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Review

Adult type diffuse gliomas in the new 2021 WHO Classification

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Summary
Adult-type diffuse gliomas represent a group of highly infiltrative central nervous system tumors with a prognosis that significantly varies depending on the specific subtype and histological grade. Traditionally, adult-type diffuse gliomas have been classified based on their morphological features with a great interobserver variability and discrepancy in patient survival even within the same histological grade. Over the last few decades, advances in molecular profiling have drastically changed the diagnostic approach and classification of brain tumors leading to the development of an integrated morphological and molecular classification endowed with a more clinically relevant value. These concepts were largely anticipated in the revised fourth-edition of WHO classification of central nervous system tumors published in 2016. The fifth-edition (WHO 2021) moved molecular diagnostics forward into a full integration of molecular parameters with the histological features into an integrative diagnostic approach. Diagnosis of adult type diffuse gliomas, IDH mutant and IDH-wildtype has been simplified by introducing revised diagnostic and grading criteria. In this review, we will discuss the most recent updates to the classification of adult-type diffuse gliomas and summarize the essential diagnostic keys providing a practical guidance to pathologists.

Key words: astrocytoma, glioblastoma, IDH-WT, IDH mutant

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Introduction

Diffuse gliomas represents the most frequent tumors of the central nervous system (CNS) in adults with an overall survival that greatly varies depending on the specific subtype and histological grade [1]. Traditionally, classification of adult-type diffuse gliomas has been primarily based on tumor histology, resulted in a great interobserver variability and discrepancy in patient survival even within the same histological grade. During the last two decades, identification of key molecular alterations allowed to the development of an integrated morphological and molecular classification endowed with a more clinically relevant value. Since the previous 2016 updated 4th edition of the World Health Organization (WHO) classification of tumors of the CNS, adult-type diffuse gliomas have been classified in two different major categories depending on presence or absence of Isocitrate Dehydrogenase 1 and 2 (IDH1 and IDH2) mutations, the latter considered to have a worst prognosis [2]. In addition, chromosome 1p and 19q codeletion, along with IDH mutations in the absence of ATRX alterations, has been defined as molecular hallmarks of oligodendrogliomas [3]. Thus, determination of the IDH status has already become a standard practice in the diagnosis of gliomas. Increasing advances in the understanding of molecular pathogenesis of

gliomas and their impact on clinical practice warrants a more comprehensive integration of these information into the WHO classification, previously anticipated by publications of the cIMPACT-NOW (Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) [4,5] and now incorporated within the 5th edition of the WHO classification of CNS tumors (CNS WHO 2021) [6]. Adult-type diffuse gliomas are now assigned to three different tumor types: Astrocytoma, IDH-mutant; Oligodendroglioma, IDH-mutant and 1p/19q-codeleted; Glioblastoma, IDH-wildtype. IDH-mutant diffuse astrocytic tumors are considered a single entity graded as CNS WHO grade 2, 3, or 4 depending on different histological and molecular features. Grading is no longer entirely histological and the finding of CDKN2A/B homozygous deletion results in a CNS WHO grade 4 IDH-mutated astrocytoma or grade 3 oligodendroglioma, even in the absence of microvascular proliferation or necrosis [7]. Of note, the absence of IDH mutation predict an aggressive behavior along with the concurrent combination of other molecular features, such as gain of whole chromosome 7, loss of whole chromosome 10, TERT promoter mutations and EGFR amplification that assign the CNS WHO grade 4 to the tumor, independently from the histological features [5]. Therefore, the WHO 2021 classification of CNS tumors integrate molecular features into a revised grading criteria as critical events for improving prediction of prognosis and possible response to treatment[8]. In the present review, we discuss the implications of each update of the current WHO classification in order to provide key guidelines and general advice for the diagnosis of adult-type diffuse gliomas.

Adult type IDH1 mutant astrocytomas

Adult type astrocytoma IDH-mutant are diffusely infiltrating gliomas harboring a mutation in either *IDH1* or *IDH2* genes with absence of 1p and 19q codeletion and frequent inactivating mutations in *ATRX* and *TP53* genes [9]. They are currently defined as either astrocytomas CNS WHO grade 2, 3 or 4 depending on variable combination of histological and molecular features [6]. Previous designations such as IDH-mutant diffuse astrocytoma, anaplastic astrocytoma or glioblastoma are no longer accepted. The large majority of adult-type IDH-mutant astrocytomas occur in young adults with a median age of 40 years with a slight predominance in male and frequently presenting as supratentorial CNS WHO grade 2 or 3 lesions, even if they may be found in older patients and in other CNS regions, the latter being frequently CNS WHO grade 4 tumors. However, astrocytoma IDH-mutant are extremely rare in patients older than 60 years. The clinical picture varies greatly depending on topography and extension of the lesion [8] and MRI features commonly suggest a CNS WHO grade 2 or grade 3. However, when present, gadolinium enhancement suggests a CNS WHO grade 4. Recent advances of magnetic resonance spectroscopy (MRS) sequences at 3 Tesla (3T) has allowed detectability of the oncometabolite D-2-Hydroxyglutarate (2HG), a marker of IDH1 and IDH2 mutation [10], a reliable and useful information in the diagnostic work-up of these tumors.

