the past 10 years than in all prior years combined [5]. A more recent and significant achievement in DIPG research is sample collection at autopsy. This has provided invaluable insights into understanding of the biology [71, 72]. Both autopsy and biopsy samples have allowed development of *in-vitro* (neurospheres) and *in-vivo* models (allograft and xenograft) [73–75].

8.1. Cell of origin

Pontine precursor-like cells (PPC), found in the ventral pons region, which are positive for the markers for the primitive neural precursor cells, nestin and vimentin, are postulated as the candidate cell of origin for DIPG [35]. Approximately half of PPC also expressed Olig2, a transcription factor which is associated with oligodendroglial precursors. This cell type was morphologically distinct from the nestin positive cells seen in the dorsal brainstem. PPC are present in all ventral brainstem structures during infancy and wane by 2 years of age. The ventral pontine and medullary nestin⁺ cells show a second peak at 6 years, corresponding to the age of presentation of DIPG. Thus, temporal and spatial distribution of these cells correlates closely with the incidence of DIPG suggesting that tumors arise secondary to dysregulation of a postnatal neurodevelopmental process [35]. Expression of SOX2, a transcription factor with activity during embryogenesis, and Olig2 in another model supports the disordered neurodevelopmental origin of DIPG [76].

8.2. The genomic landscape

DIPG biopsy and autopsy samples have undergone extensive genomic profiling and major breakthroughs have been achieved in identifying key oncogenic pathways [77]. The drivers for DIPG tumorigenesis include epigenetic changes, gene mutations, deletions or overexpression and chromosomal number changes.

8.3. Epigenetic changes

8.3.1. Histone mutations

The DNA is packaged by histone proteins into a chain of nucleosomes which are the basic building blocks of the chromatin fiber [78]. In a single nucleosome, 147 base pairs (bp) of DNA wrap around histone octamers containing two copies each of histones H2A, H2B, H3 and H4 [79]. The N-terminal ends of histones containing lysine (K) and arginine (R) residues are post-translationally modified by acetylation or methylation and regulate DNA repair, replication and transcription. The histone H3 family consists of a number of related proteins. Histone H3 isoforms H3.1 and H3.2 (also called as canonical H3) help in packaging newly replicated DNA. While H3.3 can function much the same as canonical H3 as a core part of the nucleosome, it is also deposited into transcriptionally active regions to replace histones lost during processes disrupting nucleosomes [80].

8.3.1.1. H3 mutations and DIPG

8.3.1.1.1. H3K27M mutations

H3F3A and *H3F3B* produce identical H3.3 proteins whereas *HIST1H3B* is one of the many genes encoding H3.1 [81, 82]. Distinct and recurrent mutations in H3 have been implicated in 70–80% of pediatric gliomas [83]. Lysine to methionine missense mutation at position 27 (K27M) was present in 78% of DIPG patients, with most of these mutations in *H3F3A* and up to 25% in *HIST1H3B* [84, 85]. H3K27M mutations are restricted to the midline structures [86] and H3.1 and H3.3 mutations involve two different oncogenic pathways resulting in distinct clinicopathological variants. H3.1 mutated tumors are exclusively linked to DIPG and exhibit a mesenchymal/astrocytic phenotype, a pro-angiogenic/hypoxic signature and are co-segregated with *ACVR1* mutations. Clinically, these tumors are less aggressive when compared to H3.3 mutant tumors, metastasize less frequently and respond better to radiation therapy (RT) with a median overall survival (OS) of 15 months. H3.3 mutated tumors are located in the midline structures including the brainstem, thalamus and spinal cord. They have a proneural/oligodendroglial phenotype, a pro-metastatic gene expression signature with *PDGFRA* activation. They behave more aggressively, responding poorly to RT with a median OS of 9 months and metastasize more frequently [87]. The gain-of-function H3K27M alterations are exclusive to pediatric high-grade gliomas and any H3 mutation is associated with a dismal outcome but identification of a specific mutation may help in developing specific therapeutic targets.

8.3.1.1.2. G34R/V mutations

H3F3A mutations encoding a glycine 34 to arginine or valine G34R/V comprise a smaller proportion of H3.3 mutations [88]. G34R/V mutations are seen in cerebral hemispheres of slightly older patients (9–42 years) as compared to K27M mutations (5–29 years) [86, 88, 89].

K27M and G34R/V mutations are mutually exclusive and heterozygously expressed, with one wild-type *H3F3A* allele [89].

8.3.1.1.3. Other novel mutations

H3F3A mutation resulting in lysine-to isoleucine substitution at K27 has been rarely seen in DIPG [87]. A mutation in the gene encoding the H3.2 variant, HIST2H3C, resulting in a novel K27 M mutation has been described [87].

8.3.1.2. Downstream effects of H3 mutations and gliomagenesis

8.3.1.2.1. K27 M mutations

K27M (and to a lesser extent K27I) is the only amino acid substitution which can ablate trimethylation (H3K27me3) [90]. The mutant H3K27M binds to the enhancer of zeste homolog 2 (EZH2) component of PRC2 interfering with methyltransferase activity of EZH2 which results in generalized hypomethylation. The downstream effect is derepression of targets of PRC2, upregulation of gene expression and gliomagenesis [91]. In addition, K27M mutation contributes to altered cell cycle control, inhibition of autophagy pathways and potentially increased resistance to radiotherapy [92]. Even though the mutant histone forms only 3.6–17.6% of the total cellular H3 pool, there is a near-absolute loss of H3K27me3. This represents a *trans*-dominant-negative effect across all three isoforms of the wild-type H3 protein [90, 91, 93, 94]. However, the exact role of H3K27M in DIPG tumorigenesis remains unknown as it does not induce the tumors on its own *in vivo* [95]. One of the postulates of gliomagenesis is H3.3 K27M and G34R/V acting as driver mutations followed by a second hit by another mutation like *TP53*, *PPM1D*, *ACVR1* or *PI3KR1* [83, 95, 96].

In summary, H3K27 mutations have a great significance irrespective of whichever histone H3 variant (H3.1-HIST13B, H2-HIST2H3C and H3.3-H3F3A) is targeted and will result in loss of H3K27me3 and development of DIPG.

8.3.1.2.2. G34R/V mutations

The role of H3.3G34R/V in gliomagenesis is less clear. It may act by disrupting K36me3 levels and activating potential oncogenes [90, 93]; inducing *MYCN* upregulation [97, 98] and disrupting interaction between H3.3 and ATRX/DAXX leading to aberrant deposition of H3.3 near telomeric regions and leading to alternate lengthening of telomerase [88, 89, 99].

8.3.1.3. Co-mutations associated with H3 mutations in DIPG

Other mutations associated with H3K27M mutation include α thalassemia/mental retardation syndrome X-linked (*ATRX*) or death-domain associated protein (*DAXX*) (30%), *TP53* (60%) and *NF-1*, *PDGFRA*, *BRAF*, *KRAS*, and *FGFR1* at lower frequencies [88, 99, 100]. G35R/V mutations coexpress with mutations in *TP53*, *ATRX/DAXX* and *PDGFRA* [83].

8.3.1.4. Mutations of chromatin modifiers

Chromatin writers or erasers are the enzymes which catalyze the post-translational modifications of histone tails like methylation, acetylation and ubiquitylation of lysine residues,

phosphorylation of serine or threonine residues and methylation of arginine residues. The effector proteins called readers are recruited to the chromatin by the resultant histone code which helps in localization of functional complexes that affect transcriptional regulation [101]. Numerous recurrent mutations are observed in chromatin writers, erasers, readers and remodelers in DIPG and other tumors [102].

The discovery of histone mutations which are present in up to 80% of DIPG is one of the most remarkable breakthroughs in terms of understanding DIPG biology and identification of actionable targets [90, 92, 99].

8.3.2. Polycomb repressive complex (PRC) abnormalities

Polycomb group proteins remodel chromatin enabling epigenetic silencing of genes. There are two main Polycomb group complexes found in mammals-PRC1 and PRC2. PRC1 catalyzes the monoubiquitylation of histone H2A and PRC2 catalyzes the methylation of H3K27 [103]. Some PRC1 complexes also act independent of enzymatic activity to regulate gene expression by compacting chromatin [104]. PRC1 functions downstream of PRC2 by binding specifically to H3K27me3 [103]. By inducing such sequential histone modifications, PRC1 and PRC2 achieve stable silencing of gene expression [105]. Dysregulation of PRC and its downstream targets has been implicated in many cancers [83]. B cell-specific Moloney murine leukemia virus integration site 1 (BMI-1) is a component of PRC1 complex. It was found to be highly expressed in DIPG tumor cells and its downregulation inhibited various cellular processes like cell proliferation, cell cycle signaling, telomerase expression and activity, and cell migration [105].

8.4. Gene abnormalities

Molecular profiling of DIPG samples has provided new insights [61]. In the past, candidate gene approaches were utilized to identify gene abnormalities associated with adult high-grade gliomas (HGG) [106]. Although, these studies were limited in defining the biology of DIPG due to their small numbers, they still highlighted some differences between adult HGG and DIPG. Recently, studies performed with next-generation sequencing approaches have confirmed that DIPG are molecularly distinct from adult HGG and non-DIPG pediatric HGG [18]. The current technologies utilize whole-genome sequencing (WGS), whole-exome sequencing (WES), and RNA-sequencing in addition to copy number, gene expression, and methylation profiles and histopathology.

8.4.1. Mutational burden of DIPG compared to other tumors

The genomic signatures of the most pediatric HGG are complex and involve significant copy number alterations (CNAs), single nucleotide variants (SNVs) and structural variants [107–109]. HGG have a higher mutation burden than many other pediatric cancers but it is still lower than common adult cancers [77]. HGG commonly show structural variants like simple rearrangements and abnormalities caused by chromothripsis [84]. But there is a wide range of genomic complexity in pediatric HGG. At one end of the spectrum is infant non-brainstem HGG (NBS-HGG) arising in children less than 3 years old. These tumors have significantly

lower mutation burden [84]. At the other end of the spectrum are HGG from patients with inherited mutations in mismatch repair genes. Germline mutations in tumor suppressor genes like *TP53* and neurofibromin 1 (*NF1*) predispose to the development of HGG [110]. The hypermutated tumors arising in the context of these germline mutations show a very high number of somatic SNVs; these may be more than 100-fold higher than in 95% of pediatric HGG [84]. DIPG show similar mutation burden as other pediatric HGG and their genomic complexity is indicative of multiple genetic mechanisms generating numerous mutations which provide the tumor with diverse potential pathways to therapeutic resistance [77].

