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Paediatric type diffuse high grade gliomas in the WHO CNS5 classification: What the pathologist needs to know?

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Abstract

Pediatric-type of diffuse high-grade gliomas (HGG) are classified as a distinct group in the current fifth edition of WHO classification. This group of high-grade tumors is no more called as glioblastoma (GBM), which has been reserved for adult isocitrate dehydrogenase (*IDH*)-wild type HGG. These tumors are uncommon as compared to embryonal tumors and low-grade gliomas (LGG). Pediatric-type of diffuse HGG biologically differs from their adult counterparts in that they are therapeutically less sensitive to alkylating chemotherapies. They comprise a heterogeneous group of molecularly defined tumors – predominantly histone gene altered, less common receptor tyrosine kinase (RTK)-mediated, and syndrome-associated. This review provides an overview of these uncommon tumors and discusses the diagnostic approach of this heterogeneous group of tumors.

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Full Text

Introduction

Brain tumors are the most common solid tumors in children and account for a majority of cancer mortality and morbidity. Gliomas are the most common primary central nervous system (CNS) tumors in children accounting for approximately 51.6% and 31.1% of all the tumors in the age group of 0–14 years and 15–19 years, respectively.[1] Gliomas have been traditionally classified into a low grade (LGG; WHO grade 1 and 2) and high grade (HGG; WHO grade 3 and 4) based upon the histomorphological features.[2] LGGs are relatively common in children whereas HGGs are frequent in adults. The expansive HGG in adult and pediatric age-group show apparent histological similarities but over the past few years, significant molecular differences have been established between the clinical and biological parameters of HGG in adults and children. Pediatric HGGs have shown frequent alterations in the histone genes causing epigenetic dysregulation, in contrast to the more known IDH mutations and epidermal growth factor receptor (EGFR) amplification in adult HGG.[3],[4] The identification of these key driver molecular alterations enabled by high-throughput molecular assays delivers robust distinctive biological and prognostic information. This compelling molecular evidence emphasizing the distinctive nature of pediatric HGG (despite histological similarities with the adult counterparts) formed the basis of delineation of pediatric type of HGG as a distinctive group of tumors in the current fifth edition of WHO classification of the central nervous system (WHO CNS5) tumors.[5],[6] The point to be remembered is that the underlying tumorigenic molecular mechanism characterized whether the HGG is of pediatric and adult type; it is not determined by the age of the patient. However, it has to be noted that the predominant HGG in the pediatric population shows molecular alteration typical of the pediatric type of HGG barring very few exceptions (especially in older children) which show the adult type of molecular alterations. Here, the authors aim to provide a comprehensive review of pediatric HGG along with a brief discussion of common histological features, molecular markers, and therapeutic perspectives.

Current Classification of Pediatric Type of HGG

Pediatric HGG though mechanisms and clinico-biological features are distinct, thus necessitating the need to identify them as a distinct group of tumors. Unlike in adults, HGG in children are de novo and do not progress from low-grade counterparts. The major differences have been summarized in [Table 1].{Table 1}

Subsequently, distinct genetic alterations were shown to be associated with location (midline/hemispheric and supra/infratentorial) and different pediatric age-groups (infants/children /adolescent), which were later shown to have distinctive prognostic and therapeutic implications.[7] In view of the overwhelming literature, WHO CNS5 has defined four subtypes of pediatric-type diffuse HGG. In keeping with the notion of restricting the term “glioblastoma” for only adult IDH wild-type of HGG, the WHO CNS5 recommends not to use the term “glioblastoma” anymore for the pediatric type of HGG. The four pediatric-type diffuse HGG include –

Diffuse midline glioma (DMG), H3K27-altered;Diffuse hemispheric glioma, H3 G34-mutant;Diffuse pediatric type HGG, H3-wild type and IDH-wild type;Infant type hemispheric glioma [Table 2].[5]{Table 2}

Diffuse midline glioma, H3K27-altered

An infiltrative pediatric HGG specifically arising in midline structures, commonly brainstem, thalamus, spinal cord, and rarely cerebellum. These are highly fatal gliomas, previously referred to as diffuse intrinsic pontine glioma (DIPG) or brain stem glioma based on site.[2] It was defined by histone mutations/alterations with two epigenetic subtypes; H3.3/3.1 K27.[5]

It has been recently identified that there are three epigenetically distinct molecular classes with K27M mutation in this group. Apart from the known H3.3/3.1 K27M mutation, it comprises of a subset of thalamic tumors with EGFR mutations and tumors showing enhancer of zest homolog inhibitory protein (EZHIP) overexpression.[8] Hence, the WHO fifth edition has renamed the entity from H3 K27-”mutant” to -“altered.”[5],[8] Incidence is 1–2 cases per 1, 00,000 population with a slight male predilection.[9] The median age of diagnosis is 9.8 years, with pontine tumors arising earlier (at a median age of 6.5 years).[10] Common clinical features depending upon site include ataxia, motor weakness, increased intracranial pressure, long tract signs, and multiple cranial neuropathies.[11] On magnetic resonance imaging (MRI), DMGs are usually T1-hypointense and T2-hyperintense. Contrast enhancement, necrosis,

or hemorrhage may be present.[2] Diffuse tumor invasion of the brainstem is seen, especially in tumors arising from the pons. Approximately 25% of these tumors invade the thalamus and upper cervical cord while 40% show leptomeningeal spread.[12] The postulated cell of origin is unknown; however, studies have shown tumor cell resemblances with nestin and OLIG2-expressing neural precursor-like cells and oligodendrocyte precursor cells.[13],[14]

Histomorphology [Figure 1]: Diffusely infiltrating glial tumor, frequently of astrocytic morphology. These group of tumors can also show oligodendroglial-like morphology composed of small and monomorphic cells (this can sometimes may lead to erroneous histological classification as oligodendroglioma; one need to remember that canonical type of oligodendroglioma is extremely rare in the pediatric age group and a diagnosis of oligodendroglioma should only be made after molecular confirmation). Nuclear pleomorphism can also be seen. Mitotic activity is a consistent feature; microvascular proliferation and necrosis can be variable.[15] However, in a diffusely infiltrating tumor with H3K27 alteration, the diagnosis of H3K27-altered DMG, CNS WHO Grade 4 can be rendered even in absence of histologic high-grade features. The diffuse infiltrating nature of the tumor should be confirmed on radiology.[12] One should be aware that H3K27M mutation has been rarely reported in other tumors, including pilocytic astrocytomas, ependymomas, and gangliogliomas; so tumors with histological features of the aforementioned entities should not be overinterpreted as DMG, H3K27-altered.[16],[17],[18],[19]{Figure 1}