Morphological features

Astrocytomas IDH-mutant are diffusely infiltrating tumors that histologically show variable morphological features depending on tumor grade. They are commonly composed of astrocytic cells with scarce cytoplasm and elongated or irregularly shaped hyperchromatic nuclei with indistinct nucleoli (Fig. 1, panel A). Microcystic changes, oligodendroglial-like appearance and presence of gemistocytic differentiation may be present. Interestingly, oligodendroglioma-like morphological features are more common in IDH-mutant astrocytoma than in the IDH-wildtype counterpart [11]. Depending on tumor grade, histological features vary from low-cell-density tumors with mild nuclear atypia and low mitotic rate, referring to CNS WHO grade 2 tumors, to lesions with increased cell density, atypia and higher mitotic rate, referred to CNS WHO grade 3 or 4 (Fig. 1, panel B and C). Indeed, WHO does not clearly delineate define cut-off for mitotic count and proliferative index and criteria does not substantially differ from the previous WHO recommendations [12]. Mitotic activity remains, for the CNS WHO grade 2 tumors barely detected with increasing proliferation rate in CNS WHO grade 3 and 4. Thus, increased mitotic activity and nuclear atypia still represents a key factor in the differential diagnosis between CNS WHO grade 2 and grade 3 astrocytomas. Albeit without clear established criteria for a reliable threshold, a mitotic rate ≥ 2-3 mitoses within the single tumor specimen and a Ki-67 proliferation index up to 10% have been associated to lower survival and with CNS WHO grade 3 astrocytomas [7]. These parameters may considerably vary in WHO grade 4 lesions. Morphological evidence of necrosis and/or glomeruloid microvascular proliferation identify CNS WHO grade 4 astrocytoma (Fig. 1, panel C), previously defined as Glioblastoma, a term now reserved exclusively to IDH-wildtype astrocytomas. Of note, despite the absence of histological criteria defining a WHO grade 4 tumor, the presence of homozygous deletion of CDKN2A and/or CDKN2B

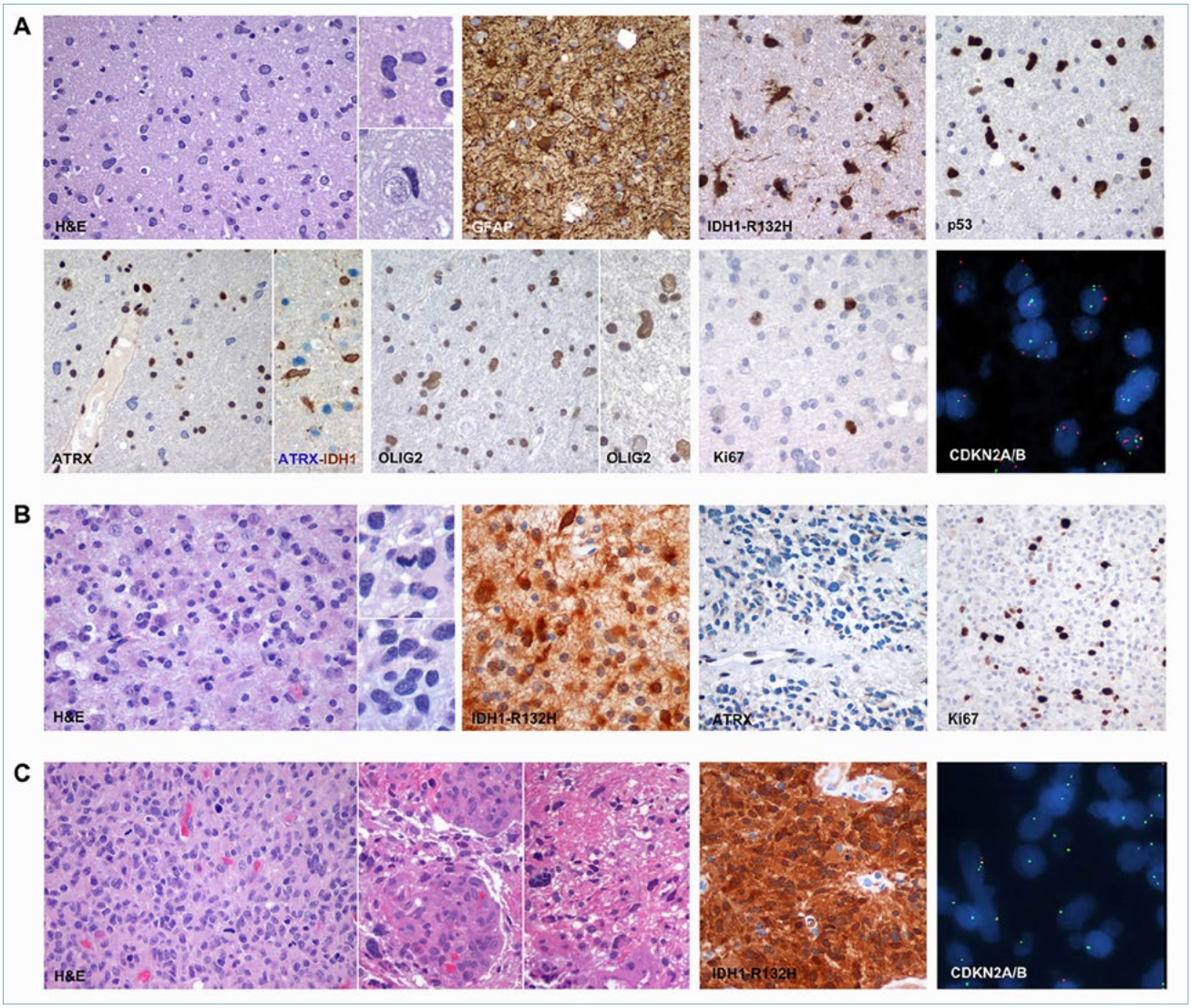

Figure 1. (A) Astrocytoma, IDH-mutant, WHO grade 2. Images show a diffusely infiltrating astrocytoma with low cell density and low mitotic rate (left panel; H&E). Tumor cells display elongated irregularly shaped hyperchromatic nuclei with scarce cytoplasm; perineuronal satellitosis is evident (upper and lower insets, respectively; H&E). Tumor cells are diffusely immunoreactive for GFAP, IDH1-R132H and p53 (upper right panels), while exhibiting loss of ATRX expression, confirmed by double immunostaining revealing positive nuclei of resident cells (blue), while IDH1-R132H positive tumor cells are ATRX negative (brown) (lower left panel and inset). OLIG2 is usually expressed both in tumor cells and in normal oligodendrocytes (middle panel and inset). Tumor display a low proliferating index and no CDKN2A/B deletion, as assayed by FISH analysis (rigth panels). (B) Astrocytoma, IDH-mutant, WHO grade 3. Increased cell density, atypia and higher mitotic rate characterize these tumors (left panel and insets; H&E). Immunohistochemical stains are positive for IDH1 R132H, negative for ATRX and reveal an elevated proliferation index (middle and right panels). (C) Astrocytoma, IDH-mutant, WHO grade 4. Increased cell density, pleomorphism, anaplasia, necrosis and/or glomeruloid microvascular proliferation are typical features of WHO grade 4 tumors (left panel and insets; H&E), along with immunoreactivity for IDH1-R132H and homozygous deletion of CDKN2A/B, considered sufficient to grade the tumor as WHO grade 4 (right panels). All images are from 40x and 60x original magnification.

is now sufficient to grade the tumor as CNS WHO grade 4 IDH-mutant astrocytoma [13] (see further description below).