8.4.2. Abnormalities of cellular proliferation pathways

8.4.2.1. Bone morphogenetic protein (BMP) signaling and *ACVR1* mutations

ACVR1, also known as *ALK2* encodes the serine kinase, Activin receptor type 1 A (*ACVR1*) [111]. It is a type 1 BMP receptor which belongs to the mammalian TGF- β signaling family [112]. *ACVR1* binds to a diverse set of ligands, including TGF- β , activins and multiple BMP [113]. *ACVR1* is essential for signaling and after ligand it is phosphorylated by *ACVR2* with formation a stable *ACVR1/2* complex [114]. This results in the phosphorylation and activation of growth promoting genes through SMAD transcription factors [77]. *ACVR1* mutations are constitutionally activating, leading to increased expression of activin signaling targets *ID1* and *ID2* [115].

8.4.2.1.1. *ACVR1* mutations and DIPG

Germline *ACVR1* mutations cause the congenital malformation syndrome fibrodysplasia ossificans progressive (FOP) [116]. Seven somatic mutations of this gene have been identified in 13–32% of DIPG samples leading to either ligand-independent kinase activation or gain-of-function effects [84, 111, 112, 115]. However, there is no increased cancer risk in FOP despite having similar germline mutations as those seen in DIPG which suggests that *ACVR1* mutations on their own are not tumor initiating and lead to DIPG only in the presence of other mutations [77, 116].

8.4.2.1.2. *ACVR1* co-mutations

ACVR1 mutant tumors commonly co-segregate with *HIST1H3B* mutations [115]. *ACVR1*-*HIST1H3B* co-segregating tumors do not show *TP53* loss or *PDGFRA* amplifications but around 60% have mutations in the PI3K signaling pathway [114].

8.4.2.1.3. Clinical implications

ACVR1 mutations signify a distinct subset of DIPG patients. They occurred more frequently in females (F:M ratio of 1.75:1) and are associated with younger age and longer survival (median OS of 14.9 months) [84, 111]. There was a significant pharmacologic inhibition of *ACVR1* by a selective *ALK2* inhibitor, LDN-193189, leading to dose-dependent cytotoxicity across all the tested DIPG cell lines [111]. Due to its role in DIPG pathogenesis and targetable potential, *ACVR1* inhibition represents a novel therapeutic option.

8.4.2.2. Receptor tyrosine kinase (RTK) pathway

RTKs are transmembrane protein receptors containing intrinsic enzymatic activity. Their ligands include growth factors, hormones and cytokines [117] and they play an critical role in mediating key signaling pathways involving cell proliferation, differentiation, survival and migration [118]. The human RTK family has 20 subfamilies and 58 known members including platelet-derived growth factor receptors (PDGFR), epidermal growth factor receptors (EGFR) and fibroblast growth factor receptors (FGFR) [118–120]. Upon ligand binding, the RTKs are activated leading to signal transduction to the nucleus and subsequent protein transcription. This is achieved by downstream activation of various RTK substrates like mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K) [121].

8.4.2.2.1. RTK pathway aberrations and DIPG

Amplifications and mutations in components of RTK-RAS-PI3K pathway are seen in up to 60% of DIPG [18]. The most common affected component is *PDGFRA*. Other abnormalities involve *AKT1*, *AKT3*, *c-MET*, epidermal growth factor receptor (*EGFR*), erythroblastic leukemia viral oncogene homolog-4 (*ERBB4*), hepatocyte growth factor, Kirsten rat sarcoma viral oncogene homolog (*KRAS*), *PIK3CA*, *PIKC2G*, *PIK3R1*, *PTEN*, insulin-like growth factor 2, and insulin-like growth factor receptor [18, 45, 53, 111].

8.4.2.2.1.1. *PDGFRA* amplifications

Whole-genome profiling of DIPG tumors have identified recurrent amplifications of *PDGFA* and *PDGFRA* with overexpression of PDGFR- α in 28–50% tumors [47, 48, 122, 123].

8.4.2.2.1.2. *PDGFRA* mutations

Somatic activating mutations including missense mutations and in-frame deletions and insertions were identified in 4.7% DIPG tumors and were found to be oncogenic *in vivo* [124]. Concurrent amplification was seen in 40% of tumors with mutations and 60% had heterozygous mutations [124]. Similar mutations were identified in other studies in 8.8–25% samples [54, 123]. Downstream activation of the PDGFR pathway has been shown by phospho-mammalian target of rapamycin (m-TOR) immunopositivity [47] as well as activation of MAPK and PI3K pathways [124]. PDGFR- α is expressed by the oligodendrocyte precursor cell derived from the candidate cell of origin PCC [35] and hence the precursor cell may be responsive to PDGF. Also, human DIPG cell culture yield was better after the addition of PDGF [35] and upregulation of PDGF pathway was associated with dorsal pontine glioblastoma in mouse models [125, 126].

8.4.2.2.1.3. *EGFR* aberrations

EGFR immunopositivity and gene amplification were seen in about 27% [47] and 7–9% [48] of cases respectively.

8.4.2.2.2. *RTK pathway co-mutations in DIPG*

PDGFRA gains and amplifications co-segregate with H3.3 mutations [88, 99] and *TP53* mutations [124].

8.4.2.2.3. *Clinical significance*

RTK signaling dysregulation, particularly PDGFR pathway either overexpression or mutation, may have an important role in the pathophysiology of DIPG and provide therapeutic targets [47]. Identification of *PDGFRA* mutations may be beneficial in developing targeted therapies. But some particular mutations like PDGFR D842V in the gastrointestinal stromal tumors confers resistance to imatinib [127]. Others may have only cytostatic effects so they may not be effective on their own [124].

8.4.2.3. *MYC and MYCN aberrations*

MYCN proto-oncogene is a member of the *MYC* family encoding the protein MYCN. MYCN plays a critical role during embryogenesis and is involved in cellular proliferation and differentiation [128]. *MYCN* amplification is seen in DIPG [84, 92, 99] and is associated with hypermethylation, high-grade histology and chromothripsis on chromosome 2p [115]. *MYCN* amplifications are transcriptional regulators that affect the epigenetic landscape by enhancing gene expression across the whole genome [84]. *MYCN* pathway maybe induced by H3.3 K27 M [99] or H3.3G34V [83, 102] but may act independent of H3 mutations [92].

8.4.2.4. *Hedgehog (Hh) signaling*

Hh signaling pathway plays a major role in regulation of developmental processes like cell proliferation, cell differentiation, tissue polarity and stem cell maintenance. Aberrant activation of Hh pathway has been implicated in the pathogenesis of cancers like medulloblastoma. No structural mutation involving this pathway resulting in the development of DIPG has been identified so far. However, in pre-clinical murine models [35] upregulation of Hh pathway activity resulted in ventral pontine hyperplasia. Hh pathway is essential for the normal development of PPC in humans. Also, Hh pathway activation stimulates and blockade reduces the self-renewal capacity of DIPG neurosphere cells. These findings indicate that Hh signaling, which drives the development of neural precursors in the ventral pons, may play a role in tumor formation in a subset of DIPG. Patients with Gorlin syndrome, a genetic entity occurring secondary to unregulated Hh activity, usually do not develop DIPG [35]. So, in addition to unregulated Hh pathway activity, a second “hit” may be necessary for DIPG transformation. Hh signaling role in the pathogenesis of DIPG was further investigated in a study [92] which identified upregulation of Hh signaling. DIPG samples showed upregulation of Patched (PTCH) and nuclear translocation of Glioma Associated Oncogene 1 (GLI1); both PTCH and GLI1 are key Hh pathway molecules. In summary, Hh pathway may play a significant role in DIPG tumorigenesis by stimulating PPC and transforming them into potential DIPG cancer stem cells (CSC).

8.4.3. Abnormalities of cell cycle regulation pathways

8.4.3.1. TP53 pathway

The TP53 pathway is a complex network of genes which respond to diverse internal and external stress signals and have an impact on the normal cellular homeostasis [129]. The p53 protein is activated by stress signals transmitted as post-translational modifications leading to apoptosis [130]. In addition, the TP53 pathway produces proteins which aid directly in DNA repair processes and alter cellular environment enabling inter-cellular communication [131]. In the critical role of safeguarding the genomic integrity, it functions as a tumor suppression pathway [132]. *TP53* is the most commonly mutated gene found in a broad variety of human cancers [129, 133].

8.4.3.1.1. TP53 mutations and DIPG

TP53 mutations are commonly found in DIPG with the reported incidence between 9 and 77% [45, 99]. They are more common in higher grade histology tumors (grades III and IV) [53]. About 50% of *TP53* wild-type grade II DIPG show presence of *PPM1D* mutations [134]. *PPM1D* is an oncogene associated with cancers like neuroblastoma [135] and lung cancer [136] which codes for wild-type p53-induced phosphatase 1D (WIP1). WIP1 is a negative regulator of *TP53* as it inactivates p53 and promotes termination of stress-induced responses. So *PPM1D* mutations have the same functional significance as *TP53* mutations [137]. *PPM1D* and *TP53* mutations are mutually exclusive and may ultimately lead to dysregulated homeostasis and tumorigenesis [134].

8.4.3.1.2. TP53 co-mutations in DIPG

TP53 mutations more commonly co-segregate with H3.3 K27 M than H3.1 K27 M [112] and frequently occur in the setting of *PDGFRA* aberrations [124].

8.4.3.2. The RB pathway

Cyclins and cyclin-dependent kinases (CDKs) control the G₁/S transition of the cell cycle [138]. The abnormalities involving these regulators observed in DIPG include cyclin-dependent kinase inhibitor 2A or 2B (*CDKN2A* or *CDKN2B*) deletions [48, 122] and *CDK4*, *CDK6* or cyclin D1 (*CCND1*), *CCND2*, and *CCND3* amplifications [48, 122, 139].

8.4.3.3. Aurora kinase pathway

Aurora kinase family include three highly homologous serine/threonine kinases required during mitosis and which are linked to many cancers [140]. *AURKB* forms the catalytic component of the chromosomal passenger complex (CPC) which plays a critical role during mitosis [141]. Almost 70% of DIPG have demonstrated overexpression of *AURKB* [142].