Therefore, the given mutation is not exclusive to DMGs; hence, its interpretation in conjunction with histomorphology is very important. Tumors with EZHIP overexpression and bithalamic tumors with EGFR A 289 mutation also have similar clinico-histological features as that of H3K27M-mutant tumors. Tumor cells express S100 protein, OLIG-2, NCAM1, and MAP2. Immunohistochemically, positivity for mutation-specific antibody for H3F3A K27M with concomitant loss of expression of H3K27me3 (synchronous with H3K27M positivity) is now an established way of diagnosing this H3K27M-mutant entity. While the other two molecular subsets of DMG (i.e., tumors with EZHIP overexpression and EGFR-mutation) show loss of H3K27me3 expression without the positivity for H3K27M. Thus, any pediatric diffuse/infiltrative glial tumor (with no histological reminiscence to pilocytic astrocytoma or any other distinctive tumor) with loss of H3K27me3 expression should alert for this unified entity of DMG, H3K27-altered, grade 4. These tumors usually show a p53 immunoreactivity pattern of TP53 mutant phenotype (pilocytic astrocytoma and gangliogliomas rarely show a TP53 pattern of p53 immunopositivity). In about 15% of cases, loss of nuclear expression of Alpha Thalessmia/mental Retardation syndrome X-linked (ATRX) may be seen, suggesting an association of ATRX mutation.[2]

Genetic Profile: It is characterized by heterozygous mutations in H3F3A, HIST1H3B, and HIST1H3C genes, which encodes the replication-independent histone variants H3.3 and H3.1, resulting in replacement of lysine by methionine at 28th position in the N-terminal end of histone.[20] K27M mutations involving H3.3 (encoded by H3F3A) are at least three times more frequent than the same mutation in histone variant H3.1 (encoded by HIST1H3B and HIST1H3C).[21] Post-translational changes at this histone site alter gene expression, DNA repair, and telomere length maintenance. This mutation is also associated with epigenetic alteration, including a decrease in H3K27 trimethylation and global DNA hypomethylation, due to inhibition of polycomb repressive complex 2 (PRC2) activity. Sievers et al.[8] emphasized that there are three molecular subgroups responsible for H3K27me3 loss: (1) H3K27M substitution mutation, (2) EZHIP overexpression; EZHIP along with H3K27M interact with allosterically stimulated PRC2 and impedes its spreading, and (3) EGFR alterations; EGFR mutations along with H3K27M or EZHIP overexpression has equivalent tumorigenic capacity. Therefore, H3K27M mutation is not necessary for the loss of H3K27me3.[22],[23] It is also noted that EGFR-altered tumors largely resemble bi-thalamic gliomas and show distinct methylation profiles similar to pGBM_RTK2.[8],[24],[25] The most common EGFR mutation is hotspot missense substitution in exon 7 (p.A289V/T), followed by in-frame insertions in exon 20. Somatic mutations in the protein complex of H3.3 and ATRX/DAXX chromatin remodeling pathway are seen in 44% of the cases and are shown to be associated with TP53 mutation and alternative lengthening of telomeres (ALT).[20] The overlap between H3F3A and TP53 mutations in pediatric HGG is almost similar to the association of TP53 and IDH mutations in adult HGG.[20],[26] It is important to note that IDH and H3F3A mutations are mutually exclusive.[20] Theoretically, increased levels of 2-hydroxyglutarate secondary to IDH mutations can interfere with K27 methylation, thus simulating the effects of H3F3A mutations. This could account for an alternate pathway in HGG seen in older children and young adults.[3] Other oncogenic pathways involved in the pathogenesis of HGG are RTK/RAS/PI3K pathway (PDGFRA, PIK3CA, or PTEN mutations), p53 pathway (TP53, PPM1D, or ATM mutations), and rarely retinoblastoma tumour suppressor (RB) pathway. Considerably, more copy number variations are detected in RB pathway with amplification of CCND1-3, CDK4, and CDK6 and deletions of CDKN1C. Mutations and fusions of the FGFR1 gene are identified in thalamic HGG, whereas recurrent mutation of ACVR1 is seen in DIPG.[2],[3],[4],[15] A small proportion of children with HGG have a germline predisposition to Li-Fraumeni syndrome or biallelic mismatch repair (MMR) deficiency. The most common inherited etiology is still neurofibromatosis type I.[27]

Treatment and outcome: Patients who harbor K27M-H3.3 alterations are associated with a worse prognosis than wild type cases. The 2-year overall survival is less than 10% for these patients.[2],[10] Treatment predominantly comprises of maximal safe resection followed by radiation therapy. Thalamic gliomas are not easily accessible for subtotal/total resection, thereby conferring a poorer survival. ACVR1 mutations are associated with younger age and longer survival.[28]

Diffuse hemispheric glioma, H3G34-mutant

A cerebral hemispheric diffusely infiltrative HGG is characterized by a missense mutation in the 34th codon (reassigned as 35th codon) on the H3F3A gene.[12] It is predominantly seen in adolescents and young adults (age <30 years) with a median age of approximately 19 years and slight male preponderance.[29] It comprises of approximately 20% of the pediatric HGG located in the cerebral hemispheres.[10] Clinical presentation includes generalized tonic–clonic seizures, neurological deficits, and features of increased intracranial tension.[30] On MRI, unlike other HGG, more than 50% of the cases may show minimal to nil post-contrast enhancement, which may lead to an erroneous diagnosis of an LGG or a non-neoplastic diagnosis. It can diffusely and extensively invade large parts of the brain showing a “gliomatosis cerebri-like” pattern on radiology.[30] More than 80% of the cases are located in the temporal and parietal hemispheres followed by fronto-parietal region. These tumors sometimes show involvement of basal ganglia, deep gray matter, and corpus callosum but typically with a striking absence of brainstem or thalamic involvement.[20],[29],[30] The postulated cell of origin based on expression pattern correlates to early embryonic and fetal development of the neocortex, amygdala, inferior temporal cortex, and ganglionic eminences.[31]

Histomorphology [Figure 2]: Cellular diffusely infiltrating glial tumor with astrocytic morphology. Admixed areas of oligo-like and patchy areas of relative undifferentiated morphology (i.e., primitive neuroectodermal tumor (PNET)-like morphology) can be seen. Mitoses are usually noted but sometimes they may not be as frequent as typically seen in HGG (thereby giving a histological impression of intermediate grade type of tumor). Microvascular proliferation and/or necrosis are noted. Interestingly, tumors with areas of undifferentiated morphology may not show microvascular proliferation and necrosis. Homer-Wright rosettes, ganglionic differentiation, and rarely high-grade pleomorphic xanthoastrocytoma-like morphology are also reported.[16],[29],[32],[33]{Figure 2}

Tumor cells express the glial fibrillary acidic protein (GFAP), diffusely in “GBM-like” morphology and focal in “PNET-like” morphology. Both variants lack OLIG2 expression with the expression of FOXG1, which is a hallmark of this entity.[22] The tumor cell nuclei frequently show strong diffuse positivity for p53 (90%) and loss of ATRX (95%) expression, suggesting TP53 and ATRX mutations.[20],[22],[29] Loss of expression for ATRX protein is an important feature to distinguish from embryonal tumors. Immunohistochemistry (IHC) specific to H3.3G34-mutant proteins can help in the diagnosis of the tumor. IHC for H3K4me3 and H3K9me3 show retained expression.[30],[34]

Genetic profile: It is characterized by heterozygous mutation at codon 35 of the H3F3A gene.