Immunophenotype

Immunohistochemistry is a useful tool used as surrogates for investigating genetic alterations. IDH-mu-

tant astrocytomas are usually immunoreactive for the antibody recognizing the IDH1(p.R132H) somatic missense mutation, present in more than 90% of cases (Fig. 1). Immunostaining is highly sensitive and specific for the detection of the IDH1-R132H mutation and may also help to distinguish neoplastic cells from reactive gliosis, particularly in small hypocellular biopsies. Low-grade lesions, particularly in young patients, with no immunoreactivity for IDH1-R132H deserve to be investigated by molecular analysis to search for rare non-canonical IDH1 or IDH2 somatic mutations, including IDH1(p.R132C), IDH1(p.R132G), IDH1(p.R394T) and IDH2(p.R172K) [14]. Concurrent mutations in TP53 and ATRX gene are also a typical feature of IDH-mutated astrocytomas, molecular features that are usually related to strong and intense p53 immunoreactivity and loss of ATRX expression [9]. IDH-mutant astrocytomas usually show a reliably expression of GFAP, even if some lesions may have a subset of GFAP negative cells, particularly in tumors with an oligodendroglial-like appearance [11]. In the latter case, Synaptophysin may be focally expressed. Oligodendrocyte transcription factor 2 (OLIG2), a transcription factor with diffuse nuclear immunoreactivity in most of diffuse gliomas, is also usually expressed in IDH-mutant astrocytomas. Proliferation index, as assayed by Ki-67/MIB-1 immunostaining, vary according to the tumor grade (Fig. 1, panel A and B).

Molecular findings

By definition, the assessment of the IDH mutational status represents the major molecular hallmark of the IDH-mutant astrocytomas. Investigation on IDH1 mutational status is generally feasible in the routine neuropathological work-up using the specific antibody recognizing the IDH1(p.R132H) mutation. In cases with negative immunostaining for IDH1-R132H or in which immunohistochemistry is not feasible, particularly in young patients with p53 overexpressing tumors and loss of ATRX expression, the molecular assessment of IDH1 and IDH2 mutation is mandatory for the diagnosis [4]. Beyond the common IDH1 mutations at codon 132, tumors exhibit other IDH1 rare mutations (e.g. at codon 394) or mutation of the IDH2 gene, commonly at codon 172, the IDH2 (p.R172K) [14]. Infratentorial astrocytomas show a predominance of non-canonical IDH-mutation variants that are exceptional in supratentorial tumors. Moreover, infratentorial IDH-mutant are frequently ATRX retained tumors. These evidence highlight to be awareness of this distinct subtype and the importance to consider it in diagnostic workflows of infratentorial gliomas [15]. IDH1 or IDH2 missense mutations result in the accumulation of the oncometabolite 2-HG that plays a crucial role in promoting oncogenesis [16] and contributes to the acquisition of the CpG island hypermethylator phenotype (G-CIMP), a distinctive molecular feature of IDH-mutated astrocytomas [17]. Accordingly, the rate of O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in IDH-mutated astrocytomas is commonly observed [8]. Interestingly, different IDH mutations were reported to have different prognostic values due to variable genome-wide DNA-methylation levels [18]. As described, IDH-mutant astrocytomas also harbor concurrent TP53 and ATRX mutations. ATRX mutations account for an abnormal telomere maintenance leading to alternative lengthening of telomeres (ALT), which is mutually exclusive with activating mutations of the telomerase reverse transcriptase (TERT) gene that are conversely present in the majority of IDH-mutant oligodendrogliomas and IDH-wild-type glioblastomas [12]. Of note, ATRX deficiency is also associated with genomic instability that may favor the co-occurrence of TP53 mutations [19]. Detection of intense and diffuse p53 immunoreactivity is suggestive of TP53 mutation supporting the diagnosis of IDH-mutant astrocytoma. However, albeit the large majority of immunoreactive cases are TP53 mutated, nonsense mutations may be associated with a complete absence of immunostaining. Thus, it is important to correlate p53 expression with coherent morphology and other immunohistochemical findings, such as loss of ATRX and presence of IDH-mutations. Additional molecular alterations involving different pathways (e.g. RB1, CDK4, MET, PDGFRA, PIK3R1) have been described in IDH-mutant astrocytomas and may be associated to tumour progression [20]. However, for diagnostic purposes, the most important and well-recognized molecular alteration is the homozygous deletion of CDKN2A/B that has been associated with tumor grade and prognosis [13]. Identification of homozygous deletion of CDKN2A/B by FISH analysis (Fig. 1, panel A and C), that represents the most commonly used technical approach, is considered sufficient to classify IDH-mutant astrocytomas as WHO grade 4, independently of the histological features of the tumor [4,12].

Diagnosis

In young patients presenting with a non-enhancing supratentorial lesion that lacks ATRX nuclear expression, with strong immunoreactivity for p53 and evidence of IDH1 R132H immunoreactivity the diagnosis of IDH-mutant CNS WHO grade 2 astrocytoma should be considered. In case of lack of IDH1-R132H immunoreactivity IDH1/IDH2 DNA sequencing will be required. Increased mitotic activity, cellularity and nuclear atypia, along with evidence of microvascular proliferation and/or necrosis remains histological fea-

tures of IDH-mutant CNS WHO grade 3 or 4 astrocytomas. However, independently of the histological features, the presence of homozygous deletion of CDKN2A/B classified tumors as CNS WHO grade 4 (Fig. 2). A wide range of differential diagnosis has to be considered, including IDH-mutant and 1p/19q-codeleted oligodendroglioma, diffuse midline glioma H3.3 K27-altered, H3 G34-mutant astrocytomas, MYB- or MYBL1-altered and MAPK pathway-altered low-grade gliomas and high-grade astrocytoma with piloid features. However, albeit with some overlapping histological features, the assessment of the molecular profile will allow a definitive diagnosis.