8.4.3.4. WEE1 kinase pathway

WEE1 kinase is an important part of G₂ checkpoint. DIPG cells, unlike normal cells, have aberrations in genes regulating the G₁ checkpoint, including *TP53*, *MDM2*, *CDKN2A*, and *ATM*

[122, 133, 143] causing a dysfunctional G₁ arrest. So, these cells rely heavily on G₂ checkpoint to repair DNA damage caused by irradiation. WEE1 protein is significantly overexpressed in post-mortem DIPG samples [73]. Abrogation of the G₂ checkpoint achieved by WEE1 kinase inhibition pushes DIPG cells with unrepaired DNA damage into mitotic catastrophe resulting in cell death.

8.4.3.5. Poly (ADP-ribose) polymerase (PARP)-1 abnormalities

PARP-1 is a nuclear protein involved in the DNA damage repair processes [144]. PARP-1 activity provides an escape mechanism for cancer cells to avoid apoptosis and its overexpression may be associated with temozolomide and radiation resistance [47]. Gain of *PARP-1* is seen in DIPG tumors and provides a potentially targetable therapeutic option [47].

8.5. Chromosomal number abnormalities

Copy number abnormalities (CNAs) reported in DIPG include gain in chromosomes 1q, 2q, 8q, 9q, 7p/7q and loss in chromosomes 16q, 17p, 20p, 21q, 10q and 4q [47, 48, 122, 123, 139, 145]. The CNAs may represent the initial mutations responsible for DIPG tumorigenesis as well as the treatment effect.

8.6. Immune checkpoint abnormalities

8.6.1. B7-H3 abnormalities

B7-H3 or CD276, a member of the B7-CD28 family, is a type I transmembrane glycoprotein [146]. Many malignant neuroectodermal tumors including adult HGG over-express B7-H3. B7-H3 was found to be overexpressed in a small panel of DIPG samples obtained at autopsy [147]. A monoclonal antibody 8H9 recognizes it and binds specifically to the tumor cells [148, 149] enabling therapeutic cell selectivity. B7H3 was targeted safely in the salvage therapy of stage IV neuroblastoma using intrathecal ¹³¹I-8H9 [150].

8.7. Tumor microenvironment abnormalities

Therapies targeted at intrinsic cellular pathways have yielded poor results in DIPG. Tumor microenvironment plays a vital role in tumorigenesis and progression, so studies have looked into investigating microenvironment alteration for better results.

8.7.1. Neuroligin-3 (NLGN3) role

Neuroligin-3 (NLGN3) is a synaptic adhesion molecule which is cleaved from neurons and oligodendrocyte precursor cells via the ADAM10 sheddase and released into the tumor microenvironment. This important neuronal activity promotes many types of brain cancers including DIPG, pediatric and adult HGG and anaplastic oligodendroglioma. NLGN3 release activates oncogenic pathways like focal adhesion kinase activation resulting in the downstream PI3K-mTOR pathway induction. This in turn causes upregulation of several synapse-related genes resulting in the proliferation of glioma cells [151]. HGG glioma growth in xenograft models was blocked by ADAM10 inhibitors by preventing NLGN3 release into

the tumor microenvironment. Similarly, patient-derived orthotopic xenografts fail to grow in *Nlgn3* knockout mice [152].

8.7.2. Pleiotrophin (PTN) role

PTN is a growth factor secreted by neural precursor cells in the lateral ventricle subventricular zone (SVZ). It has an important role in normal neurodevelopment, plasticity and regeneration. PTN acts as a glioma chemoattractant. Through autocrine/paracrine actions, it activates Rho/Rho kinase (ROCK) signaling pathway enabling migration of DIPG cells to the SVZ [153].

9. DIPG subgroups

Proteomic, methylation and mRNA analyses have identified interesting subgroups of DIPG.

PDGFRA amplification is seen in association with H3.3 mutation and ACVR1 mutation is mainly seen in H3.1 mutant tumors [87, 115].

Upregulation of N-Myc and Hh signaling pathways [92].

Upregulation of N-Myc (H3 wild-type), silent genome and H3K27 M [115].

Mesenchymal transition with stem cell-like phenotype and oligodendroglial differentiation and PDGFRA amplification/mutation.

10. Novel therapeutic approaches

10.1. Molecularly targeted agents

10.1.1. RTK-RAS-PI3K pathway

Given DIPG tumors show aberrant activation of growth factor receptor-mediated signal transduction pathways, using drugs targeting these pathways is a rational approach. *In-vitro* studies confirmed the efficacy of tyrosine kinase inhibitors (TKI) like dasatinib in reducing tumor proliferation and inhibition of *PDGFRA* activity [154]. Phase I studies of PDGFR pathway inhibition by imatinib [155] and dasatinib [156], VEGFR2 inhibition by vandetanib [156, 157], EGFR inhibition by gefitinib [158] and erlotinib [49] revealed the safety of using these drugs in children and provided the doses for phase II studies. Temsirolimus is an mTOR inhibitor [159] and its combination with perifosine, an Akt inhibitor, was shown to be safe and feasible in a phase I study in children with recurrent/refractory solid tumors including DIPG [160]. This study tested the hypothesis of dual targeting of PI3K-Akt-mTOR pathway. Phase II studies of multi-tyrosine kinase inhibitor sunitinib [161] and farnesyl transferase inhibitor tipifarnib, which inhibits farnesylation of Ras, [162] did not show activity. BIOMEDE, a phase II study, is stratifying patients based on overexpression of EGFR and/or loss of PTEN following diagnostic biopsy. The patients are randomized or assigned to different treatment arms with erlotinib, everolimus and dasatinib [163].

10.2. Epigenetic modifying agents

Histone deacetylases (HDAC) regulate the histone acetylation in nucleosomes, which mediates changes in chromatin conformation, leading to gene expression regulation [164]. HDAC gene mutations, downregulation and altered expression are linked to tumorigenesis. Histone deacetylase (HDAC) inhibitors modify histone activity to increase expression of previously silenced genes thereby leading to cell death [165]. Panobinostat is a multi-HDAC inhibitor which increases global H3 acetylation and H3K27 M methylation and reduces oncogene expression. Panobinostat-induced polyacetylation of the H3 N-terminal tail has been shown to reverse PRC2 inhibition caused by K27 M and rescue the H3K27 hypomethylation phenotype [166]. Panobinostat has shown promising results in *in vitro* [164] and some *in vivo* [167] studies although a narrow therapeutic index causing dose limiting toxicities at the required antitumor concentrations being the likely reason for lack of efficacy [164]. Its efficacy was further enhanced by histone demethylase inhibitor GSK-J4 which increase H3K27me3 [168]. DIPG patients showed no objective responses to single agent vorinostat in a phase I clinical trial [169]. Vorinostat is currently being investigated in combination with temsirolimus and RT in DIPG in a phase I trial [170]. Pediatric Brain Tumor Consortium (PBTC) is undertaking a phase I trial of single agent Panobinostat in patients with DIPG [171].

10.3. Targeting cell cycle regulation

DIPG tumors contain cell cycle regulatory gene abnormalities like amplification of D-type cyclins and CDK4/6 or loss of Ink4a-ARF resulting in cellular proliferation [172]. Targeting the cyclin/CDK/RB pathway was investigated in a preclinical trial. PD-0332991 (PD), a CDK4/6 inhibitor, was used in a genetically engineered, PDGF-B overexpressed, Ink4a-ARF and p53 deficient brainstem glioma mouse model. PD induced cell cycle arrest both *in vitro* and *in vivo*. The survival of mice treated with PD alone or in combination with RT was significantly more than untreated or mice treated with only RT [172]. Inhibition WEE1 kinase, which is overexpressed in DIPG, was shown to increase the RT response *in vitro* and *in vivo* [73].

10.4. Immunotherapy

Interferon, IL-12 and anti-glioma monoclonal antibody have shown efficacy in mouse models of non-brainstem malignancies [173]. Recently, subcutaneous vaccinations with glioma-associated antigen epitope peptides (EphA2, IL13-R α 2 and survivin) were investigated in children with DIPG. The vaccine was tolerated well and the results were encouraging for future trials [174]. IL13-R α 2 is of particular significance as it is highly and differentially expressed in DIPG [175, 176] compared to the normal brain tissue which makes it a suitable immunotherapy target.

10.5. Human neural and mesenchymal stem cells

Neural stem cells (NSC) have been utilized as the vehicle for therapeutic agents in gene therapy for experimental malignant brain tumors [177, 178]. NSC have robust tumor tropism and this property can be used to deliver therapeutic agents to the inoperable DIPG tumors. NSC are difficult to harvest and use due to their inherent immunogenicity [179]. Mesenchymal

stem cells (MSC) have been found to be a suitable alternative as they have all the properties of NSC and are more practical to use [180]. In a rat model, brainstem glioma directed migratory capacities of NSC and MSC from different sources was found to be similar *in vitro* and *in vivo*. This study showed promise as 20–30% of stem cells migrated from the site of injection in the right forebrain to the brainstem glioma cells. In addition, the group treated with genetically engineered NSC encoding cytosine deaminase (CD) suicide gene and IFN β proinflammatory cytokine and systemic 5-fluorouracil resulted in 59% reduction in the tumor size [181].

10.6. Delivery methods to overcome blood-brain barrier (BBB)

The vast majority of brain tumors are characterized by the loss of BBB integrity due to the disordered and highly permeable tumor neovasculature [182]. In DIPG, however, the BBB integrity is normal and the drug permeability is reduced as the tumor makes use of the existing brain vasculature [183]. Traditional cytotoxic drugs have shown good efficacy against DIPG cells *in vitro* but they likely fail to penetrate the BBB which may explain the contrasting results with clinical trials [28]. To overcome the disappointing results associated with systemic therapy, methods utilizing localized and targeted drug delivery have been investigated.