G34R mutation (p.Gly35Arg;c.103 G > A) is commoner than G34V mutation (p.Gly35Val; c.104 G > T). On methylation profiing, it is identified that the G34-mutant amino acid itself is not post-translationally modified, but the mutant histone epigenetically alters the lysine residue at position 36 (K36), methylation of which is responsible for transcription activation and elongation. Therefore, H3.3 G34 mutation is associated with a decrease in H3K36 methylation along with global DNA hypomethylation (65.1%).[10] There is literature suggesting mutations affecting H3K36me3 exhibit a MMR deficient-like phenotype.[35] The differential distribution of H3K36me3 is associated with overexpression of the MYCN gene but loci of hypermethylation are also noted. Hypermethylation of the OLIG1/2 gene is responsible for the loss of OLIG2 expression on IHC. Notably, almost all these tumors demonstrate O6-methylguanine-DNA methyl transferase (MGMT) promoter methylation.[7],[10] The tumor has a uniform epigenetic signature, viz. loss of function of TP53 and ATRX alterations (both found in nearly all the cases). Loss of function of ATRX causes telomeric instability and results in telomerase independent maintenance through ALT. Chromosomal aberrations in the form of 4q loss (70%) and 2q loss (67%) are also identified.[10],[20] Most frequently reported focal amplification, especially in “GBM-like” HGG is Platelet-Derived Growth Factor Receptor- Alpha (PDGFRA) amplification (33%).[22] CDKN2A homozygous deletions are seen in 75% of cases.[29] IDH and B-rapidly accelerated fibrosarcoma (BRAF) mutations have not been reported.[7]

Treatment and Outcome: Most patients receive limited field radiotherapy and chemotherapy including temozolomide after surgical resection. Even though these tumors are surgically accessible, the resection is limited by the need for the preservation of eloquent neurological functions. No difference in outcomes is found between “GBM-like” and “PNET-like” morphology. The overall survival is slightly better than other pediatric HGG with a median of 22 months and 2-year survival in 27.3% cases. MGMT promoter methylation confers with improved response to temozolomide therapy.[29] However, a few of the studies have demonstrated no difference in outcome between patients with H3K27M-altered and H3 G34-mutant tumors.[10],[30]

Diffuse pediatric type HGG, H3-wild type and IDH-wild type

This is a heterogenous group of tumors lacking H3/IDH mutation.[12],[15],[30] It is seen in children with a median age of 10 years and marginal male predominance.[25] WHO CNS5 recognizes the following three subtypes of H3/IDH wild type pediatric HGG based on DNA methylation profile (i) Diffuse pediatric-type HGG RTK2 which is enriched in EGFR

amplification and telomerase (TERT) promotor mutation; (ii) diffuse pediatric-type HGG RTK1 which is enriched in PDGFRA amplification; and (iii) diffuse pediatric-type HGG which is enriched in Myelocytomatosis -N (MYCN) amplification.[36] Tumors associated with constitutional mismatch repair syndrome (CMMRD) or Lynch syndrome (LS) are typical of the RTK1 subtype. These tumors can be located both in the supra and infratentorial compartments. Morphologically, they show features of either glioblastoma (GBM) or primitive undifferentiated tumors.

Infant type hemispheric gliomas

In infants, LGGs are more common whereas HGGs are comparatively rare accounting for 23% of all gliomas. Infantile LGG and HGG not only are morphologically dissimilar but have distinguishable genetic profiles and contrasting clinical courses. Guerreiro Stucklin et al.[37] described that infant gliomas can be grouped as (i) hemispheric, RTK driven; (ii) hemispheric, RAS/MAPK driven; and (iii) midline, RAS/MAPK driven. Interestingly, all the infantile HGGs are restricted to the hemispheric, RTK driven group, which includes ALK/ROS1/NTRK/MET alterations. Infant-type hemispheric HGG is seen in patients younger than 1 year (median age 2.8 months) with no sex predilection. The frontal lobe is the most common site followed by the parietal and temporal lobes.[38] The onset of the symptoms is delayed due to the elasticity of the infants' skull which allows the tumor to grow in size significantly before manifesting any symptoms. The symptoms are macrocephaly, nausea and vomiting, irritable behavior, and failure to thrive.[39] On MRI, tumors appear as irregular margin, heterogeneous texture, and intense contrast enhancement. Tumors in the superficial cortex can involve the meninges. Neurotropic tyrosin receptor kinase (NTRK) fusion cases are associated with an embryonic, neuronal development program, and ALK fusion cases with AMPA receptor synaptic plasticity gene signatures.[38]

Histomorphology: Highly cellular infiltrating astrocytic tumors arranged in sheets. Mild to moderate nuclear pleomorphism, microvascular proliferation, frequent mitoses, and necrosis are common. A focal spindled appearance may also be seen. Focal mini-gemistocytes and occasional ganglionic cell differentiation may be identified.[10],[38] Ki-67 labeling index is variable with a median of 14.66%. Antibodies against ALK, ROS, and c-MET protein can be used as a surrogate marker to identify the respective gene rearrangements.[37],[40],[41]

Genetic profile: Among the hemispheric-RTK driven gliomas, 96.7% of cases were HGG. ALK, ROS1, NTRK1/2/3 gene rearrangements lead to fusion of various 5′ partners with 3′ end of the truncated RTK containing the tyrosine kinase domain. ALK-driven HGG was diagnosed at a median age of 1.6 months. Interstitial microdeletions at chromosome 2p can result in ALK fusion with CCDC88A or PPP1CB, intrachromosomal copy number gains lead to ALK-EML4 fusion (known in lung adenocarcinomas), and interchromosomal copy number gains cause ALK fusion with various novel partners like MAD1L1, MSI2, etc.[37],[38] Tumors with ALK fusion might show ependymal differentiation. ROS1/MET/NTRK alterations are seen in almost all the infant hemispheric HGG. Interstitial microdeletions at 6q result in the fusion of ROS1 and GOPC genes. ZCCHC8-ROS1 fusion tumors have been reported to have mini-gemistocytic rich morphology.[38],[42] The NTRK genes have numerous interchromosomal partners and are associated with a predominance of spindle cell morphology. ETV6-NTRK3 fusions are also reported.[38],[43] Ki-67 labeling index is significantly higher in patients with NTRK fusions. Focal DNA copy number loss at 7q results in MET gene alterations. It corresponds to its counterpart in lung adenocarcinomas. Interestingly, NTRK-fusion-positive HGG, post-therapy can also show areas of LGG morphology, probably representing LGG/HGG continuum. Infant-type hemispheric gliomas show features of maturation on therapy similar to neuroblastoma.