Prognosis and treatment

Prognosis varies widely according to patient age, tumor grade and extent of resection. Patients with IDH-mutated CNS WHO grade 2 astrocytomas are significantly younger and survive significantly longer with a median survival up to 10 years, reduced to 5 years for grade 3 and even lower for grade 4 tumors with either histological features of malignancy or CDKN2A/B homozygous deletion. Standard treatment includes surgery and alkylating chemotherapy and/or radiotherapy for higher grade lesions. A variety of IDH-mutant-specific targeting strategies are emerging from preclinical research and clinical trials [21].

Oligodendrogliomas, IDH1 mutant and 1p/19q-codeleted

Oligodendroglioma are diffusely infiltrating tumours currently defined on a molecular level as IDH-mutant tumours with concurrent complete deletion of both 1p and 19q chromosomal arms. Incomplete or partial deletions are not compatible with the diagnosis of IDH-mutant 1p/19q-codeleted oligodendroglioma and demonstration of codeletion on whole 1p and 19q chromosomal arms losses by FISH analysis or molecular testing is required for the diagnosis [12]. They present either as CNS WHO grade 2 or grade 3 tumors, reflecting significant survival differences[1]. The term anaplastic oligodendroglioma is no longer recommended. Oligodendroglioma most commonly occur supra-tentorial with the frontal lobe accounting for about 60% of the cases followed by the temporal, parietal and occipital lobes. They are rarely observed within midline structures, brainstem, posterior fossa or spinal cord. Leptomeningeal spread and multifocality, in the contest of gliomatosis cerebri, are only occasionally observed, particularly at recurrence [22]. Oligodendrogliomas comprises about 5% of all brain tumour in adult with the CNS WHO grade 3 lesions being about one third of the cases. They are extremely rare in children with a peak incidence within the fourth and fifth decades of life and a slight male predominance [1]. Clinical presentation is non-specific with symptoms being

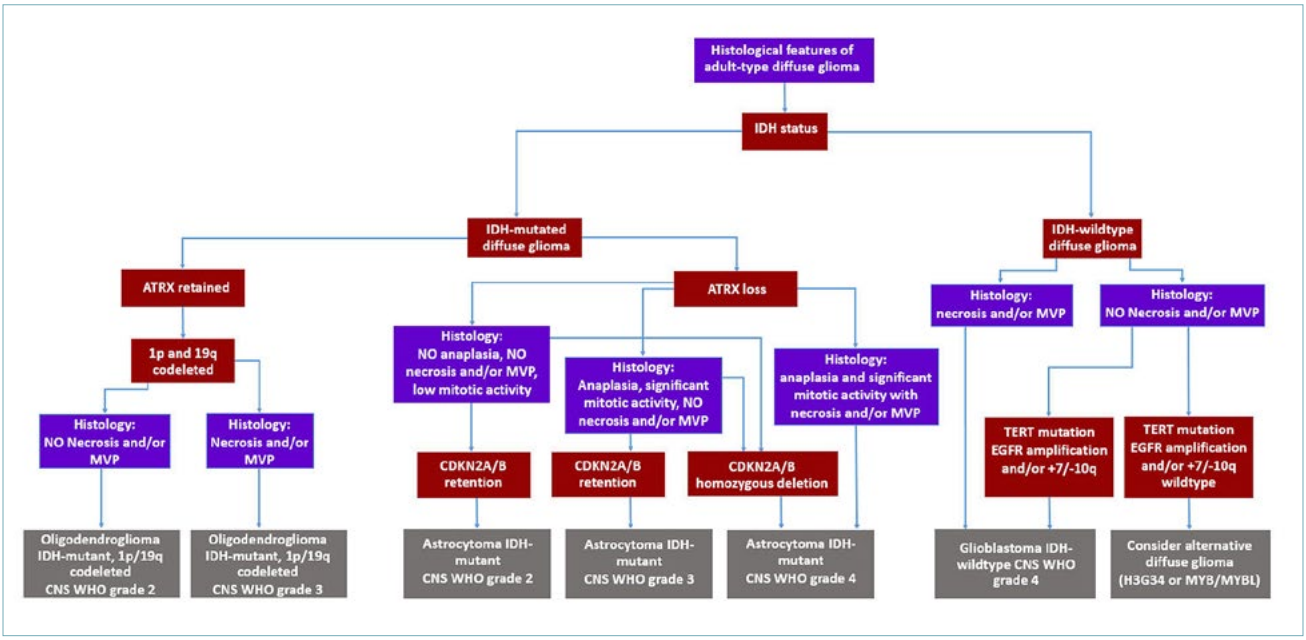

Figure 2. Flowchart shows the diagnostic algorithm of adult-type diffuse glioma, based on the most relevant histopathological features and molecular markers.

related to tumor location and increased intracranial pressure. On MRI imaging, oligodendroglioma present as a heterogeneous mass commonly involving the cortex or subcortical white matter and frequently associated with calcification, cystic degeneration and hemorrhage. Gadolinium contrast enhancement is a common feature but not a reliable indicator of tumor grade. They usually have an elevated cerebral blood volume (rCBV) due to increased vascularity. As described, MRS sequences has allowed detectability of the 2HG oncometabolite as a marker of IDH mutations [10]. In addition, the MRS detection of a cystathionine peak represents a reliable marker suggesting the presence of 1p deletion. However, these techniques currently have a limited use.

