Indian Journal of Pathology & Microbiology

REVIEW ARTICLE
Year : 2022 | **Volume** : 65 | **Issue** : 5 | **Page** : 14--23

A review of adult-type diffuse gliomas in the WHO CNS5 classification with special reference to Astrocytoma, IDH-mutant and Oligodendroglioma, IDH-mutant and 1p/19q codeleted

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Abstract

The fifth edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (WHO CNS5) features several changes in the classification, diagnostic criteria, nomenclature, and grading of diffuse gliomas. Adult-type diffuse gliomas are genetically defined and include astrocytoma, isocitrate dehydrogenase (IDH)-mutant, oligodendroglioma, IDH-mutant and 1p/19q codeleted, and glioblastoma, IDH-wildtype. This review briefly discusses two tumor types: astrocytoma, IDH-mutant, and oligodendroglioma, IDH-mutant and 1p/19q codeleted, with emphasis on relevant changes in their classification and defining molecular genetic alterations. A simplified approach to the diagnosis of these tumors is provided.

How to cite this article:
Santosh V, Rao S. A review of adult-type diffuse gliomas in the WHO CNS5 classification with special reference to Astrocytoma, IDH-mutant and Oligodendroglioma, IDH-mutant and 1p/19q codeleted.Indian J Pathol Microbiol 2022;65:14-23

How to cite this URL:
Santosh V, Rao S. A review of adult-type diffuse gliomas in the WHO CNS5 classification with special reference to Astrocytoma, IDH-mutant and Oligodendroglioma, IDH-mutant and 1p/19q codeleted. Indian J Pathol Microbiol [serial online] 2022 [cited 2022 May 25];65:14-23
Available from: https://www.ijpmonline.org/text.asp?2022/65/5/14/345055

Full Text

Introduction

Gliomas are the most common primary intrinsic tumors of the central nervous system (CNS) and include a wide range that demonstrates varied clinical and biological behaviors. They are basically divided into two subgroups: diffuse gliomas and circumscribed (non-diffuse) gliomas. Diffuse gliomas are primarily characterized by diffuse infiltration of the tumor into the neuroparenchyma. They include entities that are histologically astrocytic or oligodendroglial in morphology. The circumscribed gliomas show a more delineated growth pattern. The remarkable expansion of knowledge and understanding of the molecular underpinnings of gliomas has brought about a major change in the classification of these tumors. It is now known that adult-type and pediatric-type gliomas have distinctly different underlying molecular alterations. This review briefly discusses the evolution in the classification of adult-type diffuse gliomas, their histomorphological features, molecular genetic alterations, and a simplified approach to the diagnosis of these tumors.

Classification of Adult-Type Diffuse Gliomas - Evolution

The earliest classification of gliomas was based on the historic work by Bailey and Cushing, in which the nomenclature of gliomas was based on the histological resemblance of the neoplastic cells to their normal cellular counterpart, which was presumed to be the cell of origin of the tumor.[1] Thus, astrocytomas were believed to originate from astrocytic cells, oligodendrogliomas from oligodendroglial cells, and ependymomas from ependymal cells. In addition, the adjective “anaplastic” was applied to tumors exhibiting histological atypia and increased mitoses. Therefore, this classification had a dual-axis scale; one which referred to histological lineage and the other to the status of biological behavior based on “anaplastic” features.[2] The Bailey and Cushing classification was extensively used as it proved to be clinically and prognostically relevant and helped in the management of patients with gliomas. Several modifications were proposed in the subsequent classifications, but all of them adopted the basic principle proposed by the Bailey and Cushing classification. Subsequently, Russell and Rubinstein modified and updated the Bailey and Cushing classification, following which the World Health Organization (WHO) took over the brain tumor classification.[3]

Starting with the first edition of the WHO classification of CNS tumors (1979) and through the second (1993), third (2000), and fourth (2007) editions, all the gliomas irrespective of being diffuse or circumscribed were combined as a single category. In the first edition, glioblastoma was included as an entity under the “Poorly Differentiated and Embryonal Tumours” category and was later shifted to “Astrocytic Tumours” in the second edition. Later, in the 2007 classification, anaplastic oligoastrocytoma with necrosis was designated as “glioblastoma with oligodendroglial component.”[4] Until the 2016 update of the WHO classification of CNS tumors, histomorphology played a crucial role in the diagnosis and classification of gliomas.[5]

The challenges faced were not only in the classification of CNS tumors but also in arriving at prognostically meaningful grades. The four-tiered Kernohan grading system was first described in the year 1949, which correlated with patient prognosis.[6] Later, Zülch proposed a five-tiered grading system.[3] In the year 1988, the St. Anne Mayo four-tiered grading classified infiltrative astrocytic tumors into grades 2–4; based on four important histological criteria which include nuclear atypia, mitotic activity, microvascular proliferation, and necrosis.[7] In the year 1993, WHO adopted the St. Anne Mayo method of grading CNS tumors, particularly for diffuse gliomas.

Over the past two decades, the genomic and epigenomic landscape of diffuse gliomas has been mapped using several high-throughput technologies such as whole-genome sequencing, whole-exome sequencing, copy number alterations, and genome-wide methylation status.[2] Among the various genetic alterations, only a few molecular markers have been used in the classification of diffuse gliomas. These markers have taken an edge over morphology and now form the basis for the diagnosis, classification, and prognosis of gliomas, although morphology still forms the foundation for the classification. A major shift was witnessed in the WHO 2016 update classification of CNS tumors, where molecular information got incorporated into the classification. It was the International Society of Neuropathology (ISN)-Haarlem consensus that formed the fundamental basis for this classification. It was recognized that histological classification was unable to provide precise prognostic information and was subjective to inter-observer variability. Thus, the ISN-Haarlem guidelines suggested an integrated diagnosis in the form of a layered diagnosis to be uniformly adopted worldwide. The layering included integrated diagnosis, histological classification, WHO

grade, and molecular information.[8] Following the recommendation by ISN-Haarlem guidelines and considering the fact that clinically relevant molecular markers have a significant role in gliomas, the WHO 2016 update classification of CNS tumors saw major restructuring in the glioma classification.[5] Diffuse gliomas (including astrocytomas, oligodendrogliomas, glioblastoma) were grouped together under “Diffuse astrocytic and oligodendroglial tumors” and circumscribed gliomas (pilocytic astrocytoma, pleomorphic xanthoastrocytoma, subependymal giant cell astrocytoma) which had distinct molecular alterations were grouped under “Other astrocytic tumors.” However, at this point of time, adult and pediatric gliomas were still not recognized as distinct groups in the classification.