10.6.1. Convection-enhanced delivery (CED)

CED is a relatively novel delivery modality which utilizes the properties of bulk flow to achieve homogeneously distributed infusions into the brain parenchyma [184]. It is a neurosurgical technique in which one to several catheters are stereotactically placed within or around the tumor mass and drug is delivered locally, bypassing the BBB and reducing systemic toxicity [185, 186]. The local concentrations achieved are significantly higher than the systemic administration and the drug distribution occurs along the patterns of the glioma cell invasion along the white matter tracts [187]. Many *in vivo* feasibility studies have confirmed that drugs can be safely delivered into the rodent brainstem [188]. CED of carmustine [74, 189], carboplatin [190, 191], temozolomide [192], small-molecule kinase inhibitors [193], cisplatin [194] and gemcitabine [190, 195] have been evaluated for safety and distribution parameters. Topotecan CED in two pediatric patients with DIPG showed initial reduction in tumor size [196]. MRI guided and robotically placed catheters were used for CED of carboplatin in DIPG patients. Three out of eight patients survived beyond 15 months and the procedure was tolerated well [197, 198]. Administration of ^{124}I -8H9, a radioactively labeled antibody and a chimeric toxin with B7-H3 specificity, is currently being explored in a phase I clinical trial for DIPG patients [199]. A patient with DIPG received CED infusion of the recombinant cytotoxin IL13-PE38QQR with GD-diethylenetriamine but the tumor progresses within a few weeks [200]. IL13-PE38QQR is a recombinant *Pseudomonas aeruginosa* toxin and some early phase clinical trials investigating its CED efficacy are being conducted or recently completed in patients with DIPG and HGG [185]. These studies have established the feasibility of CED. However, more data is required to prove safety and efficacy and CED models are undergoing rigorous investigations into the physical properties of the catheters and infusion rates [173]. CED targets the primary tumor only and not the metastatic sites. In DIPG, local and distant metastases in the brain or spine are seen in 13–17% patients. Therefore, in situations where disseminated disease is present, CED therapy alone, directed toward the primary tumor site, will be inadequate and should be complemented with therapies for the secondaries [28, 32].

10.6.2. Nanoparticle delivery

Another alternative drug delivery technique involves encapsulation of cationic substances into capsules termed micro- and nanoparticles. This reduces their tissue affinity and increases the volume of distribution [28, 201].

10.6.3. Cell-mediated delivery

Bacterial cell-derived vehicles to transport chemotherapy agents across the BBB was utilized with bi-specific antibodies recognizing cell wall and EGFR moieties [202]. Similar EGFR-targeted vehicles loaded with doxorubicin resulted in significant tumor regression in canine brain cancer models [203]. ECREST (A Study of Intravenous EEDVsMit in Children With Recurrent/Refractory Solid or CNS Tumors Expressing EGFR) is a phase I study using mitoxantrone loaded nanocells in EGFR positive relapsed/refractory solid and CNS tumors including DIPG [204].

10.6.4. BBB disruption techniques

BBB can be temporarily disrupted by ultrasound methods. This results in enhanced uptake of systemically delivered drugs [28].

11. Conclusion

DIPG remains the main cause of death in pediatric patients with brain tumors. Despite many clinical trials, minimal improvement has been achieved as compared with RT. Most of the clinical trials in the past did not have sound biological bases due to a lack of biopsy specimens. In the last few years, international collaborative research has helped to identify new molecular aberrations in DIPG. These have provided insight into the pathogenesis of DIPG and may help in identifying new targeted therapies.

Author details

Santosh Valvi* and Nicholas G. Gottardo

*Address all correspondence to: santosh.valvi@health.wa.gov.au

Perth Children's Hospital, Perth, Australia

References

- [1] Scheurer ME, Bondy ML, Gurney JG. Epidemiology of childhood Cancer. In: Pizzo PA, Poplack DG, editors. Principles and Practice of Pediatric Oncology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2011. p. 2

- [2] Stiller CA, Nectoux J. International incidence of childhood brain and spinal tumours. *International Journal of Epidemiology*. 1994;**23**(3):458-464
- [3] Ostrom QT, Gittleman H, Liao P, Vecchione-Koval T, Wolinsky Y, Kruchko C, et al. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014. *Neuro-Oncology*. 2017;**19**(suppl_5):v1-v88
- [4] Freeman CR, Farmer JP. Pediatric brain stem gliomas: A review. *International Journal of Radiation Oncology, Biology, Physics*. 1998;**40**(2):265-271
- [5] Warren KE. Diffuse intrinsic pontine glioma: Poised for progress. *Frontiers in Oncology*. 2012;**2**:205
- [6] Bleyer A, O'Leary M, Barr R, Ries LAG, editors. *Cancer Epidemiology in Older Adolescents and Young Adults 15 to 29 Years of Age, Including SEER Incidence and Survival: 1975-2000*. NIH Pub. No. 06-5767. Bethesda, MD: National Cancer Institute; 2006
- [7] Smith MA, Seibel NL, Altekruse SF, Ries LA, Melbert DL, O'Leary M, et al. Outcomes for children and adolescents with cancer: Challenges for the twenty-first century. *Journal of Clinical Oncology*. 2010;**28**(15):2625-2634
- [8] Cohen KJ, Heideman RL, Zhou T, Holmes EJ, Lavey RS, Bouffet E, et al. Temozolomide in the treatment of children with newly diagnosed diffuse intrinsic pontine gliomas: A report from the Children's oncology group. *Neuro-Oncology*. 2011;**13**(4):410-416
- [9] Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: Critical review of clinical trials. *The Lancet Oncology*. 2006;**7**(3):241-248
- [10] Jansen MH, van Vuurden DG, Vandertop WP, Kaspers GJ. Diffuse intrinsic pontine gliomas: A systematic update on clinical trials and biology. *Cancer Treatment Reviews*. 2012;**38**(1):27-35
- [11] Donaldson SS, Laningham F, Fisher PG. Advances toward an understanding of brain-stem gliomas. *Journal of Clinical Oncology*. 2006;**24**(8):1266-1272
- [12] Littman P, Jarrett P, Bilaniuk LT, Rorke LB, Zimmerman RA, Bruce DA, et al. Pediatric brain stem gliomas. *Cancer*. 1980;**45**(11):2787-2792
- [13] Berger MS, Edwards MS, LaMasters D, Davis RL, Wilson CB. Pediatric brain stem tumors: Radiographic, pathological, and clinical correlations. *Neurosurgery*. 1983;**12**(3):298-302
- [14] Grimm SA, Chamberlain MC. Brainstem glioma: A review. *Current Neurology and Neuroscience Reports*. 2013;**13**(5):346
- [15] Fangusaro J. Pediatric high-grade gliomas and diffuse intrinsic pontine gliomas. *Journal of Child Neurology*. 2009;**24**(11):1409-1417
- [16] Albright AL, Guthkelch AN, Packer RJ, Price RA, Rourke LB. Prognostic factors in pediatric brain-stem gliomas. *Journal of Neurosurgery*. 1986;**65**(6):751-755
- [17] Fisher PG, Breiter SN, Carson BS, Wharam MD, Williams JA, Weingart JD, et al. A clinicopathologic reappraisal of brain stem tumor classification. Identification of pilocystic astrocytoma and fibrillary astrocytoma as distinct entities. *Cancer*. 2000;**89**(7):1569-1576

- [18] Schroeder KM, Hoeman CM, Becher OJ. Children are not just little adults: Recent advances in understanding of diffuse intrinsic pontine glioma biology. *Pediatric Research*. 2014;**75**(1-2):205-209
- [19] Fischbein NJ, Prados MD, Wara W, Russo C, Edwards MS, Barkovich AJ. Radiologic classification of brain stem tumors: Correlation of magnetic resonance imaging appearance with clinical outcome. *Pediatric Neurosurgery*. 1996;**24**(1):9-23
- [20] Bartels U, Hawkins C, Vezina G, Kun L, Souweidane M, Bouffet E. Proceedings of the diffuse intrinsic pontine glioma (DIPG) Toronto Think Tank: Advancing basic and translational research and cooperation in DIPG. *Journal of Neuro-Oncology*. 2011;**105**(1):119-125
- [21] Warren KE. Novel therapeutic delivery approaches in development for pediatric gliomas. *CNS Oncology*. 2013;**2**(5):427-435
- [22] Tzika AA, Astrakas LG, Zarifi MK, Zurakowski D, Poussaint TY, Goumnerova L, et al. Spectroscopic and perfusion magnetic resonance imaging predictors of progression in pediatric brain tumors. *Cancer*. 2004;**100**(6):1246-1256
- [23] Phillips NS, Sanford RA, Helton KJ, Boop FA, Zou P, Tekautz T, et al. Diffusion tensor imaging of intraaxial tumors at the cervicomedullary and pontomedullary junctions. Report of two cases. *Journal of Neurosurgery*. 2005;**103**(6 Suppl):557-562
- [24] Helton KJ, Phillips NS, Khan RB, Boop FA, Sanford RA, Zou P, et al. Diffusion tensor imaging of tract involvement in children with pontine tumors. *AJNR. American Journal of Neuroradiology*. 2006;**27**(4):786-793
- [25] Hipp SJ, Steffen-Smith E, Hammoud D, Shih JH, Bent R, Warren KE. Predicting outcome of children with diffuse intrinsic pontine gliomas using multiparametric imaging. *Neuro-Oncology*. 2011;**13**(8):904-909
- [26] van Dongen GA, Poot AJ, Vugts DJ. PET imaging with radiolabeled antibodies and tyrosine kinase inhibitors: Immuno-PET and TKI-PET. *Tumour Biology*. 2012;**33**(3):607-615
- [27] Jansen MH, Veldhuijzen van Zanten SEM, van Vuurden DG, Huisman MC, Vugts DJ, Hoekstra OS, et al. Molecular drug imaging: (89)Zr-Bevacizumab PET in children with diffuse intrinsic Pontine Glioma. *Journal of Nuclear Medicine*. 2017;**58**(5):711-716
- [28] El-Khouly FE, van Vuurden DG, Stroink T, Hulleman E, Kaspers GJL, Hendrikse NH, et al. Effective drug delivery in diffuse intrinsic Pontine Glioma: A theoretical model to identify potential candidates. *Frontiers in Oncology*. 2017;**7**:254
- [29] Mantravadi RV, Phatak R, Bellur S, Liebner EJ, Haas R. Brain stem gliomas: An autopsy study of 25 cases. *Cancer*. 1982;**49**(6):1294-1296
- [30] Grigsby PW, Garcia DM, Ghiselli R. Analysis of autopsy findings in patients treated with irradiation for thalamic and brain stem tumors. *American Journal of Clinical Oncology*. 1989;**12**(3):255-258
- [31] Sethi R, Allen J, Donahue B, Karajannis M, Gardner S, Wisoff J, et al. Prospective neuraxis MRI surveillance reveals a high risk of leptomeningeal dissemination in diffuse intrinsic pontine glioma. *Journal of Neuro-Oncology*. 2011;**102**(1):121-127