Morphological features

As reported, histological features do not formally contribute anymore to the diagnosis of oligodendroglioma since the tumor must fulfill molecular criteria. However, morphology still has the important role of addressing the molecular workup. Histologically, ol-

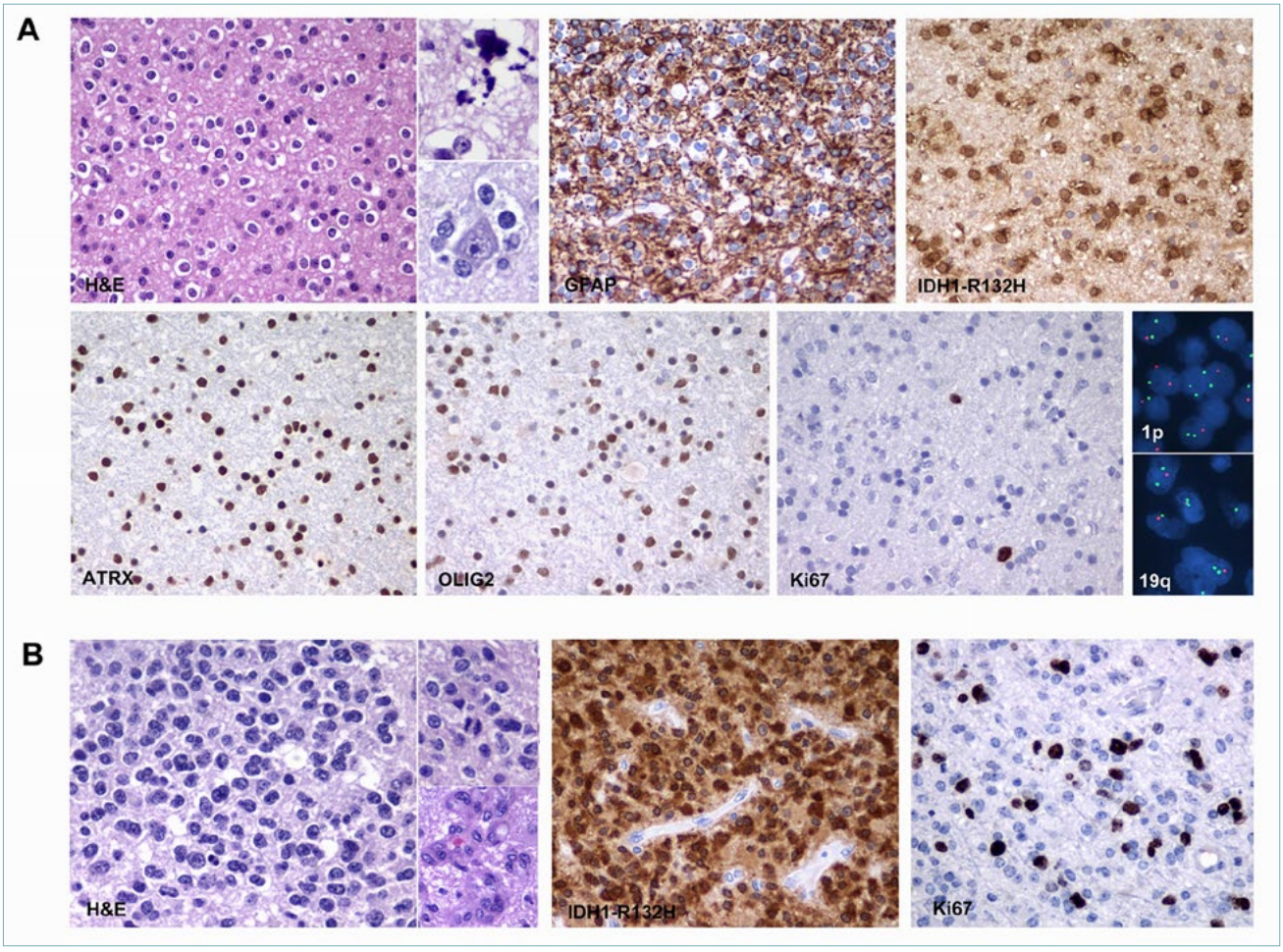

Figure 3. (A) Oligodendroglioma, IDH-mutant and 1p/19q codeleted, WHO grade 2. Representative images showing a diffusely infiltrating tumor characterized by round-to-oval monomorphic nuclei with perinuclear clearing. Microcalcifications and perineuronal satellitosis is a common feature (left panel and insets; H&E). Immunostains for GFAP is negative, while IDH1-R132H is diffusely positive (upper right panels). Tumor cells retain expression of ATRX and are diffusely immunoreactive for OLIG2 (lower left and middle panel). Proliferation index is low and FISH analysis reveals 1p/19q codeletion (right panels). (B) Oligodendroglioma, IDH-mutant and 1p/19q codeleted, WHO grade 3. Increased cellularity with nuclear anaplasia, mitotic figures, microvascular proliferation and/or necrosis are distinctive features of grade 3 tumors (left panel and insets; H&E). Immunohistochemistry reveals intense and diffuse IDH1-R132H immunoreactivity and a high proliferation index (middle and right panels). All images are from 40x and 60x original magnification.

igodendrogliomas consist of densely packed cells with round nuclei with perinuclear clearing, so-called "fried egg appearance", representing a technical artifact not seen on frozen sections or cytological smears. They have a dense and thin branching blood vessel network, defined as chicken wire vasculature, and microcalcifications are frequently seen (Fig. 3, panel A). CNS WHO grade 3 oligodendrogliomas show increased cellularity with obvious nuclear anaplasia, frequent mitotic figures, microvascular proliferation and necrosis (Fig. 3, panel B). Hypercellular nodules with occasional mitoses and moderate nuclear atypia are still consistent with grade 2 designation. A standard cut-off for the mitotic count has not been clearly defined, albeit more than 2.5 mitoses/mm^2 has been associated to a worst prognosis [6,12]. The presence of neuronal satellitosis and perivascular or subpial neoplastic cell distribution is a common finding. The presence of astrocytic differentiation does not preclude the diagnosis of oligodendroglioma. Small gemistocytes with eosinophilic cytoplasm and eccentrically placed nuclei are often seen, particularly in CNS WHO grade 3 tumors. The designation of oligoastrocytoma, referring to mixed tumors with both oligodendroglioma and astrocytoma features, has been already removed from the previous WHO 2016 since the introduction of the molecular diagnostic criteria indicating that the large majority of these tumors have a molecular profile of either oligodendroglioma or astrocytoma [23]. However, rare cases exhibiting a true mixed phenotype with both morphologically appearing oligodendroglial regions displaying 1p/19q codeletion and astrocytic areas harboring TP53 and ATRX mutations have been reported, suggesting an evolutionary differentiation from common progenitor cells [24].