Treatment and outcome: Safe surgical resection followed by molecular characterization and targeted inhibitor chemotherapy is the line of management. Several ALK/ROS inhibitors including crizotinib have proven to be efficacious. NTRK inhibitor larotrectinib has shown a significant decrease in tumor size. The survival of infant hemispheric HGG is heterogeneous and shows paradoxical outcomes. The association between tumor grade and clinical outcome is unpredictable.[10] About 43% of patients with ALK-fused HGG have less than 3-year overall survival. Five-year overall survival is 25% and 42.9% for patients with ROS1 and NTRK fusion, respectively.[37]

IDH-mutant pediatric HGG

IDH-mutant comprises of approximately 6% of all pediatric HGG. The median age of diagnosis is 15.5 years and is exclusively located in a hemispheric location.[7] IDH-mutant HGG is morphologically indistinguishable from IDH-wild type HGG. None of the IDH-mutant tumors show any evidence of a precursor LGG, as often identified in adult GBM.

Nearly all the IDH mutations are located at the second base of codon 132 resulting in R132H (CGT > CAT) heterozygous point mutation.[20] It is frequently associated with 10q loss (70%) and homozygous deletion of 9p (60%). MGMT promoter methylation (90%) is found in almost all tumors.[7] It expresses a proneural gene signature, along with a large overlap with TP53 mutations. The patients show a significantly longer overall survival than the other subgroups. IDH mutation, 9p deletion, and MGMT methylation are associated with a favorable outcome.[7],[22]

Pediatric HGGs associated with cancer predisposition syndromes

Germline and inherited alterations predisposing to numerous neoplasms are common among the pediatric age group. The most common syndromic associations of pediatric HGG are Li-Fraumeni syndrome (LFS), constitutional mismatch repair deficiency (cMMR), and neurofibromatosis type 1 (NF1). Among these LFS, attributed to TP53 loss of function mutations, is the most frequent (approximately 10% of all CNS tumors) with up to 50% of patients showing TP53 mutations.[44] The prognosis of these tumors remains dismal in spite of aggressive management. Patients with monoallelic mutations of MMR genes develop LS. Patients present within the first two decades of life with a history of a high degree of consanguinity. Initial screening can be performed by IHC showing loss of nuclear expression of the MMR proteins. PD-L1 inhibitor pembrolizumab is approved for the treatment of MMR deficient cancers.[44] Gene involved in the pathogenesis of NF1 is a tumor suppressor gene that encodes for a protein neurofibromin. Most of the NF1 associated tumors are LGG; on the contrary, it is frequently present as a somatic alteration in sporadic pediatric HGG. Patients should be diligently screened for the features suggestive of any of the aforementioned syndromes and genetic evaluation for the same is obligatory. Germline heterozygous (monoallelic) mutations in MMR genes result in LS, while homozygous (biallelic) mutations in MMR genes result in CMMRD.[45] Other names for CMMRD are brain tumor predisposition syndrome type 1 (BTPS1), mismatch repair cancer syndrome (MMRCS), or biallelic mismatch repair deficiency (BMMRD). LS is an autosomal dominant syndrome, characterized mainly by gastrointestinal and genitourinary malignancies in mid to late adulthood (third to sixth decade). Primary brain tumors are a rare feature of LS.[46] The most common genes affected in LS are MLH1 followed by MSH2 and PMS2. If both parents have LS, the chances of having a child with LS are 50%, while with CMMRD are ~25%. Earlier these tumors were considered under the umbrella of Turcot syndrome.[47] CMMRD is a rare, often underdiagnosed, autosomal recessive childhood cancer syndrome affecting children and young adults with an incidence of CMMRD in approximately 1 per million patients. PMS2 gene is the most commonly altered gene (63%) followed by MSH6 (25%), MLH1 (9%), and MSH2 (3%).[48] A spectrum of brain tumors can be found in CMMRD, i.e., HGGs, medulloblastoma, and supratentorial primitive neuroectodermal tumors. However, it has to be noted that MMR deficiency in HGG can be due to sporadic or germline mutations. Café-au-lait spots are the most common non-neoplastic feature of CMMRD, which is a hallmark of neurofibromatosis 1. Thus, defining CMMRD phenotype is a challenge. However, NF1 is characterized by mainly LGGs, whereas CMMRD-associated gliomas are usually high grade. The incidence of HGG in NF1 is much lower. A European consortium, named as “Care for CMMRD” has developed a scoring system based on clinical features to identify the cases needed to undergo testing for microsatellite instability (MSI). The various clinical features that suggest the presence of CMMRD include any child or young adult with an LS-associated tumor, adenomatous polyposis, presence of Café-au-lait macules in the absence of NF1, history of consanguinity, and patients with hematolymphoid malignancy in the absence of radiation exposure. A combined score of ≥3 is an indication for testing for MMR deficiency.[49] However, these guidelines are difficult to follow due to the rarity of CMMRD and the diversity of clinical presentation. Histologically, in addition to the typical HGG features that often show the presence of highly pleomorphic astrocytic cells, numerous bizarre multinucleated giant cells admixed with smaller cells with round or spindle cell morphology prominence of intratumoral lymphocytic infiltrate may also be seen. On IHC, the loss of expression of one or more MMR proteins in tumor cells as well as native cells is characteristic. Thus, wherever clinically indicated, MMR proteins should be included in the diagnostic IHC panel. The identification of MMR deficient glial tumors is crucial for patient management and afflicted family members. The diagnosis of CMMRD can be done by immunohistochemical analysis of MMR proteins, analysis of MSI by PCR, or germline sequencing analysis by next-generation sequencing (NGS). Among these, the definitive diagnostic test is to confirm biallelic mutations in MMR genes by germline sequencing; however, IHC is an efficient method with 93.2% sensitivity.[50] The prognosis of MMR-deficient patients with glial tumors is extremely poor due to the aggressive phenotype of tumors. The median survival is <30 months after the first diagnosis. Therefore, identification of these cases is imperative, as close surveillance can markedly reduce cancer-related morbidity and mortality. As the MMR deficiency-associated tumors show a high mutational burden, there is a potential role of therapy with immune checkpoint inhibitors.

Conclusion

Our knowledge of pediatric HGG has increased enormously over the last decade. Identification of key genetic alterations and specific epigenetic signatures with a consequent refinement of molecular classification has been a major breakthrough. While we have some idea of the role of epigenetic alterations in oncogenesis, we are still far from a clear picture. In order to unveil the intricate mechanisms, the development of refined models is imperative. Based on the current knowledge, a screening algorithm can be devised with a summary of molecular methods, testing and clinical factors, illustrated in [Table 3] and [Figure 3]. The current knowledge has helped in the construction of a “patient-tailored” therapeutic approach too. Pediatric HGGs, even though rare, have an aggressive course of disease with a plethora of unexplored diagnostic and treatment options.{Table 3}{Figure 3}

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Conflicts of interest

There are no conflicts of interest.