Beyond the WHO 2016 classification, the rapid unearthing of promising biomarkers and new drug targets has resulted in exponential growth in the field, and to keep pace with these advancements, the cIMPACT-NOW (Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) was created to provide updates and recommendations for future WHO classifications.[9] Seven updates of the cIMPACT-NOW have been published till date.[10],[11],[12],[13],[14],[15],[16] The recommendations of these updates are incorporated in the fifth edition of the WHO classification of CNS tumors (WHO CNS5), wherein a major revamp of the classification of gliomas is seen.[17] However, this classification will also adhere to the routine methods of tumor characterization such as histology and immunohistochemistry (IHC) and therefore the new classification is expected to be a “hybrid” one.

General changes in the WHO CNS5 are with respect to CNS tumor taxonomy, nomenclature, and grading. The terms “entity” and “variant” are replaced by “type” and “subtype”. As the recommendations of cIMPACT-NOW are to use an integrated diagnosis with the layered format, liberal use of NOS (not otherwise specified) and NEC (not elsewhere classified) is allowed to facilitate diagnosis at all the centers. NOS suffix is used when a diagnostic test required for assigning a WHO diagnosis is not carried out or the results are inconclusive. NEC suffix is used when the essential diagnostic tests have been carried out, but the results do not conform to a defined WHO diagnosis.

Adult-Type Diffuse Glioma (WHO CNS5)

Some of the major changes in the classification of diffuse gliomas include segregation of diffuse gliomas into adult-type and pediatric-type, simplifying the classification of adult-type diffuse gliomas including nomenclature and grading, and division of pediatric diffuse gliomas into pediatric-type low- and high-grade gliomas. The grading of adult-type diffuse gliomas has also undergone major changes. Arabic numerals replace Roman numerals and grading is within tumor types. WHO CNS5 also recommends the use of “CNS WHO grade” to distinguish it from other WHO tumor grading systems.[17]

Adult-type diffuse gliomas are a genetically defined group and include three tumor types:

(i) astrocytoma, isocitrate dehydrogenase (IDH)-mutant, (ii) oligodendroglioma, IDH-mutant and 1p/19q codeleted, and (iii) glioblastoma, IDH-wildtype.

Astrocytoma, IDH-mutant

Astrocytoma, IDH-mutant is a diffusely infiltrating astrocytic glioma. This tumor harbors IDH1 or IDH2 mutations. In addition, alpha-thalassemia mental retardation syndrome X-linked (ATRX) and/or Tp53 mutations are seen in the majority of these tumors and 1p/19q codeletion is not observed. A combined histological and molecular grading is applied to this tumor type, wherein CDKN2A/B homozygous deletion is considered along with histology for grading. The three grades, which are referred to as “subtypes,” are astrocytoma, IDH-mutant CNS WHO grade 2; astrocytoma, IDH-mutant CNS WHO grade 3; and astrocytoma, IDH-mutant CNS WHO grade 4; and these replace the earlier tumor entities diffuse astrocytoma, anaplastic astrocytoma, and IDH-mutant glioblastoma.

Localization

These tumors are usually located in the cerebral hemispheres, most often in the frontal lobes, but can occur anywhere in the neuraxis including the brain stem and spinal cord. Recently, a molecularly distinct form of astrocytoma, IDH-mutant has been described in the infratentorial compartment.[18]

Clinical presentation

Patients frequently present with seizures of insidious onset, often accompanied by features of raised intracranial tension such as headache and vomiting. Other neurological abnormalities such as speech or language difficulties, sensory or motor function abnormalities, or visual disturbances can be present. Occasional grade 2 tumors are incidentally detected when neuroimaging is performed for headaches or following trivial trauma.[19]

Neuroimaging

Findings on neuroimaging can vary depending on the location and grade of these tumors. On magnetic resonance imaging (MRI), astrocytoma IDH-mutant CNS WHO grade 2 tumors are expansile, ill-defined cerebral hemispheric lesions, hypointense on T1 weighted images, and hyperintense on T2 weighted images. Although there is T2 weighted hyperintensity, this is often accompanied by relative hypointensity on fluid-attenuated inversion recovery (FLAIR) sequences (T2–FLAIR mismatch sign). This feature is seen in grade 2 and grade 3 tumors.[20] Post-contrast enhancement is absent in grade 2 tumors. Perilesional edema, midline shift, and post-contrast enhancement are more prominent in higher-grade tumors. In addition, grade 4 tumors can show central necrosis with rim enhancement.

Macroscopy

Astrocytoma, IDH-mutant tumors are diffusely infiltrating, expansile astrocytic gliomas arising in the white matter and invading normal anatomical structures causing blurring of the gray–white junction. Secondary changes such as microcyst or macrocyst formation, sometimes filled with gelatinous material, may be observed. With increasing grades, there can be areas of hemorrhage and grade 4 tumors can show focal areas of necrosis.