- [32] Gururangan S, McLaughlin CA, Brashears J, Watral MA, Provenzale J, Coleman RE, et al. Incidence and patterns of neuraxis metastases in children with diffuse pontine glioma. *Journal of Neuro-Oncology*. 2006;**77**(2):207-212
- [33] Donahue B, Allen J, Siffert J, Rosovsky M, Pinto R. Patterns of recurrence in brain stem gliomas: Evidence for craniospinal dissemination. *International Journal of Radiation Oncology, Biology, Physics*. 1998;**40**(3):677-680
- [34] Maria BL, Rehder K, Eskin TA, Hamed LM, Fennell EB, Quisling RG, et al. Brainstem glioma: I. Pathology, clinical features, and therapy. *Journal of Child Neurology*. 1993;**8**(2):112-128
- [35] Monje M, Mitra SS, Freret ME, Raveh TB, Kim J, Masek M, et al. Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**(11):4453-4448
- [36] Schumacher M, Schulte-Monting J, Stoeter P, Warmuth-Metz M, Solymosi L. Magnetic resonance imaging compared with biopsy in the diagnosis of brainstem diseases of childhood: A multicenter review. *Journal of Neurosurgery*. 2007;**106**(2 Suppl):111-119
- [37] Edwards MS, Wara WM, Urtasun RC, Prados M, Levin VA, Fulton D, et al. Hyperfractionated radiation therapy for brain-stem glioma: A phase I-II trial. *Journal of Neurosurgery*. 1989;**70**(5):691-700
- [38] Laigle-Donadey F, Doz F, Delattre JY. Brainstem gliomas in children and adults. *Current Opinion in Oncology*. 2008;**20**(6):662-667
- [39] Bugiani M, Veldhuijzen van Zanten SEM, Caretti V, Schellen P, Aronica E, Noske DP, et al. Deceptive morphologic and epigenetic heterogeneity in diffuse intrinsic pontine glioma. *Oncotarget*. 2017;**8**(36):60447-60452
- [40] Hawkins C, Ellison DW, Sturm D. Diffuse midline glioma, H3K27M-mutant. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, editors. *WHO Classification of Tumours of the Central Nervous System*. Revised 4th ed. Lyon: International Agency for Research on Cancer (IARC); 2016. pp. 57-59
- [41] Jenkin RD, Boesel C, Ertel I, Evans A, Hittle R, Ortega J, et al. Brain-stem tumors in childhood: A prospective randomized trial of irradiation with and without adjuvant CCNU, VCR, and prednisone. A report of the Childrens Cancer Study Group. *Journal of Neurosurgery*. 1987;**66**(2):227-233
- [42] Epstein F, Wisoff JH. Intrinsic brainstem tumors in childhood: Surgical indications. *Journal of Neuro-Oncology*. 1988;**6**(4):309-317
- [43] Albright AL, Packer RJ, Zimmerman R, Rorke LB, Boyett J, Hammond GD. Magnetic resonance scans should replace biopsies for the diagnosis of diffuse brain stem gliomas: A report from the Children's Cancer Group. *Neurosurgery*. 1993;**33**(6):1026-1029 discussion 9-30
- [44] Cartmill M, Punt J. Diffuse brain stem glioma. A review of stereotactic biopsies. *Child's Nervous System*. 1999;**15**(5):235-237 discussion 8

- [45] Grill J, Puget S, Andreiuolo F, Philippe C, MacConaill L, Kieran MW. Critical oncogenic mutations in newly diagnosed pediatric diffuse intrinsic pontine glioma. *Pediatric Blood & Cancer*. 2012;**58**(4):489-491
- [46] Roujeau T, Machado G, Garnett MR, Miquel C, Puget S, Geoerger B, et al. Stereotactic biopsy of diffuse pontine lesions in children. *Journal of Neurosurgery*. 2007;**107**(1 Suppl):1-4
- [47] Zarghooni M, Bartels U, Lee E, Buczkowicz P, Morrison A, Huang A, et al. Whole-genome profiling of pediatric diffuse intrinsic pontine gliomas highlights platelet-derived growth factor receptor alpha and poly (ADP-ribose) polymerase as potential therapeutic targets. *Journal of Clinical Oncology*. 2010;**28**(8):1337-1344
- [48] Paugh BS, Broniscer A, Qu C, Miller CP, Zhang J, Tatevossian RG, et al. Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and cell-cycle regulatory genes in diffuse intrinsic pontine glioma. *Journal of Clinical Oncology*. 2011;**29**(30):3999-4006
- [49] Geoerger B, Hargrave D, Thomas F, Ndiaye A, Frappaz D, Andreiuolo F, et al. Innovative therapies for children with cancer pediatric phase I study of erlotinib in brainstem glioma and relapsing/refractory brain tumors. *Neuro-Oncology*. 2011;**13**(1):109-118
- [50] Ogiwara H, Morota N. The efficacy of a biopsy of intrinsic brainstem lesions for decision making of the treatments. *Child's Nervous System*. 2013;**29**(5):833-837
- [51] MacDonald TJ. Diffuse intrinsic pontine glioma (DIPG): Time to biopsy again? *Pediatric Blood & Cancer*. 2012;**58**(4):487-488
- [52] Walker DA, Liu J, Kieran M, Jabado N, Picton S, Packer R, et al. A multi-disciplinary consensus statement concerning surgical approaches to low-grade, high-grade astrocytomas and diffuse intrinsic pontine gliomas in childhood (CPN Paris 2011) using the Delphi method. *Neuro-Oncology*. 2013;**15**(4):462-468
- [53] Buczkowicz P, Bartels U, Bouffet E, Becher O, Hawkins C. Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: Diagnostic and therapeutic implications. *Acta Neuropathologica*. 2014;**128**(4):573-581
- [54] Hoffman LM, DeWire M, Ryall S, Buczkowicz P, Leach J, Miles L, et al. Spatial genomic heterogeneity in diffuse intrinsic pontine and midline high-grade glioma: Implications for diagnostic biopsy and targeted therapeutics. *Acta Neuropathologica Communications*. 2016;**4**:1
- [55] Janssens GO, Gidding CE, Van Lindert EJ, Oldenburger FR, Erasmus CE, Schouten-Meeteren AY, et al. The role of hypofractionation radiotherapy for diffuse intrinsic brainstem glioma in children: A pilot study. *International Journal of Radiation Oncology, Biology, Physics*. 2009;**73**(3):722-726
- [56] Negretti L, Bouchireb K, Levy-Piedbois C, Habrand JL, Dhermain F, Kalifa C, et al. Hypofractionated radiotherapy in the treatment of diffuse intrinsic pontine glioma in children: A single institution's experience. *Journal of Neuro-Oncology*. 2011;**104**(3):773-777

- [57] Wolff JE, Driever PH, Erdlenbruch B, Kortmann RD, Rutkowski S, Pietsch T, et al. Intensive chemotherapy improves survival in pediatric high-grade glioma after gross total resection: Results of the HIT-GBM-C protocol. *Cancer*. 2010;**116**(3):705-712
- [58] Massimino M, Spreafico F, Biassoni V, Simonetti F, Riva D, Trecate G, et al. Diffuse pontine gliomas in children: Changing strategies, changing results? A mono-institutional 20-year experience. *Journal of Neuro-Oncology*. 2008;**87**(3):355-361
- [59] Wolff JE, Rytting ME, Vats TS, Zage PE, Ater JL, Woo S, et al. Treatment of recurrent diffuse intrinsic pontine glioma: The MD Anderson Cancer Center experience. *Journal of Neuro-Oncology*. 2012;**106**(2):391-397
- [60] Frappaz D, Schell M, Thiesse P, Marec-Berard P, Mottolese C, Perol D, et al. Preradiation chemotherapy may improve survival in pediatric diffuse intrinsic brainstem gliomas: Final results of BSG 98 prospective trial. *Neuro-Oncology*. 2008;**10**(4):599-607
- [61] Buczkowicz P, Hawkins C. Pathology, molecular genetics, and epigenetics of diffuse intrinsic pontine glioma. *Frontiers in Oncology*. 2015;**5**:147
- [62] Jalali R, Raut N, Arora B, Gupta T, Dutta D, Munshi A, et al. Prospective evaluation of radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *International Journal of Radiation Oncology, Biology, Physics*. 2010;**77**(1):113-118
- [63] Chassot A, Canale S, Varlet P, Puget S, Roujeau T, Negretti L, et al. Radiotherapy with concurrent and adjuvant temozolomide in children with newly diagnosed diffuse intrinsic pontine glioma. *Journal of Neuro-Oncology*. 2012;**106**(2):399-407
- [64] Jakacki RI, Cohen KJ, Buxton A, Krailo MD, Burger PC, Rosenblum MK, et al. Phase 2 study of concurrent radiotherapy and temozolomide followed by temozolomide and lomustine in the treatment of children with high-grade glioma: A report of the Children's Oncology Group ACNS0423 study. *Neuro-Oncology*. 2016;**18**(10):1442-1450
- [65] Agnihotri S, Burrell K, Buczkowicz P, Remke M, Golbourn B, Chornenkyy Y, et al. ATM regulates 3-methylpurine-DNA glycosylase and promotes therapeutic resistance to alkylating agents. *Cancer Discovery*. 2014;**4**(10):1198-1213
- [66] Molecularly Determined Treatment of Diffuse Intrinsic Pontine Gliomas (DIPG)
- [67] Bernier-Chastagner V, Grill J, Doz F, Bracard S, Gentet JC, Marie-Cardine A, et al. Topotecan as a radiosensitizer in the treatment of children with malignant diffuse brainstem gliomas: Results of a French Society of Paediatric Oncology Phase II study. *Cancer*. 2005;**104**(12):2792-2797
- [68] Kivivuori SM, Riikonen P, Valanne L, Lonnqvist T, Saarinen-Pihkala UM. Antiangiogenic combination therapy after local radiotherapy with topotecan radiosensitizer improved quality of life for children with inoperable brainstem gliomas. *Acta Paediatrica*. 2011;**100**(1):134-138
- [69] Bradley KA, Zhou T, McNall-Knapp RY, Jakacki RI, Levy AS, Vezina G, et al. Motexafin-gadolinium and involved field radiation therapy for intrinsic pontine glioma of

childhood: A children's oncology group phase 2 study. *International Journal of Radiation Oncology, Biology, Physics*. 2013;**85**(1):e55-e60