References

1 Ostrom QT, Patil N, Cioffi G, Waite K, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2013–2017. Neuro Oncol 2020;22(Supplement_1):iv1-96.
2 Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, *et al*. The 2016 World Health Organization classification of tumors of the central nervous system: A summary. Acta Neuropathol 2016;131:803-20.
3 Jones C, Perryman L, Hargrave D. Paediatric and adult malignant glioma: Close relatives or distant cousins? Nat Rev Clin Oncol 2012;9:400-13.
4 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. J Clin Oncol 2010;28:3061-8.
5 Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, *et al*. The 2021 WHO classification of tumors of the central nervous system: A summary. Neuro Oncol 2021;23:1231-51.
6 Louis DN, Wesseling P, Aldape K, Brat DJ, Capper D, Cree IA, *et al*. cIMPACT- NOW update 6: New entity and diagnostic principle recommendations of the cIMPACT- Utrecht meeting on future CNS tumor classification and grading. Wiley Online Library; 2020. p. 844-56.
7 Korshunov A, Ryzhova M, Hovestadt V, Bender S, Sturm D, Capper D, *et al*. Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers. Acta Neuropathol 2015;129:669-78.
8 Sievers P, Sill M, Schrimpf D, Stichel D, Reuss DE, Sturm D, *et al*. A subset of pediatric-type thalamic gliomas share a distinct DNA methylation profile, H3K27me3 loss and frequent alteration of EGFR. Neuro Oncol 2021;23:34-43.
9 Chen J, Lin Z, Barrett L, Dai L, Qin Z. Identification of new therapeutic targets and natural compounds against diffuse intrinsic pontine glioma (DIPG). Bioorg Chem 2020;99:103847.
10 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;32:520-37. e5.
11 Kramm CM, Butenhoff S, Rausche U, Warmuth-Metz M, Kortmann R-D, Pietsch T, *et al*. Thalamic high-grade gliomas in children: A distinct clinical subset? Neuro Oncol 2011;13:680-9.
12 Yoshimura J, Onda K, Tanaka R, Takahashi H. Clinicopathological study of diffuse type brainstem gliomas: Analysis of 40 autopsy cases. Neurol Med Chir (Tokyo) 2003;43:375-82.
13 Filbin MG, Tirosh I, Hovestadt V, Shaw ML, Escalante LE, Mathewson ND, *et al*. Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. Science 2018;360:331-5.
14 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. Proc Natl Acad Sci U S A 2011;108:4453-8.
15 Buczkowicz P, Bartels U, Bouffet E, Becher O, Hawkins C. Histopathological spectrum of paediatric diffuse intrinsic pontine glioma: Diagnostic and therapeutic implications. Acta Neuropathol 2014;128:573-81.
16 Gessi M, Capper D, Sahm F, Huang K, von Deimling A, Tippelt S, *et al*. Evidence of H3 K27M mutations in posterior fossa ependymomas. Acta Neuropathol 2016;132:635-7.
17 Hochart A, Escande F, Rocourt N, Grill J, Koubi-Pick V, Beaujot J, *et al*. Long survival in a child with a mutated K27M-H3. 3 pilocytic astrocytoma. Ann Clin Transl Neurol 2015;2:439-43.
18 Kleinschmidt-DeMasters B, Donson A, Foreman NK, Dorris K. H3 K27M mutation in gangliogliomas can be associated with poor prognosis. Brain Pathol 2017;27:846-50.
19 Louis DN, Giannini C, Capper D, Paulus W, Figarella-Branger D, Lopes MB, *et al*. cIMPACT-NOW update 2: diagnostic clarifications for diffuse midline glioma, H3 K27M-mutant and diffuse astrocytoma/anaplastic astrocytoma, IDH-mutant. Acta Neuropathol 2018;135:639-42.
20 Schwartzentruber J, Korshunov A, Liu X-Y, Jones DT, Pfaff E, Jacob K, *et al*. Driver mutations in histone H3. 3 and chromatin remodelling genes in paediatric glioblastoma. Nature 2012;482:226-31.
21 Rodriguez FJ, Vizcaino MA, Lin M-T. Recent advances on the molecular pathology of glial neoplasms in children and adults. J Mol Diagn 2016;18:620-34.
22 Sturm D, Witt H, Hovestadt V, Khuong-Quang D-A, Jones DT, Konermann C, *et al*. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell 2012;22:425-37.
23 Lewis PW, Allis CD. Poisoning the “histone code” in pediatric gliomagenesis. Cell Cycle 2013;12:3241-2.
24 Mondal G, Lee JC, Ravindranathan A, Villanueva-Meyer JE, Tran QT, Allen SJ, *et al*. Pediatric bithalamic gliomas have a distinct epigenetic signature and frequent EGFR exon 20 insertions resulting in potential sensitivity to targeted kinase inhibition. Acta Neuropathol 2020;139:1071-88.
25 Korshunov A, Schrimpf D, Ryzhova M, Sturm D, Chavez L, Hovestadt V, *et al*. H3-/IDH-wild type pediatric glioblastoma is comprised of molecularly and prognostically distinct subtypes with associated oncogenic drivers. Acta Neuropathol 2017;134:507-16.
26 Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, *et al*. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765-73.
27 Genetic insights into familial tumors of the nervous system. In: Melean G, Sestini R, Ammannati F, Papi L, editors. American Journal of Medical Genetics Part C: Seminars in Medical Genetics. Wiley Online Library; 2004.
28 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. Nat Genet 2014;46:444-50.
29 Korshunov A, Capper D, Reuss D, Schrimpf D, Ryzhova M, Hovestadt V, *et al*. Histologically distinct neuroepithelial tumors with histone 3 G34 mutation are molecularly similar and comprise a single nosologic entity. Acta Neuropathol 2016;131:137-46.
30 Yoshimoto K, Hatae R, Sangatsuda Y, Suzuki SO, Hata N, Akagi Y, *et al*. Prevalence and clinicopathological features of H3. 3 G34-mutant high-grade gliomas: a retrospective study of 411 consecutive glioma cases in a single institution. Brain Tumor Pathol 2017;34:103-12.
31 Haase S, Nuñez FM, Gauss JC, Thompson S, Brumley E, Lowenstein P, *et al*. Hemispherical pediatric high-grade glioma: Molecular basis and therapeutic opportunities. Int J Mol Sci 2020;21:9654.
32 Sasaki S, Tomomasa R, Nobusawa S, Hirato J, Uchiyama T, Boku E, *et al*. Anaplastic pleomorphic xanthoastrocytoma associated with an H3G34 mutation: A case report with review of literature. Brain Tumor Pathol 2019;36:169-73.
33 Andreiuolo F, Lisner T, Zlocha J, Kramm C, Koch A, Bison B, *et al*. H3F3A-G34R mutant high grade neuroepithelial neoplasms with glial and dysplastic ganglion cell components. Acta Neuropathol 2019;7:1-4.
34 Haque F, Varlet P, Puntonet J, Storer L, Bountali A, Rahman R, *et al*. Evaluation of a novel antibody to define histone 3.3 G34R mutant brain tumours. Acta Neuropathol 2017;5:1-9.
35 Li F, Mao G, Tong D, Huang J, Gu L, Yang W, *et al*. The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutSα. Cell 2013;153:590-600.
36 Capper B, Johns DTW, Tabori U, Varlet P. Diffuse pediatric type high grade glioma, H3 wild and IDH wild type. The 5th Edition of WHO classification of tumors of the central nervous system; 2021. p. 77-80.
37 Guerreiro Stucklin AS, Ryall S, Fukuoka K, Zapotocky M, Lassaletta A, Li C, *et al*. Alterations in ALK/ROS1/NTRK/MET drive a group of infantile hemispheric gliomas. Nat Commun 2019;10:4343.
38 Clarke M, Mackay A, Ismer B, Pickles JC, Tatevossian RG, Newman S, *et al*. Infant high-grade gliomas comprise multiple subgroups characterized by novel targetable gene fusions and favorable outcomes. Cancer Discov 2020;10:942-63.
39 Reed C, Rosemberg S, Gherpelli JLD, Matushita H, de Almeida GGM, Diament AJ. Brain tumors in the first two years of life: a review of forty cases. Pediatr Neurosurg 1993;19:180-5.
40 Kwak Y, Kim S-I, Park C-K, Paek SH, Lee S-T, Park S-H. C-MET overexpression and amplification in gliomas. Int J Clin Exp Pathol 2015;8:14932-8.
41 Davare MA, Henderson JJ, Agarwal A, Wagner JP, Iyer SR, Shah N, *et al*. Rare but recurrent ROS1 fusions resulting from chromosome 6q22 microdeletions are targetable oncogenes in glioma. Clin Cancer Res 2018;24:6471-82.
42 Coccé MC, Mardin BR, Bens S, Stütz AM, Lubieniecki F, Vater I, *et al*. Identification of ZCCHC8 as fusion partner of ROS1 in a case of congenital glioblastoma multiforme with at (6; 12)(q21; q24. 3). Genes Chromosomes Cancer 2016;55:677-87.