Microscopy

Astrocytoma, IDH-mutant, CNS WHO grade 2 tumors are well-differentiated, mild to moderately cellular astroglial tumors, composed of fibrillary, protoplasmic, and gemistocytic astrocytes in varying proportions. These cells diffusely infiltrate the neuroparenchyma over a microcystic, finely fibrillated glial matrix. A predominant gemistocytic pattern is not restricted to IDH-mutant astrocytomas and can be noted in IDH wildtype tumors as well. Occasionally, an oligodendrocyte-like morphology can be seen. The tumor cells exhibit mild to moderate nuclear atypia, in the form of nuclear enlargement, irregular nuclear contours and chromatin patterns, and hyperchromasia. The nucleolus is generally inconspicuous. Mitotic activity is usually absent or inconspicuous. A single mitosis or low mitotic count in a resected tumor is suggestive of a grade 2 tumor.[14] There is an absence of microvascular proliferation and necrosis. Astrocytoma, IDH-mutant, CNS WHO grade 3 tumors are more cellular, show anaplastic features including multinucleated tumor cells, nuclear atypia, and significant mitosis. The defining feature of these tumors is an absence of microvascular proliferation and necrosis. The earlier entity, glioblastoma, IDH-mutant is now replaced by astrocytoma, IDH-mutant, CNS WHO grade 4.[14] These tumors show necrosis and/or microvascular proliferation along with histological features of grade 3 tumors. Several times, they have focal oligodendroglioma-like areas. Necrosis is usually focal in astrocytoma, IDH-mutant, CNS WHO grade 4 tumors, while large areas of ischemic necrosis or palisading necrosis which are hallmarks of glioblastoma, IDH-wildtype, CNS WHO grade 4, are less common in these tumors. In addition, an astrocytoma, IDH-mutant, CNS WHO grade 2 or 3 tumor harboring a homozygous deletion of CDKN2A/B is also designated as astrocytoma, IDH-mutant CNS WHO grade 4, despite the absence of microvascular proliferation and/or necrosis. [Table 1] summarizes the grading criteria for astrocytoma, IDH-mutant tumors.{Table 1]

Immunophenotype

Astrocytoma, IDH-mutant tumors show immunopositivity for glial fibrillary acidic protein (GFAP), vimentin, and Olig2. The surrogate molecular diagnostic panel for routine testing includes the following: IDH1p.R132H, ATRX, p53 along with MIB-1 (Ki67). The majority of IDH-mutant astrocytomas show IDH1p.R132H immunopositivity along with loss of ATRX expression and p53 positivity. Only in cases where IDH1p.R132H is immunopositive and there is ATRX retained expression, testing for 1p/19q codeletion is advised to exclude oligodendroglioma.[11] In cases that are negative for IDH1p.R132H, DNA sequencing for the other non-canonical IDH1 and 2 mutations is advised. However, if DNA sequencing is not performed or the results are inconclusive, the diagnosis of “Astrocytoma, NOS” is offered. The Ki-67 proliferation index is very low, generally <4% in CNS WHO grade 2 IDH-mutant astrocytomas, and ranges from 4% to 10% in CNS WHO grade 3 tumors. However, these values are arbitrary since the lower values can overlap with grade 2 astrocytomas and the higher values can overlap grade 4 tumors. [Figure 1] shows the histological, IHC, and diagnostic molecular features of astrocytoma, IDH-mutant tumors.{Figure 1}

Diagnostic molecular alterations

IDH1 and 2 mutations

Mutations in IDH1 and 2 genes are the important driver mutations in the initiation and progression of most adult diffuse gliomas. IDH mutations were first described in 2008 by Parsons et al.[21] in a subset of glioblastomas. Later, landmark studies established that IDH1 mutations occur early in IDH-mutant diffuse gliomas.[22],[23] Astrocytoma, IDH-mutant tumors are defined by IDH1 or IDH2 mutations. IDH1 mutation initiates gliomagenesis which is followed by 1p and 19q codeletion in oligodendroglioma and ATRX/TP53 mutation in astrocytoma. Further studies showed that IDH mutations also had a significant prognostic role in these tumors.[24],[25],[26] IDH mutation is a single hot spot mutation, and is heterozygous, wherein one copy of the wildtype is preserved. This mutation affects amino acid 132 in IDH1 or 172 in IDH2. The most common mutations are the CGT to CAT transition at codon 132 of the IDH1 gene leading to arginine being replaced by histidine (R132H), which is caused by G395A nucleotide change (IDH1:c395G>A, p.R132H mutation). This is seen in 80–90% of IDH-mutant tumors. Other less common mutations are p.R132C (arginine to cysteine), p.R132G (arginine to glycine), p.R132S (arginine to serine), p.R132L (arginine to leucine), p.R132V (arginine to valine) of the IDH1 gene, and p.R172K (arginine to lysine), p.R172G (arginine to glycine), p.R172M (arginine to methionine), p.R172S (arginine to serine), and p.R172Y (arginine to tyrosine) of the IDH2 gene. Heterozygous mutations in IDH dominantly inhibit wildtype IDH and the mutant enzyme converts α-ketoglutarate (αKG) to α-hydroxyglutarate which acts as an oncometabolite that inhibits dioxygenases and demethylases, thereby affecting DNA repair mechanisms and chromatin modification, leading to hypermethylation of CpG islands throughout the genome, thereby forming a subgroup called G-CIMP (glioma-CpG island methylator phenotype).[23] G-CIMP subgroup exhibits a proneural pattern on gene expression profiling and is associated with a favorable prognosis.

DNA sequencing formed the basis for analysis of mutational status until the year 2009 when Capper and his colleagues raised a monoclonal antibody against mutant IDH1p.R132H protein.[27] This antibody is highly sensitive and specific to detect the mutant protein and therefore IDH1p.R132H immunopositivity is considered as a surrogate marker for IDH1R132H mutation. In diffuse lower-grade gliomas, if IDH1p.R132H is negative by IHC, sequencing for other non-canonical IDH1 and IDH2 should be performed.