- [70] Aquino-Parsons C, Hukin J, Green A. Concurrent carbogen and radiation therapy in children with high-risk brainstem gliomas. *Pediatric Blood & Cancer*. 2008;**50**(2):397-399
- [71] Broniscer A, Baker JN, Baker SJ, Chi SN, Geyer JR, Morris EB, et al. Prospective collection of tissue samples at autopsy in children with diffuse intrinsic pontine glioma. *Cancer*. 2010;**116**(19):4632-4467
- [72] Kambhampati M, Perez JP, Yadavilli S, Saratsis AM, Hill AD, Ho CY, et al. A standardized autopsy procurement allows for the comprehensive study of DIPG biology. *Oncotarget*. 2015;**6**(14):12740-12747
- [73] Caretti V, Hiddingh L, Lagerweij T, Schellen P, Koken PW, Hulleman E, et al. WEE1 kinase inhibition enhances the radiation response of diffuse intrinsic pontine gliomas. *Molecular Cancer Therapeutics*. 2013;**12**(2):141-150
- [74] Sewing AC, Caretti V, Lagerweij T, Schellen P, Jansen MH, van Vuurden DG, et al. Convection enhanced delivery of carmustine to the murine brainstem: A feasibility study. *Journal of Neuroscience Methods*. 2014;**238**:88-94
- [75] Misuraca KL, Barton KL, Chung A, Diaz AK, Conway SJ, Corcoran DL, et al. Pax3 expression enhances PDGF-B-induced brainstem gliomagenesis and characterizes a subset of brainstem glioma. *Acta Neuropathologica Communications*. 2014;**2**:134
- [76] Ballester LY, Wang Z, Shandilya S, Miettinen M, Burger PC, Eberhart CG, et al. Morphologic characteristics and immunohistochemical profile of diffuse intrinsic pontine gliomas. *The American Journal of Surgical Pathology*. 2013;**37**(9):1357-1364
- [77] Jones C, Baker SJ. Unique genetic and epigenetic mechanisms driving paediatric diffuse high-grade glioma. *Nature Reviews. Cancer*. 2014;**14**(10):651-661
- [78] Kepert JF, Toth KF, Caudron M, Mucke N, Langowski J, Rippe K. Conformation of reconstituted mononucleosomes and effect of linker histone H1 binding studied by scanning force microscopy. *Biophysical Journal*. 2003;**85**(6):4012-4022
- [79] Marino-Ramirez L, Kann MG, Shoemaker BA, Landsman D. Histone structure and nucleosome stability. *Expert Review of Proteomics*. 2005;**2**(5):719-729
- [80] Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell*. 2004;**116**(1):51-61
- [81] Wells D, Hoffman D, Kedes L. Unusual structure, evolutionary conservation of non-coding sequences and numerous pseudogenes characterize the human H3.3 histone multigene family. *Nucleic Acids Research*. 1987;**15**(7):2871-2889
- [82] Albig W, Bramlage B, Gruber K, Klobeck HG, Kunz J, Doenecke D. The human replacement histone H3.3B gene (H3F3B). *Genomics*. 1995;**30**(2):264-272
- [83] Yuen BT, Knoepfler PS. Histone H3.3 mutations: A variant path to cancer. *Cancer Cell*. 2013;**24**(5):567-574

- [84] Wu G, Diaz AK, Paugh BS, Rankin SL, Ju B, Li Y, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nature Genetics*. 2014;**46**(5):444-450
- [85] Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfort J, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nature Genetics*. 2012;**44**(3):251-253
- [86] Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor KR, et al. Integrated molecular meta-analysis of 1,000 pediatric high-grade and diffuse intrinsic pontine glioma. *Cancer Cell*. 2017;**32**(4):520-537 e5
- [87] Castel D, Philippe C, Calmon R, Le Dret L, Truffaux N, Boddaert N, et al. Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathologica*. 2015;**130**(6):815-827
- [88] Schwartzenuber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*. 2012;**482**(7384):226-231
- [89] Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell*. 2012;**22**(4):425-437
- [90] Lewis PW, Muller MM, Koletsky MS, Cordero F, Lin S, Banaszynski LA, et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science*. 2013;**340**(6134):857-861
- [91] Bender S, Tang Y, Lindroth AM, Hovestadt V, Jones DT, Kool M, et al. Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. *Cancer Cell*. 2013;**24**(5):660-672
- [92] Saratsis AM, Kambhampati M, Snyder K, Yadavilli S, Devaney JM, Harmon B, et al. Comparative multidimensional molecular analyses of pediatric diffuse intrinsic pontine glioma reveals distinct molecular subtypes. *Acta Neuropathologica*. 2014;**127**(6):881-895
- [93] Chan KM, Fang D, Gan H, Hashizume R, Yu C, Schroeder M, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes & Development*. 2013;**27**(9):985-990
- [94] Venneti S, Garimella MT, Sullivan LM, Martinez D, Huse JT, Heguy A, et al. Evaluation of histone 3 lysine 27 trimethylation (H3K27me3) and enhancer of Zest 2 (EZH2) in pediatric glial and glioneuronal tumors shows decreased H3K27me3 in H3F3A K27M mutant glioblastomas. *Brain Pathology*. 2013;**23**(5):558-564
- [95] Funato K, Major T, Lewis PW, Allis CD, Tabar V. Use of human embryonic stem cells to model pediatric gliomas with H3.3K27M histone mutation. *Science*. 2014;**346**(6216):1529-1533

- [96] Nikbakht H, Panditharatna E, Mikael LG, Li R, Gayden T, Osmond M, et al. Spatial and temporal homogeneity of driver mutations in diffuse intrinsic pontine glioma. *Nature Communications*. 2016;**7**:11185
- [97] Swartling FJ, Savov V, Persson AI, Chen J, Hackett CS, Northcott PA, et al. Distinct neural stem cell populations give rise to disparate brain tumors in response to N-MYC. *Cancer Cell*. 2012;**21**(5):601-613
- [98] Bjerke L, Mackay A, Nandhabalan M, Burford A, Jury A, Popov S, et al. Histone H3.3 mutations drive pediatric glioblastoma through upregulation of MYCN. *Cancer Discovery*. 2013;**3**(5):512-519
- [99] Khuong-Quang DA, Buczkowicz P, Rakopoulos P, Liu XY, Fontebasso AM, Bouffet E, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathologica*. 2012;**124**(3):439-447
- [100] Jones DT, Hutter B, Jager N, Korshunov A, Kool M, Warnatz HJ, et al. Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. *Nature Genetics*. 2013;**45**(8):927-932
- [101] Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001;**293**(5532):1074-1080
- [102] Plass C, Pfister SM, Lindroth AM, Bogatyrova O, Claus R, Lichter P. Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer. *Nature Reviews. Genetics*. 2013;**14**(11):765-780
- [103] Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 2011;**469**(7330):343-349
- [104] Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, et al. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Molecular Cell*. 2010;**38**(3):452-464
- [105] Kumar SS, Sengupta S, Lee K, Hura N, Fuller C, DeWire M, et al. BMI-1 is a potential therapeutic target in diffuse intrinsic pontine glioma. *Oncotarget*. 2017;**8**(38):62962-62975
- [106] Louis DN, Rubio MP, Correa KM, Gusella JF, von Deimling A. Molecular genetics of pediatric brain stem gliomas. Application of PCR techniques to small and archival brain tumor specimens. *Journal of Neuropathology and Experimental Neurology*. 1993;**52**(5):507-515
- [107] Bax DA, Mackay A, Little SE, Carvalho D, Viana-Pereira M, Tamber N, et al. A distinct spectrum of copy number aberrations in pediatric high-grade gliomas. *Clinical Cancer Research*. 2010;**16**(13):3368-3377
- [108] Qu HQ, Jacob K, Fatet S, Ge B, Barnett D, Delattre O, et al. Genome-wide profiling using single-nucleotide polymorphism arrays identifies novel chromosomal imbalances in pediatric glioblastomas. *Neuro-Oncology*. 2010;**12**(2):153-163

- [109] Barrow J, Adamowicz-Brice M, Cartmill M, MacArthur D, Lowe J, Robson K, et al. Homozygous loss of ADAM3A revealed by genome-wide analysis of pediatric high-grade glioma and diffuse intrinsic pontine gliomas. *Neuro-Oncology*. 2011;**13**(2):212-222
- [110] Kyritsis AP, Bondy ML, Rao JS, Sioka C. Inherited predisposition to glioma. *Neuro-Oncology*. 2010;**12**(1):104-113
- [111] Taylor KR, Mackay A, Truffaux N, Butterfield Y, Morozova O, Philippe C, et al. Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nature Genetics*. 2014;**46**(5):457-461
- [112] Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, Nikbakht H, Gerges N, Fiset PO, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nature Genetics*. 2014;**46**(5):462-466
- [113] Rigueur D, Brugger S, Anbarchian T, Kim JK, Lee Y, Lyons KM. The type I BMP receptor ACVR1/ALK2 is required for chondrogenesis during development. *Journal of Bone and Mineral Research*. 2015;**30**(4):733-741
- [114] Han HJ, Jain P, Resnick AC. Shared ACVR1 mutations in FOP and DIPG: Opportunities and challenges in extending biological and clinical implications across rare diseases. *Bone*. 2018;**109**:91-100
- [115] Buczkowicz P, Hoeman C, Rakopoulos P, Pajovic S, Letourneau L, Dzamba M, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nature Genetics*. 2014;**46**(5):451-456
- [116] Zadeh G, Aldape K. ACVR1 mutations and the genomic landscape of pediatric diffuse glioma. *Nature Genetics*. 2014;**46**(5):421-422
- [117] Regad T. Targeting RTK signaling pathways in Cancer. *Cancers (Basel)*. 2015;**7**(3):1758-1784
- [118] Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2010;**141**(7):1117-1134
- [119] Li E, Hristova K. Role of receptor tyrosine kinase transmembrane domains in cell signaling and human pathologies. *Biochemistry*. 2006;**45**(20):6241-6251
- [120] Hubbard SR, Miller WT. Receptor tyrosine kinases: Mechanisms of activation and signaling. *Current Opinion in Cell Biology*. 2007;**19**(2):117-123
- [121] Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2000;**103**(2):211-225
- [122] Paugh BS, Qu C, Jones C, Liu Z, Adamowicz-Brice M, Zhang J, et al. Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *Journal of Clinical Oncology*. 2010;**28**(18):3061-3068
- [123] Puget S, Philippe C, Bax DA, Job B, Varlet P, Junier MP, et al. Mesenchymal transition and PDGFRA amplification/mutation are key distinct oncogenic events in pediatric diffuse intrinsic pontine gliomas. *PLoS One*. 2012;**7**(2):e30313
- [124] Paugh BS, Zhu X, Qu C, Endersby R, Diaz AK, Zhang J, et al. Novel oncogenic PDGFRA mutations in pediatric high-grade gliomas. *Cancer Research*. 2013;**73**(20):6219-6629