43 Ziegler DS, Wong M, Mayoh C, Kumar A, Tsoli M, Mould E, *et al*. Brief report: Potent clinical and radiological response to larotrectinib in TRK fusion-driven high-grade glioma. Br J Cancer 2018;119:693-6.

44 Michaeli O, Tabori U. Pediatric high grade gliomas in the context of cancer predisposition syndromes. J Korean Neurosurg Soc 2018;61:319-32.

45 Abedalthagafi M. Constitutional mismatch repair-deficiency: Current problems and emerging therapeutic strategies. Oncotarget 2018;9:35458-69.

46 Yang C, Austin F, Richard H, Idowu M, Williamson V, Sabato F, *et al.* Lynch syndrome–associated ultra-hypermutated pediatric glioblastoma mimicking a constitutional mismatch repair deficiency syndrome. Cold Spring Harb Mol Case Stud2019;5:a003863. doi: 10.1101/mcs.a003863.

47 Turcot J, Després J-P, St Pierre F. Malignant tumors of the central nervous system associated with familial polyposis of the colon: Report of two cases. Dis Colon Rectum 1959;2:465-8.

48 Gianno F, Antonelli M, Ferretti E, Massimino M, Arcella A, Giangaspero F. Pediatric high-grade glioma: A heterogeneous group of neoplasms with different molecular drivers. Glioma 2018;1:117-24.

49 Wimmer K, Kratz CP, Vasen HF, Caron O, Colas C, Entz-Werle N, *et al*. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: Suggestions of the European consortium 'care for CMMRD'(C4CMMRD). J Med Genet 2014;51:355-65.

50 Carrato C, Sanz C, Muñoz-Mármol AM, Blanco I, Pineda M, Del Valle J, *et al*. The challenge of diagnosing constitutional mismatch repair deficiency syndrome in brain malignancies from young individuals. Int J Mol Sci 2021;22:4629.

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Figure 1: Salient features of diffuse midline glioma, H3K27-altered tumor: 10-year-old with pontine SOL. (a-d) Histomorphology shows a cellular infiltrating glial tumor of astrocytic morphology with nuclear anaplasia, mitotic activity, foci of microvascular proliferation, and spotty foci of necrosis. On immunohistochemistry, the tumor shows loss of expression for H3K27me3 protein (e), a mosaic pattern of staining for ATRX protein (f) and; the tumor is positive for p53 protein (g). MIB1 labeling index is approximately 20-25% (h). The diagnostic finding is the hotspot point mutation detected on sanger sequencing (p.Lys28Met)