ATRX mutation

ATRX mutation is one of the important molecular alterations discovered in recent years. ATRX mutation is an important molecular event in the development of diffuse glioma.[28] The gene is located on Xq21.1 and is a DNA helicase and chromatin remodeling protein. ATRX mutations are loss of function mutations.[29],[30] These include truncating mutations, seen in about three-fourths of the cases, and missense mutations in the highly conserved region of the helicase domain, seen in about one-quarter of cases. These alterations are associated with ATRX loss on IHC. ATRX along with death-domain associated protein (DAXX) functions to maintain the inactive portion of the genome in a compact structure. Loss of ATRX and DAXX are important in telomere maintenance mechanism [alternative lengthening of telomeres (ALT)], a presumed precursor to genomic instability.[29],[30] ATRX loss is seen characteristically in IDH-mutant astrocytomas, histone mutant tumors, and is mutually exclusive with 1p/19q codeletion. ATRX mutations are mutually exclusive with activating telomerase reverse transcriptase (TERT) promoter mutations. Therefore, in IDH-mutant astrocytomas, TERT promoter mutations are rare but are present in most of the IDH-mutant oligodendrogliomas which do not harbor ATRX mutation, as well as in IDH-wildtype glioblastomas.

Assessment of ATRX mutation is routinely carried out by IHC. Since the mutation results in a truncated or negative protein expression, the mutation is evidenced by the loss of ATRX expression in the tumor cells. In endothelial cells, native and reactive glial cells and entrapped neurons, retained nuclear expression of ATRX is noted. This serves as an internal control. Hence, ATRX immunoreactivity must be assessed in the tumor core rather than its infiltrating front.

TP53 mutations

TP53 mutations are noted in >50% of astrocytic gliomas, and the frequency increases with the grade of the tumor.[31],[32] There is a correlation between p53 immunopositivity and TP53 mutations although this is not linear. Studies have suggested that a strong p53 nuclear positivity in >10% of the tumor cells predicts TP53 mutations in gliomas, with a high degree of sensitivity and specificity.[33],[34] p53 immunopositivity has a high degree of correlation with missense mutations, although this is lower with truncating mutations.[34] Nonsense mutations or deletions can cause false-negative results.[33],[34] IHC interpretation of p53 needs caution as the antibody detects both wildtype and mutant p53 proteins. Any alterations resulting in an increase of half-life of the p53 protein such as cellular stress, or even alterations of mouse double minute 2 (MDM2), MDMX, or Alternative reading frame (ARF) which cause abnormal accumulation of wildtype p53 protein can result in p53 immunopositivity.[33]

CDKN2A/B deletion

Cyclin-dependent kinase inhibitor 2A (CDKN2A) gene which is located on chromosome 9 (9p21.3), encodes two proteins; p16 (or p16INK4a) and p14 (or p14ARF). P16INK4a inhibits phosphorylation of retinoblastoma protein by cyclin-dependent kinases, CDK4 and CDK6. p14ARF induces a p53-dependent cell cycle arrest by interacting with MDM2 and stabilizing p53. Recent studies have analyzed the role of CDKN2A/B deletion in prognostication of IDH-mutant astrocytomas, as histological grading has been found to be insufficient in accurately grading these tumors and providing the appropriate prognostic indication. Reis et al.[35] observed that grade 2 and 3 astrocytomas with CDKN2A deletion are associated with a poor prognosis. Shirahata et al.[36] observed that Grade 3 astrocytomas with CDKN2A deletion were associated with shorter overall survival compared to non-deleted astrocytic tumors and grade 4 glioblastomas without CDKN2A deletion. The CDKN2A/B homozygous deletions in IDH-mutant astrocytic gliomas range from 0 to 12% in WHO grade 2, 6 to 20% in WHO grade 3, and 16 to 34% in WHO grade 4 tumors (cIMPACT 5).[14] According to WHO CNS5, an astrocytoma, IDH-mutant tumor with microvascular proliferation or necrosis or CDKN2A/B homozygous deletion, or any combination of these features, is now designated as astrocytoma, IDH-mutant, CNS WHO grade 4 and replaces the term “Glioblastoma, IDH-mutant, WHO grade IV.” This results in three grades of IDH-mutant astrocytomas (CNS WHO grades 2–4) and the term glioblastoma is retained for only the IDH wildtype tumors.

Oligodendroglioma, IDH-mutant and 1p/19q codeleted, CNS WHO grades 2 and 3

Oligodendroglioma, IDH-mutant and 1p/19q codeleted, is defined as a “diffusely infiltrating glioma with IDH1 or IDH2 mutation and codeletion of chromosome arms 1p and 19q.”[5] This tumor type is graded as CNS WHO grade 2 or 3.

Localization

These tumors are also localized to the cerebral hemispheres, predominantly the frontal lobe, like the IDH-mutant astrocytomas. Rare examples of tumors localized to the posterior fossa, basal ganglia, brainstem, and spinal cord are reported.[37] Occasionally, they can have a gliomatosis cerebri-like spread or can be multifocal.[38],[39]

Clinical presentation

Patients frequently present with seizures. This can be accompanied by features of raised intracranial tension, such as headache and vomiting along with focal neurological deficits and cognitive changes. Like the IDH-mutant astrocytomas, the signs and symptoms depend on the location of the tumor. Occasionally, the tumor can be detected incidentally on neuroimaging.

Neuroimaging

On MRI, the tumors show heterogeneous hypointensity on T1 weighted and hyperintensity on T2 weighted images. Post-contrast enhancement is seen mostly in CNS WHO grade 3 oligodendrogliomas but can be focal in CNS WHO grade 2 tumors.[40] Calcification is common and can be detected on computerized tomography (CT) scans. Some tumors show cystic changes and/or hemorrhage.[41]

Macroscopy

Oligodendrogliomas are often well-defined tumors, expanding the white matter with the blurring of the gray–white junction. Occasionally, these tumors are very superficial and located in the cortex, sometimes with limited leptomeningeal involvement. Focal gritty areas (due to calcification), cystic change, and mucoid degeneration can be present. Grade 3 tumors can show areas of hemorrhage and necrosis.