Immunophenotype

Likewise IDH-mutant astrocytomas, immunoreactivity for the IDH1-R132H antibody is detected in the large majority of oligodendrogliomas. Lack of immunoreactivity warrants molecular investigations to search for the rare non-canonical IDH1 or IDH2 somatic mutations. In contrast to the IDH-mutant astrocytoma, oligodendrogliomas retain nuclear expression of ATRX and commonly lack nuclear expression of p53 (Fig. 3, panel A). IDH-mutant tumors with concurrent intense p53 immunoreactivity and loss of ATRX expression are likely to be IDH-mutated astrocytomas and molecular testing for 1p and 19q deletion may be not necessary. GFAP is usually not expressed or selectively expressed in the minigemistocytes and gliofibrillary components. OLIG2, albeit not specific, is usually widely expressed (Fig. 3, panel A). Immunoreactivity for synaptophysin may be present in neoplastic cells, in line with the pro-neuronal phenotype of these tumors. Oligodendrogliomas are frequently immunoreactive for α-internexin, supposed as a predictor of 1p/19q-codeletion [25], as well as the reduced nuclear expression of H3 p.K28me3 (K27me3) [26], but they neither sensitive nor specific and molecular analysis remains the gold standard. Finally, Ki-67/MIB1 immunostaining varies according to tumour grade being usually low in CNS WHO grade 2 oligodendrogliomas (< 5%) and more than 10% in CNS WHO grade 3 lesions (Fig. 2, panel A and B), albeit a definitive cut-off has not been established.

Molecular findings

IDH-mutated and 1p/19q codeleted oligodendrogliomas are by definition molecularly defined by the presence of concurrent IDH1 or IDH2 mutations and 1p/19q codeletion. Oligodendrogliomas harbor missense mutations in the IDH1/2 genes with more than 90% of IDH mutations being related to the canonical IDH1 p.R132H mutation. Interestingly, oligodendrogliomas have a higher proportion of non-canonical mutations as compared to IDH-mutated astrocytomas, with mutation of IDH2 on codon 172 representing the most frequent alteration. The presence of whole-arm deletions of the chromosomes 1p and 19q is a common finding and is required for the diagnosis. Incomplete or partial deletions are not compatible with the diagnosis. The most commonly used commercial probes for 1p/19q testing by FISH localize to the region 1p36 and 1q25 on chromosome 1 and 19p13 and 19q13 on chromosome 19 (Fig. 3, panel A) and does not explicitly evaluate whole-arm chromosomal deletions raising the possibility of false results. Thus, FISH analysis should be interpreted with caution and integrated within a coherent clinico-pathological and morphological contexts. In unusual situations, it may be worthwhile using alternative assays, such as next-generation sequencing or global DNA methylation profiling. In addition, nearly all oligodendroglioma harbor TERT promoter mutations [27]. However, detection of TERT mutations is not sufficient for the diagnosis since this alteration is also observed in a subset of 1p/19q-intact IDH-mutant astrocytomas. Moreover, oligodendrogliomas arising in pediatric age or in adolescents frequently lack TERT promoter mutations [28]. Other molecular alterations frequently occur in oligodendrogliomas, including mutation of CIC[29] (human orthologue of the Drosophila melanogaster capicua gene, located in 19q13.2 chromosome region) and FUBP1 [30] (far upstream element-binding protein 1, located at chromosome 1p31.1 region) and alterations of the NOTCH signaling pathway, all associated with recurrences and shorter survival [31,20]. Finally, homozy-

gous deletion of CDKN2A has been reported as an independent marker of shorter survival in a small subset of CNS WHO grade 3 oligodendrogliomas but being absent in those of CNS WHO grade 2 [13].

Diagnosis

Young adult patients presenting with a frontal mass and MRS features suggestive of IDH1/2 mutations and 1p loss are likely to have an oligodendroglioma. As previously reported, oligodendrogliomas are molecularly defined by the presence of concurrent IDH1 or IDH2 mutations and 1p/19q codeletion. Thus, the diagnostic workup requires molecular analysis of IDH1/IDH2 status by either IDH1-R132H immunohistochemistry and/or gene sequencing, as well as demonstration of whole-arm chromosomal losses on 1p and 19q by FISH analysis or other molecular techniques (Fig. 2). Of course, lack of IDH1-R132H immunoreactivity require IDH1/IDH2 DNA sequencing to exclude non-canonical mutations. Older patients aged more than 60 years are likely not to have an oligodendroglioma and IDH1/2 testing is not strictly required. In addition, oligodendrogliomas usually retains ATRX expression and p53 in barely detected. Usually, loss of nuclear ATRX expression is sufficient to exclude oligodendroglioma and favor the diagnosis of an IDH-mutant astrocytoma, even in the presence of oligodendroglioma-like histological features. In this case, additional testing for 1p/19q codeletion are not necessary. The majority of oligodendrogliomas are CNS WHO grade 2 tumors. However, MRI enhancing lesion with histological features of malignancy, such as increased cellularity with marked atypia and high mitotic count (≥ 2.5 mitoses/mm^2), microvascular proliferation and/or necrosis are consistent with the diagnosis of CNS WHO grade 3 oligodendrogliomas. TERT promoter mutations are frequently found in IDH-mutant and 1p/19q-codeleted oligodendrogliomas [28], albeit molecular testing for TERT mutations is not strictly required for the diagnosis. Of note, albeit hardly infrequent, TERT mutations have been reported in IDH-mutant astrocytomas. A wide range of morphological mimics should be considered in the differential diagnosis, including neurocytoma, IDH-mutated astrocytomas with oligodendroglioma-like features, ependymomas with clear cell morphology and low-grade gliomas such as pilocytic astrocytomas and dysembryoplastic neuroepithelial tumors. Metastatic clear cell carcinomas may also be considered. However, integration of clinical, immunophenotypical and molecular features are usually sufficient to allow a definitive diagnosis.