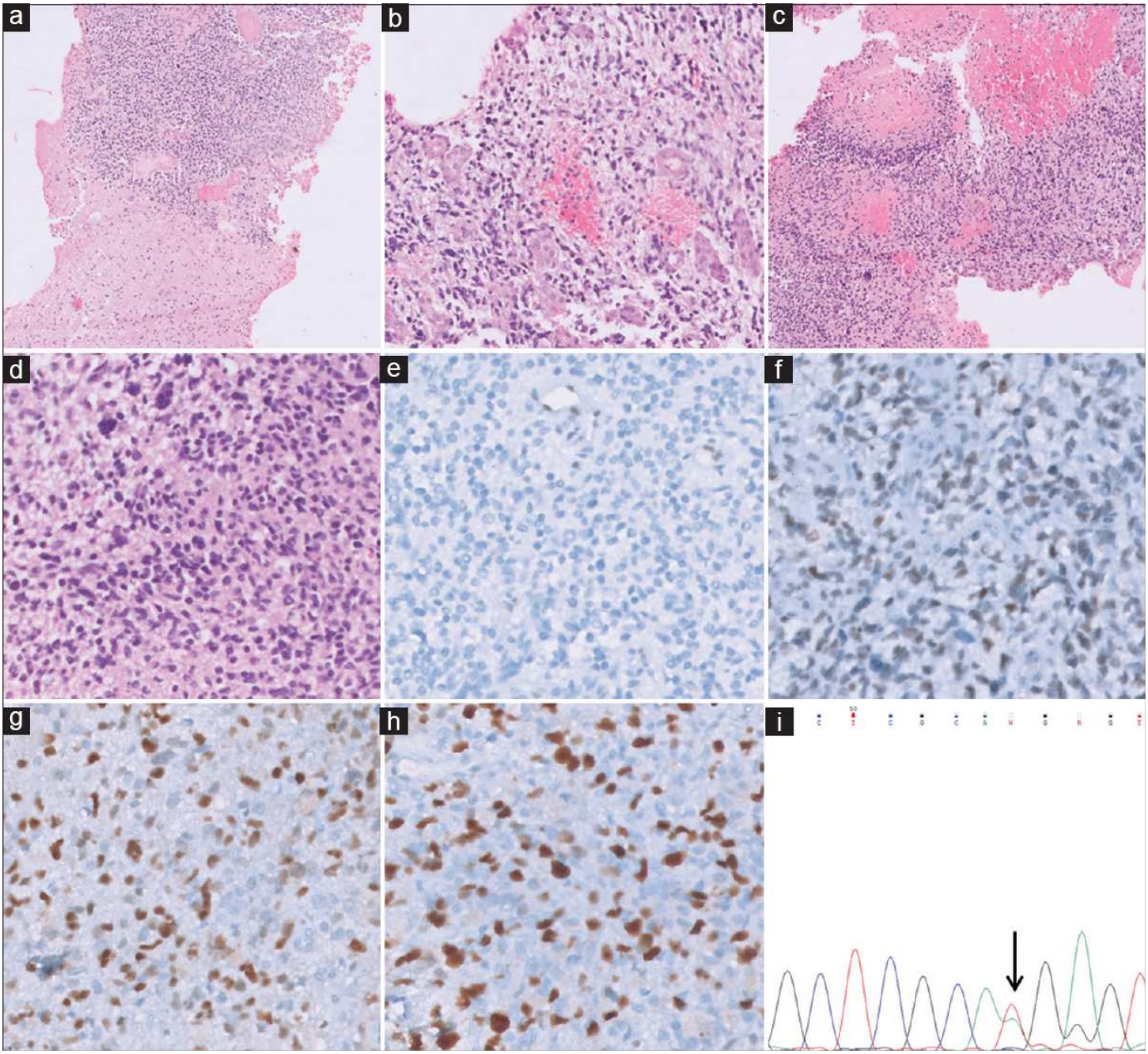

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Figure 2: Salient features of diffuse hemispheric glioma, H3G34-mutant tumor. 23-year-old male with right frontal SOL. (a-d) Histomorphology shows a cellular tumor with predominant areas of undifferentiated (PNET-like) morphology. Bizzare pleomorphic tumor giant cells noted, extremely frequent mitoses, foci of microvascular proliferation, and areas of necrosis seen. Occasional areas of low cellularity were also noted. On immunohistochemistry, the tumor shows mosaic pattern of staining for for H3K27me3 protein (e), while being negative for IDH1R132H (f); the tumor is positive for p53 protein (g); and shows loss of ATRX protein expression (h). MIB-1 labeling index is approximately 80-90%. The diagnostic finding is the hotspot point mutation detected on sanger sequencing (p.Gly35Arg)