Microscopy

Oligodendroglioma is composed of tumor cells arranged in lobules intersected by a dense network of thin-walled, branching capillaries. The cells have round uniform nuclei and perinuclear halo, which gives the cell a “fried-egg” appearance, and this imparts a “honeycomb” pattern to the tumor. Microcalcification, mineralization of blood vessels, hemorrhage, and microcystic, myxoid change are frequently noted. Rarely the stroma can be desmoplastic. Perineuronal satellitosis and subpial carpeting are characteristic features. Minigemistocytes or microgemistocytes and gliofibrillary oligodendrocytes are seen in many oligodendrogliomas, most often in CNS WHO grade 3 tumors. Sometimes the cells can have intracytoplasmic dense eosinophilic granules or a “signet ring” morphology. Some tumors can have an oligoastrocytic or even astrocytic morphology. However, these phenotypes do not impede a diagnosis of oligodendroglioma if the tumor is IDH-mutant and 1p/19q codeleted. While grade 2 tumors do not have features of anaplasia, grade 3 tumors show significant anaplastic features, including brisk mitosis (≥6 mitoses/10 high-power fields (HPFs)), and microvascular proliferation, and/or necrosis.

Immunophenotype

Oligodendrogliomas show immunopositivity for oligodendroglial transcription factors; Olig1, Olig2, and SOX10. The ensuing reactive astrocytes, minigemistocytes and gliofibrillary oligodendrocytes are GFAP positive. The intervening neuropil in the tumor can be synaptophysin positive and this should not be mistaken for a neurocytic neoplasm. Most tumors are immunopositive for IDH1p.R132H, show ATRX retained expression, and are immunonegative for p53. In a few cases harboring rare (non-canonical) IDH1 and 2 mutations, IHC for IDH1p.R132H can be negative. Alpha internexin immunopositivity and reduced expression of H3K27me3 is seen in many tumors but these are not surrogates for 1p/19q codeletion. The diagnosis is established by demonstrating IDH mutation and 1p/9q codeletion. While the grade 2 tumors have a low MIB1 labeling index, it is generally above 10% in grade 3 tumors.[42] The histological, IHC, and diagnostic molecular features of oligodendroglioma are shown in [Figure 2].{Figure 2}

Diagnostic molecular alterations

Oligodendroglioma, CNS WHO grades 2 and 3 tumors are defined by IDH mutations and 1p/19q codeletion.

1p/19q codeletion is the first biomarker in neuro-oncology discovered in the year 1994. Reifenberger analyzed molecular alterations in 37 cases of oligodendrogliomas and mixed gliomas by restriction fragment length polymorphism analysis. They observed frequent allelic deletions on 1p and 19q in oligodendroglial tumors. The loss of one hybrid chromosome results in 1p and 19q loss of heterozygosity (LOH).[43] This occurs as a result of an unbalanced whole-arm translocation between chromosomes 1 and 19 with the loss of the derivative chromosome, del (1;19) (p10; q10) which occurs early in the genesis of oligodendrogliomas. It was earlier reported that practically all the oligodendrogliomas harbor 1p/19q codeletion and IDH1R132 or IDH2R172 mutations.[44] Hence, the WHO 2016 update classification of CNS tumors and WHO CNS5 have recommended a mandatory demonstration of both the molecular alterations for the diagnosis of oligodendroglioma. Whole-exome sequencing studies have shown that more than 50% of tumors with 1p and 19q codeletion, harbor mutations in homolog of Drosophila capicua (CIC) at chromosome 19q13.2 and far upstream element-binding protein (FUBP1) at 1p31.1.[45] Another common accompanying genetic event in 1p/19q codeleted tumors is TERT gene promoter mutations. Importantly, 1p/19q codeletion codeleted tumors respond better to chemotherapy and radiation therapy and with a significantly better prognosis.[46],[47]

As 1p/19q codeletion and ATRX mutation are mutually exclusive, the cIMPACT-NOW update 2 clarified that in an IDH mutant diffuse glioma with astrocytic features exhibiting ATRX loss of expression, diagnosis of IDH-mutant astrocytoma can be offered without the need to perform testing for 1p/19q codeletion.[11]

1p/19q codeletion is routinely tested by fluorescence in situ hybridization (FISH). Although the technique has a few disadvantages, it is the most practical test for detection of 1p/19q codeletion and hence is widely used.

Oligodendroglioma, IDH-mutant and 1p/19q codeleted tumors show associated multiple CpG island hypermethylation. In addition, more than 90% of oligodendrogliomas show O(6) methylguanine DNA methyl transferase (MGMT) promoter methylation.[48] Homozygous deletion of CDKN2A/B is noted in a small subset of CNS WHO grade 3 oligodendrogliomas and is associated with worse clinical outcomes. This alteration is not seen in CNS WHO grade 2 oligodendrogliomas and hence could serve as a molecular marker of oligodendroglioma, IDH-mutant and 1p/19q codeleted CNS WHO grade 3.

Oligodendroglioma NOS, oligoastrocytoma NOS/NEC

Histologically typical oligodendrogliomas as well as oligoastrocytomas where molecular testing for IDH mutation and 1p/19q codeletion assay is not performed, or the results are inconclusive are designated as oligodendroglioma/oligoastrocytoma, NOS, respectively. However, the term “oligoastrocytoma” has been deleted from WHO CNS5 because on molecular testing, this tumor is either a genetically defined astrocytoma or oligodendroglioma.[49],[50] Therefore, in diffuse gliomas with an oligoastrocytic or ambiguous histology, IHC and molecular testing become important, and an algorithmic approach can be followed for a definitive diagnosis, as shown in [Figure 3] and [Figure 4]. Occasional oligoastrocytomas can show astrocytoma-like molecular alterations in the astrocytic areas and oligodendroglioma-like molecular alterations in the oligodendroglioma-like areas. Such tumors are designated as oligoastrocytoma, NEC.[10]{Figure 3}{Figure 4}

A practical approach for the diagnosis of adult-type diffuse gliomas, suitable for a resource-limited setting is shown in [Figure 3] and [Figure 4]. It is important to note that astrocytoma, IDH-mutant and oligodendroglioma, IDH-mutant and 1p/19q codeleted have essential and desirable diagnostic criteria. For example, a methylation profile is a desirable diagnostic criterion.