- [125] Becher OJ, Hambardzumyan D, Walker TR, Helmy K, Nazarian J, Albrecht S, et al. Preclinical evaluation of radiation and perifosine in a genetically and histologically accurate model of brainstem glioma. *Cancer Research*. 2010;**70**(6):2548-2557
- [126] Masui K, Suzuki SO, Torisu R, Goldman JE, Canoll P, Iwaki T. Glial progenitors in the brainstem give rise to malignant gliomas by platelet-derived growth factor stimulation. *Glia*. 2010;**58**(9):1050-1065
- [127] Corless CL, Schroeder A, Griffith D, Town A, McGreevey L, Harrell P, et al. PDGFRA mutations in gastrointestinal stromal tumors: Frequency, spectrum and in vitro sensitivity to imatinib. *Journal of Clinical Oncology*. 2005;**23**(23):5357-5364
- [128] Guglielmi L, Cinnella C, Nardella M, Maresca G, Valentini A, Mercanti D, et al. MYCN gene expression is required for the onset of the differentiation programme in neuroblastoma cells. *Cell Death & Disease*. 2014;**5**:e1081
- [129] Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature*. 2000;**408**(6810):307-310
- [130] Jin S, Levine AJ. The p53 functional circuit. *Journal of Cell Science*. 2001;**114**(Pt 23):4139-4140
- [131] Harris SL, Levine AJ. The p53 pathway: Positive and negative feedback loops. *Oncogene*. 2005;**24**(17):2899-2908
- [132] Aubrey BJ, Strasser A, Kelly GL. Tumor-suppressor functions of the TP53 pathway. *Cold Spring Harbor Perspectives in Medicine*. 2016;**6**(5):1-16
- [133] Zhang S, Feng X, Koga H, Ichikawa T, Abe S, Kumanishi T. p53 gene mutations in pontine gliomas of juvenile onset. *Biochemical and Biophysical Research Communications*. 1993;**196**(2):851-857
- [134] Zhang L, Chen LH, Wan H, Yang R, Wang Z, Feng J, et al. Exome sequencing identifies somatic gain-of-function PPM1D mutations in brainstem gliomas. *Nature Genetics*. 2014;**46**(7):726-730
- [135] Richter M, Dayaram T, Gilmartin AG, Ganji G, Pemmasani SK, Van Der Key H, et al. WIP1 phosphatase as a potential therapeutic target in neuroblastoma. *PLoS One*. 2015;**10**(2):e0115635
- [136] Zajkowicz A, Butkiewicz D, Drosik A, Giglok M, Suwinski R, Rusin M. Truncating mutations of PPM1D are found in blood DNA samples of lung cancer patients. *British Journal of Cancer*. 2015;**112**(6):1114-1120
- [137] Kleiblova P, Shaltiel IA, Benada J, Sevcik J, Pechackova S, Pohlreich P, et al. Gain-of-function mutations of PPM1D/Wip1 impair the p53-dependent G1 checkpoint. *The Journal of Cell Biology*. 2013;**201**(4):511-521
- [138] Duronio RJ, Xiong Y. Signaling pathways that control cell proliferation. *Cold Spring Harbor Perspectives in Biology*. 2013;**5**(3):a008904
- [139] Warren KE, Killian K, Suuriniemi M, Wang Y, Quezado M, Meltzer PS. Genomic aberrations in pediatric diffuse intrinsic pontine gliomas. *Neuro-Oncology*. 2012;**14**(3):326-332

- [140] Dar AA, Goff LW, Majid S, Berlin J, El-Rifai W. Aurora kinase inhibitors--rising stars in cancer therapeutics? *Molecular Cancer Therapeutics*. 2010;**9**(2):268-278
- [141] Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, et al. The small molecule Hesperadin reveals a role for aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *The Journal of Cell Biology*. 2003;**161**(2):281-294
- [142] Buczkowicz P, Zarghooni M, Bartels U, Morrison A, Misuraca KL, Chan T, et al. Aurora kinase B is a potential therapeutic target in pediatric diffuse intrinsic pontine glioma. *Brain Pathology*. 2013;**23**(3):244-253
- [143] Sung T, Miller DC, Hayes RL, Alonso M, Yee H, Newcomb EW. Preferential inactivation of the p53 tumor suppressor pathway and lack of EGFR amplification distinguish de novo high grade pediatric astrocytomas from de novo adult astrocytomas. *Brain Pathology*. 2000;**10**(2):249-259
- [144] Ratnam K, Low JA. Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. *Clinical Cancer Research*. 2007;**13**(5):1383-1388
- [145] Rickert CH, Strater R, Kaatsch P, Wassmann H, Jurgens H, Dockhorn-Dworniczak B, et al. Pediatric high-grade astrocytomas show chromosomal imbalances distinct from adult cases. *The American Journal of Pathology*. 2001;**158**(4):1525-1532
- [146] Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, Liu D, et al. B7-H3: A costimulatory molecule for T cell activation and IFN-gamma production. *Nature Immunology*. 2001;**2**(3):269-274
- [147] Zhou Z, Luther N, Ibrahim GM, Hawkins C, Vibhakkar R, Handler MH, et al. B7-H3, a potential therapeutic target, is expressed in diffuse intrinsic pontine glioma. *Journal of Neuro-Oncology*. 2013;**111**(3):257-264
- [148] Modak S, Kramer K, Gultekin SH, Guo HF, Cheung NK. Monoclonal antibody 8H9 targets a novel cell surface antigen expressed by a wide spectrum of human solid tumors. *Cancer Research*. 2001;**61**(10):4048-4054
- [149] Xu H, Cheung IY, Guo HF, Cheung NK. MicroRNA miR-29 modulates expression of immunoinhibitory molecule B7-H3: Potential implications for immune based therapy of human solid tumors. *Cancer Research*. 2009;**69**(15):6275-6281
- [150] Kramer K, Kushner BH, Modak S, Pandit-Taskar N, Smith-Jones P, Zanzonico P, et al. Compartmental intrathecal radioimmunotherapy: Results for treatment for metastatic CNS neuroblastoma. *Journal of Neuro-Oncology*. 2010;**97**(3):409-418
- [151] Venkatesh HS, Johung TB, Caretti V, Noll A, Tang Y, Nagaraja S, et al. Neuronal activity promotes glioma growth through neuroligin-3 secretion. *Cell*. 2015;**161**(4):803-816
- [152] Venkatesh HS, Tam LT, Woo PJ, Lennon J, Nagaraja S, Gillespie SM, et al. Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. *Nature*. 2017;**549**(7673):533-537

- [153] Qin EY, Cooper DD, Abbott KL, Lennon J, Nagaraja S, Mackay A, et al. Neural precursor-derived pleiotrophin mediates subventricular zone invasion by glioma. *Cell*. 2017;**170**(5):845-859 e19
- [154] Truffaux N, Philippe C, Paulsson J, Andreiuolo F, Guerrini-Rousseau L, Cornilleau G, et al. Preclinical evaluation of dasatinib alone and in combination with cabozantinib for the treatment of diffuse intrinsic pontine glioma. *Neuro-Oncology*. 2015;**17**(7):953-964
- [155] Pollack IF, Jakacki RI, Blaney SM, Hancock ML, Kieran MW, Phillips P, et al. Phase I trial of imatinib in children with newly diagnosed brainstem and recurrent malignant gliomas: A pediatric brain tumor consortium report. *Neuro-Oncology*. 2007;**9**(2):145-160
- [156] Broniscer A, Baker SD, Wetmore C, Pai Panandiker AS, Huang J, Davidoff AM, et al. Phase I trial, pharmacokinetics, and pharmacodynamics of vandetanib and dasatinib in children with newly diagnosed diffuse intrinsic pontine glioma. *Clinical Cancer Research*. 2013;**19**(11):3050-3058
- [157] Broniscer A, Baker JN, Tagen M, Onar-Thomas A, Gilbertson RJ, Davidoff AM, et al. Phase I study of vandetanib during and after radiotherapy in children with diffuse intrinsic pontine glioma. *Journal of Clinical Oncology*. 2010;**28**(31):4762-4768
- [158] Geyer JR, Stewart CF, Kocak M, Broniscer A, Phillips P, Douglas JG, et al. A phase I and biology study of gefitinib and radiation in children with newly diagnosed brain stem gliomas or supratentorial malignant gliomas. *European Journal of Cancer*. 2010;**46**(18):3287-3293
- [159] Holmes D. PI3K pathway inhibitors approach junction. *Nature Reviews. Drug Discovery*. 2011;**10**(8):563-564
- [160] Becher OJ, Gilheeney SW, Khakoo Y, Lyden DC, Haque S, De Braganca, KC, et al. A phase I study of perifosine with temsirolimus for recurrent pediatric solid tumors. *Pediatric Blood & Cancer*. 2017;**64**:7
- [161] Wetmore C, Daryani VM, Billups CA, Boyett JM, Leary S, Tanos R, et al. Phase II evaluation of sunitinib in the treatment of recurrent or refractory high-grade glioma or ependymoma in children: A children's Oncology Group Study ACNS1021. *Cancer Medicine*. 2016;**5**(7):1416-1424
- [162] Fouladi M, Nicholson HS, Zhou T, Laningham F, Helton KJ, Holmes E, et al. A phase II study of the farnesyl transferase inhibitor, tipifarnib, in children with recurrent or progressive high-grade glioma, medulloblastoma/primitive neuroectodermal tumor, or brainstem glioma: A Children's Oncology Group study. *Cancer*. 2007;**110**(11):2535-2541
- [163] Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication. Available from: <https://ClinicalTrials.gov/show/NCT02233049>
- [164] Hennika T, Hu G, Olaciregui NG, Barton KL, Ehteda A, Chitranjan A, et al. Pre-clinical study of panobinostat in xenograft and genetically engineered murine diffuse intrinsic pontine glioma models. *PLoS One*. 2017;**12**(1):e0169485