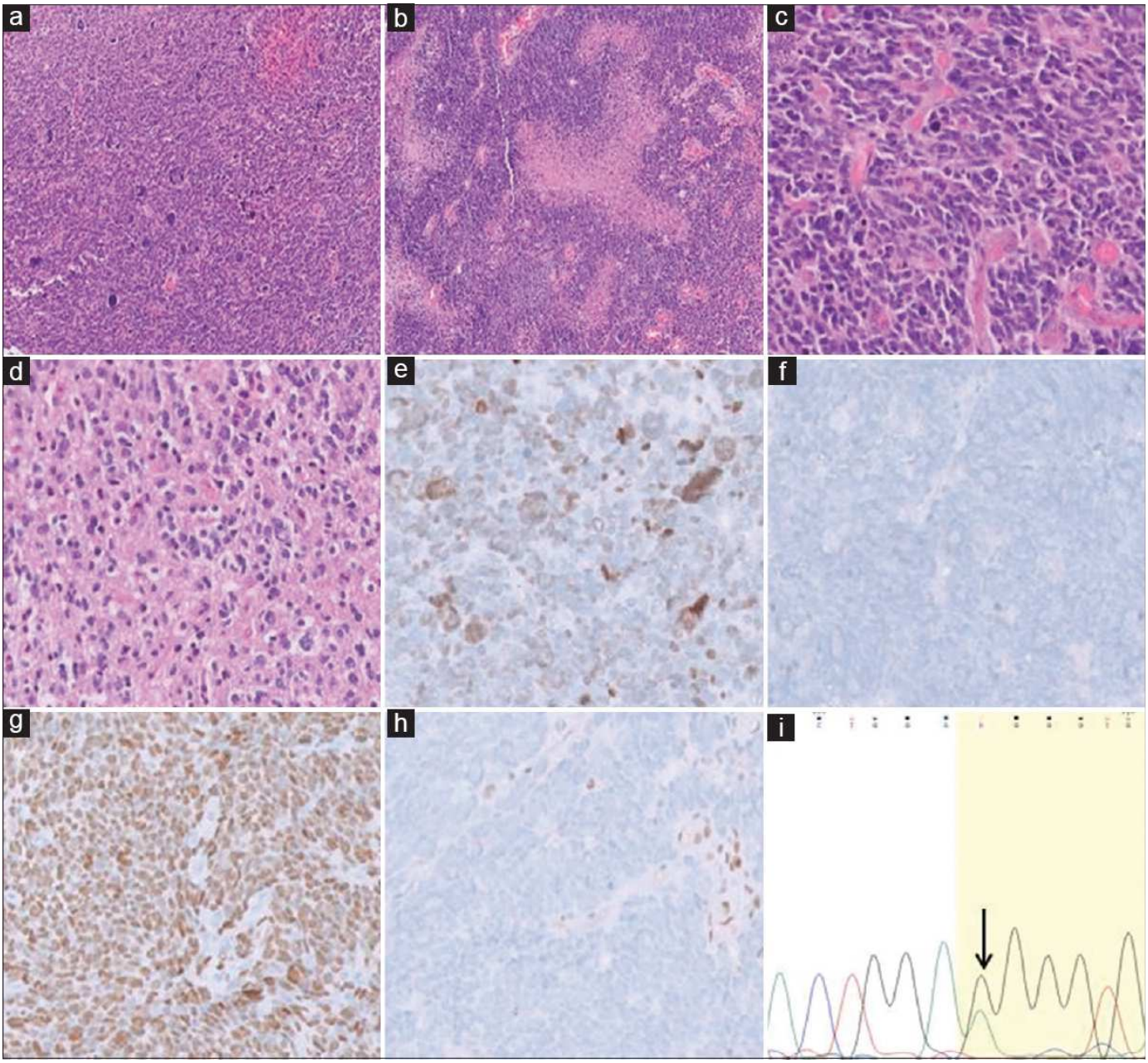

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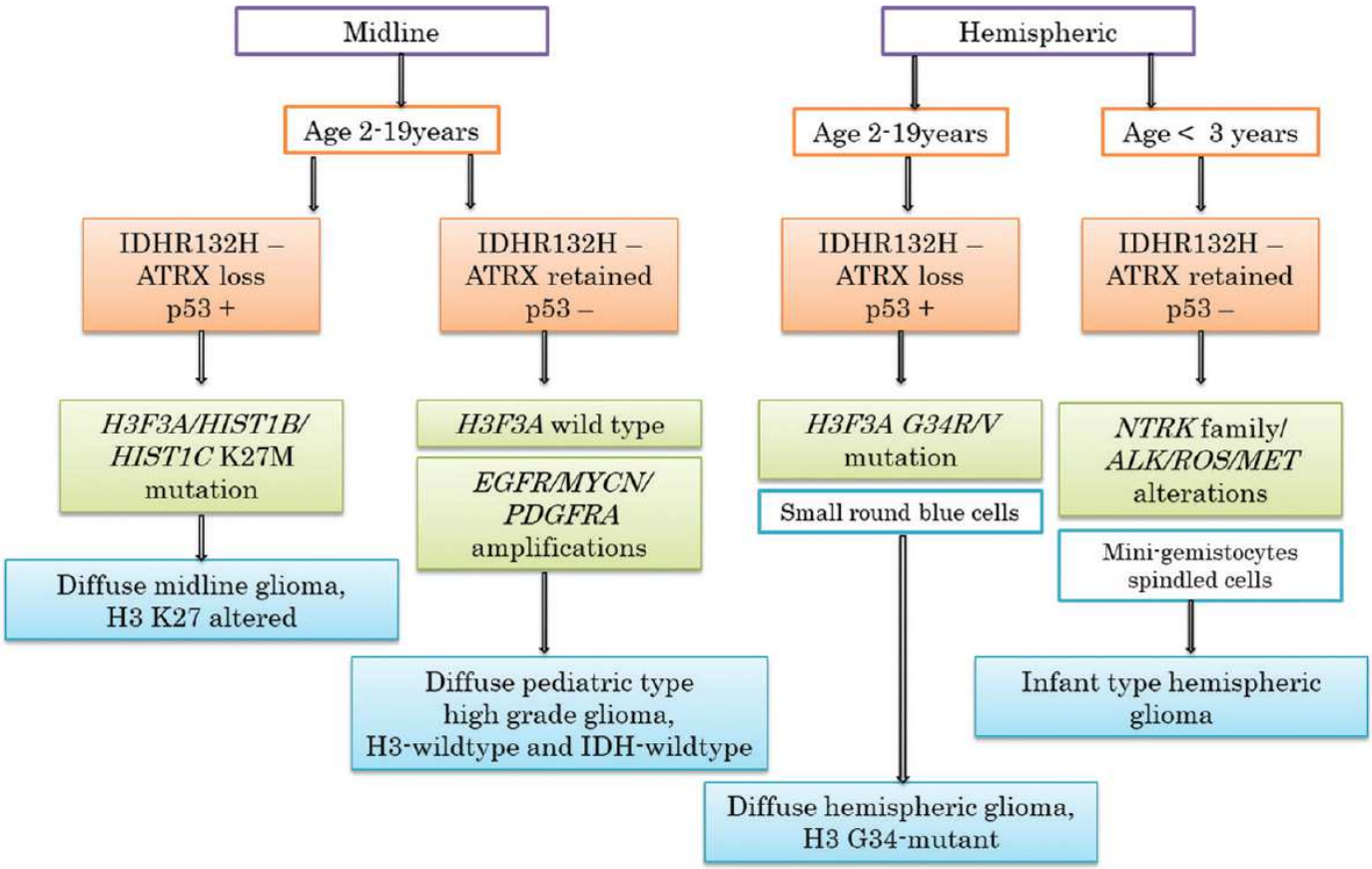
Figure 3: Clinical, immunohistochemical, and molecular algorithmic approach to pediatric high-grade gliomas



Abbreviations: - negative; + positive; ± can be positive or negative

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Table 1: Differences between pediatric and adult high-grade gliomas

Genetic Abnormality	*Pediatric HGG*				*Adult HGG*
	Infratentorial	*Supratentorial*			
		Infants	*Children*	*Adolescent*	
Chromosomal Gains					
1q	++	++	++	++	+
7	+	-	-	-	+++
Chromosomal Loss					
16q	+	++	++	++	+
10q	++	+	+	++	+++
4q	+	++	++	++	-
Amplifications					
EGFR	+	-	+	+	+++
PDGFRA	+++	-	++	++	++
Deletions					
CDKN2C	+++	+	+	++	+++
CDKN2A/B	-	+	++	++	+++
Pathway alterations					
p53 pathway	+++	+++	++	++	++
Rb pathway	++	+	+	+	+++
RTK/PI3K pathway	+++	+	++	++	+++
Gene mutations					
BRAFV600E	-	-	+	++	++
IDH1/2	-	-	-	+	+++
H3F3A K27M	+++	Ab	+++	++	++
H2F3A G34R/V	-	Ab	+	+++	++
HIST1H3B K27M	++	Ab	+	-	-
NF1	+++	+++	++	-	-
Methylation					
MGMT methyl	Ab	-	-	+++	+++
H3K27me: K27M	+++	Ab	++	++	++
H3K36me: G34R/V	-	Ab	+	+++	++

rare; +moderately common; ++frequent; +++very frequent; Ab absent

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Table 2: Overview of pediatric high-grade gliomas

Features	*H3.3/3.1 K27M altered*	*H3.3 G34 mutant*	*H3/IDH-wild type**			*Infant type hemisphe ric glioma*
			pedGBM_MYCN	*pedGBM_RTK1*	*pedGBM_RTK2*	
Median age	9.8 years	19 years	8 years	11 years	10 years	2.8 months
Location	Midline, infratentorial	Hemispheric, supratentorial	Anywhere	Hemisphe ric, midline, supratentorial	Hemisphe ric, supratentorial	Hemisphe ric, supratentorial
Morphology	Astrocytic, may show low grade morphology	"GBM-like" and "PNET-like"	"GBM-like" And "PNET-like"	Astrocytic , high grade	Astrocytic, high grade	Astrocytic, mini-gemistocytes
IHC profile	OLIG2+ FOXG1-H3K27me 3 loss	FOXG1+ OLIG2-H3K36me3 loss	FOXG1+ OLIG2+	FOXG1+ OLIG2+ ATRX loss	FOXG1+ OLIG2+	ALK + ROS1+ c-MET+
Mutations	*H3F3A, K27M, TP53, ATRX, EGFR*	*H3F3A, G34R/V, TP53, ATRX*	*TP53, hTERT*	*TP53, ATRX*	*hTERT*	*ALK, ROS1, NTRK1/2/3*
Copy number variations	*PDGFRA, EGFR* amplification	*CCND/CDK, PDGFRA* amplification	*MYCN* amplificat ion *CDK4/6* amplification	*PDGFRA* amplification, *CDKN2A* deletion	*EGFR* amplification *CDKN2A* deletion 7+, 10-	*ALK, MET* amplification
Global Methylation	Normal	Hypomethylated	Normal	Normal	Normal	Normal
MGMT methylation	Rare	Methylated	Rare	Rare	Absent	Absent
Gene expression	Proneural	Mixed	Mesenchy mal	Proneural	Classical	Proneural
Average Survival	6 months	21 months	14 months	21 months	44 months	16 months

*Korshunov *et al*. defined methylation-based classification. Mackay *et al*. defined molecular classification is described in text with overlapping features of both the classification systems

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Table 3: Key diagnostic modalities for pediatric high-grade glioma (except methylation studies)

	Immunohistochemistry	*Molecular methods*
Diffuse midline glioma, H3 K27-altered	H3K27M, H3K27me3*	*H3.3* and *H3.1, EGFR, EZHIP*
Diffuse hemispheric glioma, H3G34-mutant	H3G34, H3K36me3*, p53, ATRX	*H3.3* and *H3.1, TP53, ATRX*
Diffuse pediatric type high-grade glioma, H3-wild type and IDH-wild type	IDH1R132H, H3K27me3*, H3K36me3*	*IDH1, IDH2, H3.3* and *H3.1, PDGFRA, MYCN, EGFR*
Infant type hemispheric glioma	ALK, ROS1, c-MET	*ALK, ROS1, MET*

*Loss of expression