Conclusion

While there are obvious merits of molecular classification over the traditional histological classification in approaching adult diffuse gliomas, there are issues of technical and economical limitations in several countries. Therefore, it is important that a meaningful diagnosis is reached with the minimum number of molecular tests possible to decide on an appropriate management strategy in individual cases. Moreover, genetic alterations can be assessed by several different techniques that can vary in their sensitivity, specificity, and cost. Currently, there is no clear consensus by the WHO on a specific assay to be adopted for each genetic alteration. However, the knowledge of molecular alterations would certainly help deeper understanding of the biology of gliomas, thereby justifying the new WHO classification.

Acknowledgements

The authors acknowledge the technical staff and students of the neuro-oncology laboratory at NIMHANS for carrying out routine molecular tests for various brain tumors.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Figure 1: Depicts the histological and molecular features of astrocytoma, IDH-mutant tumors. (a-c) show histological features of grade 2 (a H&E, × 100), grade 3 (b, arrows mitosis, H&E, × 200), and grade 4 (c star focal necrosis) astrocytomas. d–f show immunohistochemical features of an astrocytoma, IDH-mutant CNS WHO grade 3. There is a homozygous deletion of CDKN2A (g FISH) and IDH1R132G mutation on sequencing (h)

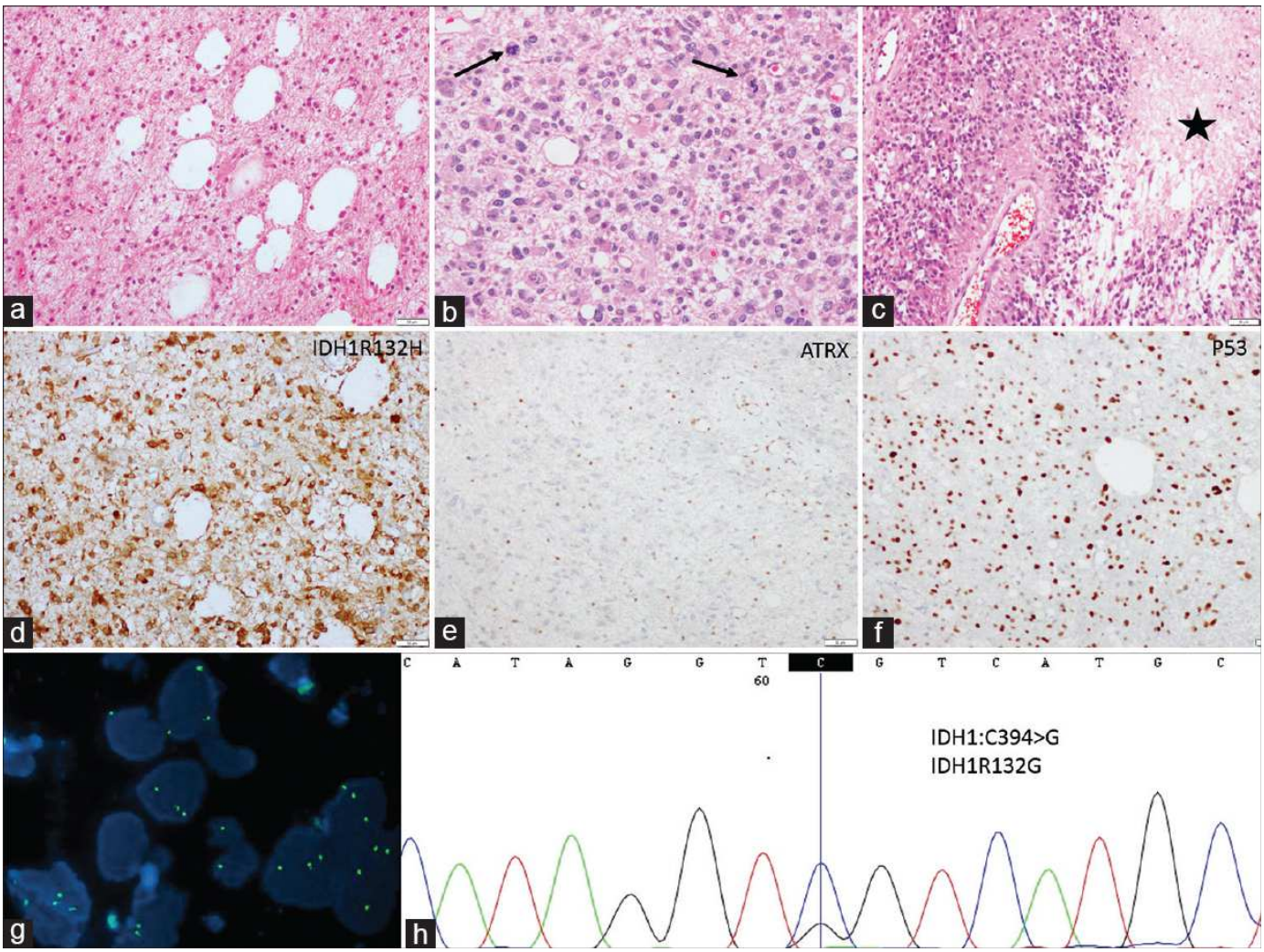

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Figure 2: Depicts the histological and molecular features of oligodendroglioma, IDH-mutant & 1p/19q codeleted tumors. (a and b) show histological features of grade 2 (a H&E × 100) and grade 3 (b, arrows mitosis, H&E × 200) tumors. (c and d) show immunohistochemical features of an oligodendroglioma with IDH1p.R132H immunopositivity (c, × 100) and retained nuclear expression of ATRX (d × 100). FISH shows codeletion of 1p (e, immunofluorescence) and 19q (f, immunofluorescence)