Prognosis and treatment

Overall, oligodendrogliomas are associated with better prognosis compared to IDH-mutant astrocytomas with an overall survival of more than 10 years [1]. Younger age at diagnosis, extent of surgical resection and frontal lobe location are associated with favorable outcome. Grading remains of utmost importance in predicting the prognosis and response to treatment. CNS WHO grade 2 oligodendroglioma frequently recur and malignant progression to CNS WHO grade 3 at recurrence is common. Homozygous CDKN2A and/or CDKN2B deletion has been reported in a subset of CNS-WHO grade 3 oligodendroglioma and is associated with poor prognosis [13]. Therapeutic options for recurrent or progressive CNS WHO grade 2 oligodendroglioma comprise adjuvant chemotherapy with Temozolomide. CNS-WHO grade 3 oligodendroglioma are usually treated with radiotherapy and/or chemotherapy.

Glioblastoma, IDH1 wildtype

The annual incidence of gliomas is approximately of six cases per 100,000 individuals worldwide. Men are 1.6-fold more likely to be diagnosed with gliomas than women [1]. IDH-wildtype diffuse astrocytic gliomas in the adult have distinct genetic alterations and clinical behaviour compared to IDH-mutant. In the WHO 2021 classification the term glioblastoma is used only for IDH-wildtype tumors and its diagnosis is based on the histologic presence of mitotic activity, microvascular proliferation and/or necrosis (Fig. 2) [12]. In the new classification molecular biomarkers are important not only to define neoplastic entities but also to reduce the dependency on morphologic features for tumor classification. Indeed, previous histopathologic classification suffered from high intra- and inter-observer variability, particularly among low grade IDH-wildtype tumors [12]. Presence of specific molecular alteration i.e., TERT promoter mutation, epidermal growth factor receptor (EGFR) amplification, and combined chromosome 7 gain/chromosome 10 loss (+7/–10), even in the context of grade 2 or 3 histopathology defined diffuse glioma are characterized by poor prognosis and are coherent with the molecular diagnosis of IDH-wildtype glioblastoma (Fig. 2) [5,12].

Morphological features

Glioblastoma is a tumor with high and heterogeneous cellularity, composed of astrocytic cells with marked nuclear atypia, ranging from a monotonous poorly differentiated cells to pleomorphic astrocytic cells with numerous giant cells, and sometimes lipidized or granular cells. Brisk mitotic activity is evident and necrosis and/or microvascular proliferation is the rule

(Fig. 4, panel A). Tumor cells show and infiltrative diffuse pattern, can accumulate in the subpial region, around neurons (satellitosis) and vessels, causing the frequent multifocality of glioblastoma. Occasionally, glioblastoma presents area of mesenchymal and epithelial metaplasia. In mesenchymal metaplasia different lineages could be present such as osseus, chondroid, adipocytic or myogenic. In glioblastoma IDH-wildtype the most frequent is sarcomatous metaplasia. In epithelial metaplasia, could be observed areas of squamous with epithelial whorls and/or glandular structures. A specific pattern can be recognized by a predominance of a specific cell type and along with the corresponding histological subtype i.e., giant cell glioblastomas (GC-GBM), gliosarcoma (GS) and epithelioid glioblastoma (E-GBM). GC-GBMs tumor contains numerous multinucleated giant cells with variable size, atypical mitotic feature and marked stromal reaction with reticulin. Osseus, chondroid, adipocytic or myogenic component could be seen (Fig. 4, panel A). GFAP and OLIG2 are positive in glial component and focally positive in mesenchymal component. Epithelioid glioblastoma (E-GBM) is characterized by a population of closely packed epithelioid cells and some rhabdoid cells with high mitotic activity, along with microvascular proliferation and focal necrosis. Most of these tumors harbor a mutation of the BRAF gene in which valine (V) is substituted by glutamic acid (E) at amino acid 600 (BRAF–V600E) [32]. Other patterns comprise small cells glioblastoma which show