- [165] New M, Olzscha H, Thangue L, NB. HDAC inhibitor-based therapies: Can we interpret the code? *Molecular Oncology*. 2012;**6**(6):637-656
- [166] Hashizume R. Epigenetic targeted therapy for diffuse intrinsic pontine glioma. *Neurologia Medico-Chirurgica (Tokyo)*. 2017;**57**(7):331-342
- [167] Anne M, Sammartino D, Barginear MF, Budman D. Profile of panobinostat and its potential for treatment in solid tumors: An update. *OncoTargets and Therapy*. 2013;**6**:1613-1624
- [168] Grasso CS, Tang Y, Truffaux N, Berlow NE, Liu L, Debily MA, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nature Medicine*. 2015;**21**(7):827
- [169] Fouladi M, Park JR, Stewart CF, Gilbertson RJ, Schaiquevich P, Sun J, et al. Pediatric phase I trial and pharmacokinetic study of vorinostat: A Children's Oncology Group phase I consortium report. *Journal of Clinical Oncology*. 2010;**28**(22):3623-3629
- [170] Study of Suberoylanilide Hydroxamic Acid (SAHA) With Temsirolimus in Children With Diffuse Intrinsic Pontine Glioma (DIPG). Available from: <https://ClinicalTrials.gov/show/NCT02420613>
- [171] Trial of Panobinostat in children with diffuse intrinsic Pontine Glioma. Available from: <https://ClinicalTrials.gov/show/NCT02717455>
- [172] Barton KL, Misuraca K, Cordero F, Dobrikova E, Min HD, Gromeier M, et al. PD-0332991, a CDK4/6 inhibitor, significantly prolongs survival in a genetically engineered mouse model of brainstem glioma. *PLoS One*. 2013;**8**(10):e77639
- [173] Goodwin CR, Xu R, Iyer R, Sankey EW, Liu A, Abu-Bonsrah N, et al. Local delivery methods of therapeutic agents in the treatment of diffuse intrinsic brainstem gliomas. *Clinical Neurology and Neurosurgery*. 2016;**142**:120-127
- [174] Pollack IF, Jakacki RI, Butterfield LH, Hamilton RL, Panigrahy A, Potter DM, et al. Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant brainstem and nonbrainstem gliomas. *Journal of Clinical Oncology*. 2014;**32**(19):2050-2058
- [175] Okada H, Low KL, Kohanbash G, McDonald HA, Hamilton RL, Pollack IF. Expression of glioma-associated antigens in pediatric brain stem and non-brain stem gliomas. *Journal of Neuro-Oncology*. 2008;**88**(3):245-250
- [176] Joshi BH, Puri RA, Leland P, Varricchio F, Gupta G, Kocak M, et al. Identification of interleukin-13 receptor alpha2 chain overexpression in situ in high-grade diffusely infiltrative pediatric brainstem glioma. *Neuro-Oncology*. 2008;**10**(3):265-274
- [177] Benedetti S, Pirola B, Pollo B, Magrassi L, Bruzzone MG, Rigamonti D, et al. Gene therapy of experimental brain tumors using neural progenitor cells. *Nature Medicine*. 2000;**6**(4):447-450
- [178] Kim SK, Kim SU, Park IH, Bang JH, Aboody KS, Wang KC, et al. Human neural stem cells target experimental intracranial medulloblastoma and deliver a therapeutic gene leading to tumor regression. *Clinical Cancer Research*. 2006;**12**(18):5550-5556

- [179] Ehtesham M, Stevenson CB, Thompson RC. Stem cell therapies for malignant glioma. *Neurosurgical Focus*. 2005;**19**(3):E5
- [180] Hamada H, Kobune M, Nakamura K, Kawano Y, Kato K, Honmou O, et al. Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy. *Cancer Science*. 2005;**96**(3):149-156
- [181] Lee DH, Ahn Y, Kim SU, Wang KC, Cho BK, Phi JH, et al. Targeting rat brainstem glioma using human neural stem cells and human mesenchymal stem cells. *Clinical Cancer Research*. 2009;**15**(15):4925-4934
- [182] Muldoon LL, Soussain C, Jahnke K, Johanson C, Siegal T, Smith QR, et al. Chemotherapy delivery issues in central nervous system malignancy: A reality check. *Journal of Clinical Oncology*. 2007;**25**(16):2295-2305
- [183] Subashi E, Cordero FJ, Halvorson KG, Qi Y, Nours JC, Becher OJ, et al. Tumor location, but not H3.3K27M, significantly influences the blood-brain-barrier permeability in a genetic mouse model of pediatric high-grade glioma. *Journal of Neuro-Oncology*. 2016;**126**(2):243-251
- [184] Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. *Proceedings of the National Academy of Sciences of the United States of America*. 1994;**91**(6):2076-2080
- [185] Zhou Z, Singh R, Souweidane MM. Convection-enhanced delivery for diffuse intrinsic pontine glioma treatment. *Current Neuropharmacology*. 2017;**15**(1):116-128
- [186] Debinski W, Tatter SB. Convection-enhanced delivery for the treatment of brain tumors. *Expert Review of Neurotherapeutics*. 2009;**9**(10):1519-1527
- [187] Groothuis DR. The blood-brain and blood-tumor barriers: A review of strategies for increasing drug delivery. *Neuro-Oncology*. 2000;**2**(1):45-59
- [188] Occhiogrosso G, Edgar MA, Sandberg DI, Souweidane MM. Prolonged convection-enhanced delivery into the rat brainstem. *Neurosurgery*. 2003;**52**(2):388-393 discussion 93-4
- [189] Souweidane MM, Occhiogrosso G, Mark EB, Edgar MA, Dunkel IJ. Interstitial infusion of carmustine in the rat brain stem with systemic administration of O6-benzylguanine. *Journal of Neuro-Oncology*. 2004;**67**(3):319-326
- [190] Degen JW, Walbridge S, Vortmeyer AO, Oldfield EH, Lonser RR. Safety and efficacy of convection-enhanced delivery of gemcitabine or carboplatin in a malignant glioma model in rats. *Journal of Neurosurgery*. 2003;**99**(5):893-898
- [191] Wu Q, Guarnieri M, Tyler B, Clatterbuck RE, Liu Y, Carson BS. Section on tumors: Young Investigator Award: Local release of carboplatin via an Alzet mini-osmotic pump prolongs survival in a rat brainstem tumor model. *Clinical Neurosurgery*. 2004;**51**:332-339
- [192] Yoshimura J, Siu IM, Thomale UW, Jallo GI. The effects of temozolomide delivered by prolonged intracerebral microinfusion against the rat brainstem GBM allograft model. *Child's Nervous System*. 2012;**28**(5):707-713

- [193] Zhou Z, Ho SL, Singh R, Pisapia DJ, Souweidane MM. Toxicity evaluation of convection-enhanced delivery of small-molecule kinase inhibitors in naive mouse brainstem. *Child's Nervous System*. 2015;**31**(4):557-562
- [194] Kroin JS, Penn RD. Intracerebral chemotherapy: Chronic microinfusion of cisplatin. *Neurosurgery*. 1982;**10**(3):349-354
- [195] Murad GJ, Walbridge S, Morrison PF, Szerlip N, Butman JA, Oldfield EH, et al. Image-guided convection-enhanced delivery of gemcitabine to the brainstem. *Journal of Neurosurgery*. 2007;**106**(2):351-356
- [196] Anderson RC, Kennedy B, Yanes CL, Garvin J, Needle M, Canoll P, et al. Convection-enhanced delivery of topotecan into diffuse intrinsic brainstem tumors in children. *Journal of Neurosurgery. Pediatrics*. 2013;**11**(3):289-295
- [197] Singleton WGB, Barua NU, Morgan J, Bienemann AS, Killick-Cole CL, Asby DJ, et al. NS-21 multi-catheter intermittent convection-enhanced delivery of carboplatin as a treatment for diffuse intrinsic pontine glioma (DIPG): Pre-clinical rationale and early clinical experience. *Neuro-Oncology*. 2016;**18**(suppl_3):iii131-iii
- [198] Barua NU, Lowis SP, Woolley M, O'Sullivan S, Harrison R, Gill SS. Robot-guided convection-enhanced delivery of carboplatin for advanced brainstem glioma. *Acta Neurochirurgica*. 2013;**155**(8):1459-1165
- [199] Convection-Enhanced Delivery of 124I-8H9 for Patients With Non-Progressive Diffuse pontine gliomas Previously Treated With External Beam Radiation Therapy. Available from: <https://ClinicalTrials.gov/show/NCT01502917>
- [200] Lonser RR, Warren KE, Butman JA, Quezado Z, Robison RA, Walbridge S, et al. Real-time image-guided direct convective perfusion of intrinsic brainstem lesions. Technical note. *Journal of Neurosurgery*. 2007;**107**(1):190-197
- [201] MacKay JA, Deen DF, Szoka FC, J. Distribution in brain of liposomes after convection enhanced delivery; modulation by particle charge, particle diameter, and presence of steric coating. *Brain Research*. 2005;**1035**(2):139-153
- [202] MacDiarmid JA, Mugridge NB, Weiss JC, Phillips L, Burn AL, Paulin RP, et al. Bacterially derived 400 nm particles for encapsulation and cancer cell targeting of chemotherapeutics. *Cancer Cell*. 2007;**11**(5):431-445
- [203] MacDiarmid JA, Langova V, Bailey D, Pattison ST, Pattison SL, Christensen N, et al. Targeted doxorubicin delivery to brain tumors via minicells: Proof of principle using dogs with spontaneously occurring tumors as a model. *PLoS One*. 2016;**11**(4):e0151832
- [204] A Study of Intravenous EEDVsMit in Children With Recurrent Refractory Solid or CNS Tumours Expressing EGFR. Available from: <https://ClinicalTrials.gov/show/NCT02687386>