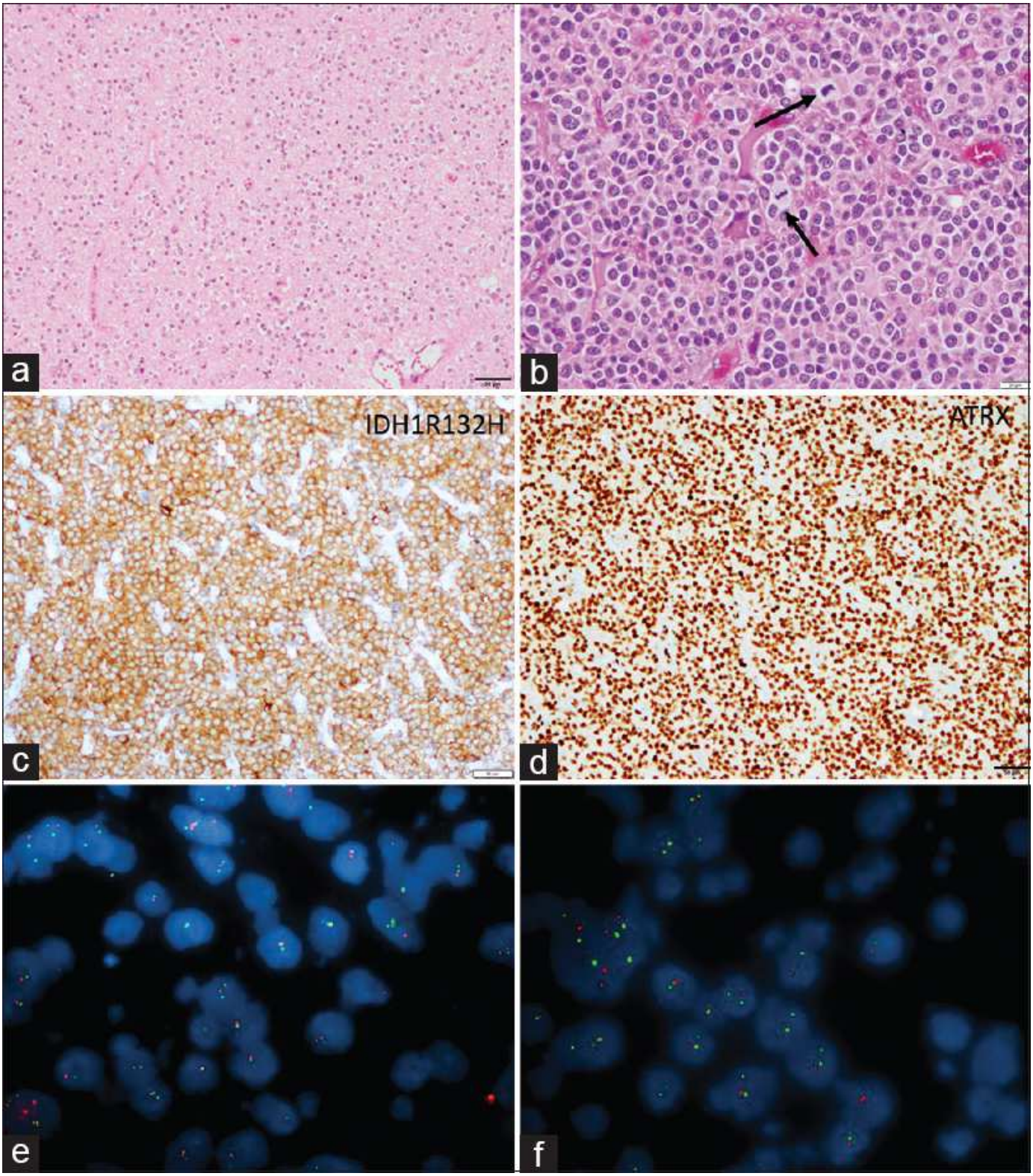
a
b
IDH1R132H
c
ATRX
d
e
f

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Figure 3: Shows an algorithm for the approach to adult-type diffuse gliomas, CNS WHO grades 2/3, IDH1p.R132H positive in a resource-constrained setting

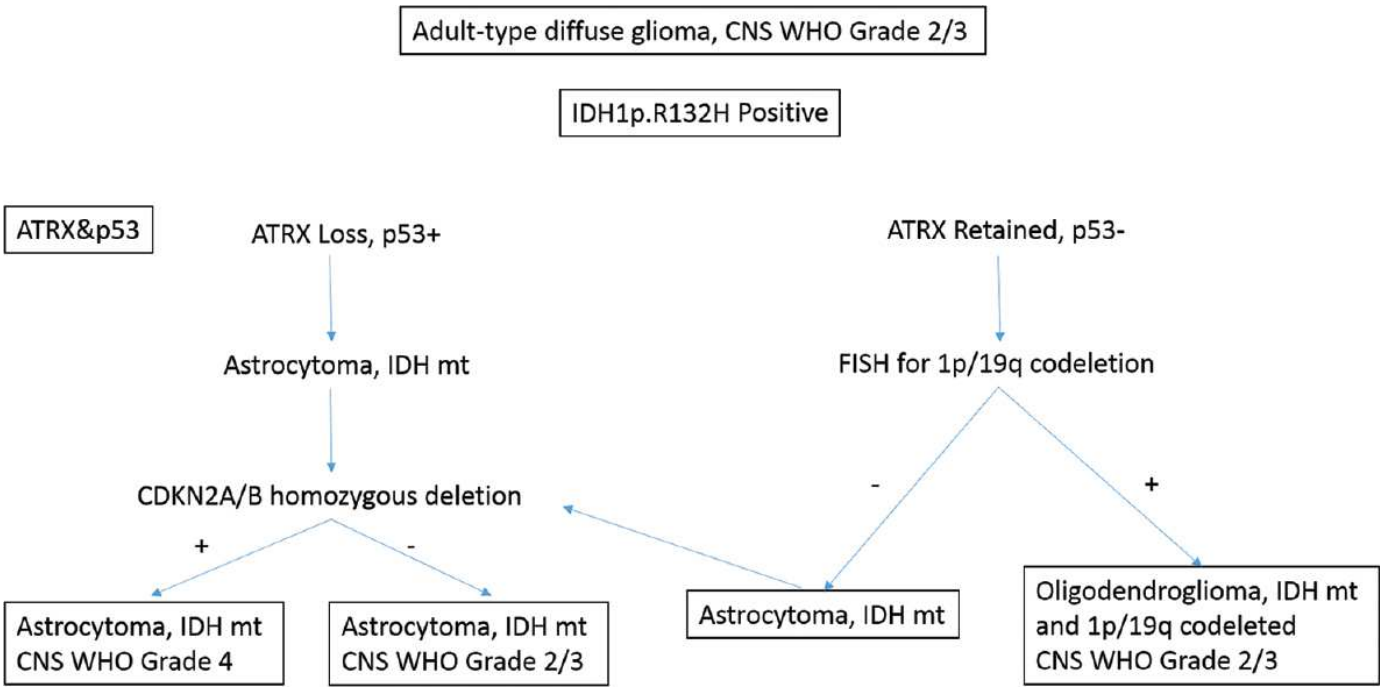

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Figure 4: Shows an algorithm for the approach to adult-type diffuse gliomas, CNS WHO grades 2/3, IDH1p.R132H negative in a resource-constrained setting

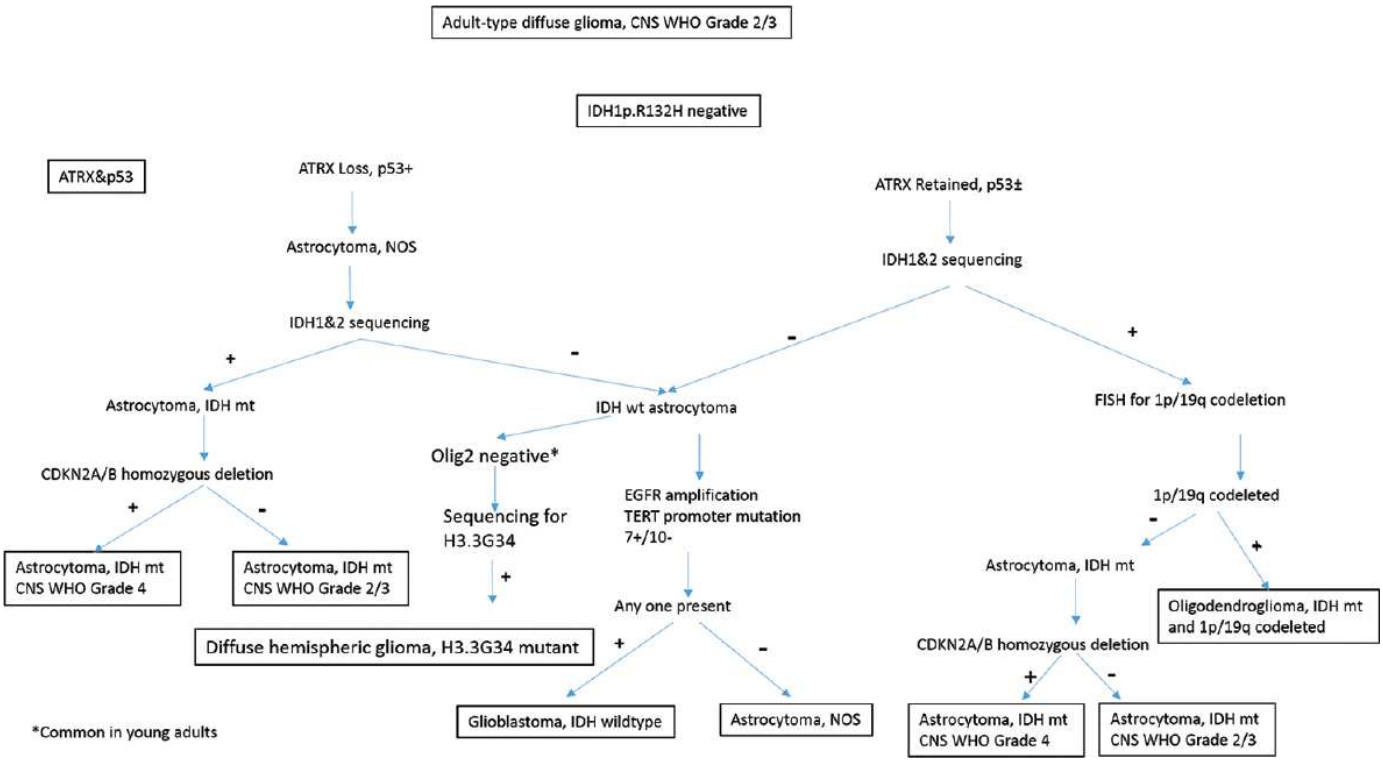

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Table 1: Shows the criteria for grading astrocytoma, IDH-mutant tumors[14]

Astrocytoma, IDH-mutant Grade	*Histology/Molecular features*
CNS WHO grade 2	A diffusely infiltrating astrocytic glioma with *IDH1* or *IDH2* mutation Well-differentiated Lacks features of anaplasia Absent or low mitosis No microvascular proliferation and/or necrosis No *CDKN2A/B* homozygous deletion
CNS WHO grade 3	A diffusely infiltrating astrocytic glioma with *IDH1* or *IDH2* mutation Significant anaplasia/mitosis No microvascular proliferation and/or necrosis No *CDKN2A/B* homozygous deletion
CNS WHO grade 4	A diffusely infiltrating astrocytic glioma with *IDH1* or *IDH2* mutation with microvascular proliferation and/or necrosis Or *CDKN2A/B* homozygous deletion Or any combinations of these features*