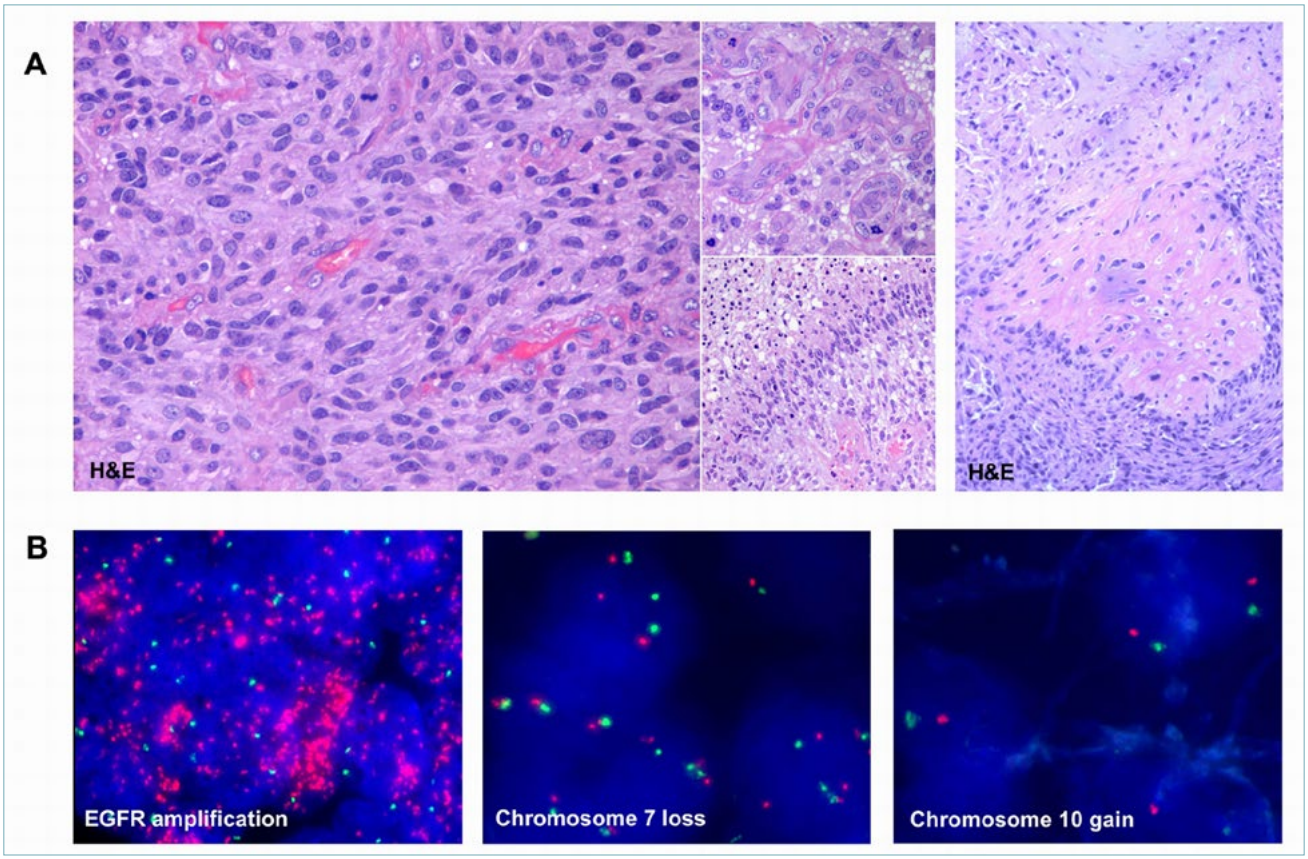

Figure 4. (A) Glioblastoma, IDH-wildtype. Photomicrograph showing neoplastic astrocytes with nuclear pleomorphism and areas of necrosis and microvascular proliferation (left panel and insets; H&E). As described, GBM may have areas of sarcomatous transformation composed of a spindle cell with round to spindled vesicular nuclei, pleomorphism and mitotic figures. Osteoid tissue with prominent osteoblastic rimming and occasional scattered osteoclastic giant cells are shown ((right panel; H&E). (B) FISH analysis with fluorescent probes for EGFR (red) and chromosome 7 centromere (green) reveals numerous and merged EGFR signals in interphase nuclei of neoplastic cells, indicative of EGFR amplification (left panel). A representative FISH analysis of interphase nuclei of histologically WHO grade 2 diffuse astrocytoma IDH wildtype are shown. Nuclei shows three signals detected with the chromosome 7 and one signal with chromosome 10 centromeric probes (middle and right panels, respectively), molecular features associated to aggressive behavior and diagnosis of glioblastoma, IDH-wildtype.

frequent EGFR amplification, oligodendrocyte-like glioblastoma which often harbor FGFR3:TACC3 fusion and have a better prognosis, and granular cells glioblastoma which are diffusely immunoreactive for OLIG2, and CD68, while being negative for GFAP.

Immunophenotype

Glioblastoma display GFAP and OLIG2 immunoreactivity. Focal GFAP positivity with CD68 stain can be observed typically in granular cells and giant cells glioblastoma. Synaptophysin immunoreactive cells may be also present in some glioblastomas. Glioblastoma with mesenchymal component have a typically biphasic tissue pattern with reticulin-rich mesenchymal and reticulin-free glial elements. Cytokeratin expression is observed in foci with squamous cell metaplasia. ATRX protein is usually retained and IDH1-R132H immunostaining is negative. Nuclear accumulation of p53 protein could be present, suggesting a TP53 mutation. Proliferation index (Ki-67/MIB-1) is usually high with a great regional variation.

Molecular findings

IDH-wildtype glioblastomas exhibit a high intra-and inter-tumoral molecular heterogeneity. Characteristic molecular alterations include mutations in genes regulating receptor tyrosine kinase (RTK)/rat sarcoma (RAS)/phosphoinositide 3-kinase (PI3K), TP53, and retinoblastoma (RB) signaling [33]. Activation of RAS/MEK/ERK and PI3K/AKT signaling pathways regulate cell proliferation and survival and could be stimulated by several alterations, i.e., amplification and/or mutation in receptor tyrosine kinase (RTK), including epithelial growth factor receptor (EGFR), platelet-derived growth factor receptor α (PDGFRA) [34], MET [35], and fibroblast growth factor receptor (FGFR) [36]. EGFR alterations are one of the most common alterations in patients with GBM, present in almost 50% of cases. Specific alterations are amplification, mutation, rearrangement, even the most frequent is amplification. Usually, the latter is associated with a mutation in the extracellular domain or in in-frame intragenic deletions encoding EGFR vIII [37]. Regarding FGFR, in a small percentage of glioblastoma FGFR1-TACC1 was detected, while FGFR3 fusions are more commonly observed [38]. Most FGFR3 fusions are with transforming acidic coiled-coil 3 (TACC3) and result from the in-frame fusion of the FGFR3 N-terminus with the TACC3 C-terminus. Histologically FGFR3-TACC3 fusion protein define a subset of IDH-wildtype glioblastoma with specific histopathology [39] and with diffuse growth pattern. FGFR aberrations detection in some cases can be useful for diagnosis. TERT promoter (TERTp) mutation is common in glioblastoma and is a negative prognostic factor [40]. Recent efforts have demonstrated associations between TERT promoter mutation, EGFR amplification, and combined losses of whole chromosome 10 and/or gains of whole chromosome 7 (+7/–10) (Fig. 4, panel B) and poor prognosis [5]. This observation led to define the molecular parameter that can assign the grade independently of histological findings. This means that is possible to define a molecular glioblastoma, IDH-wildtype CNS WHO grade 4, even in cases that otherwise appear histologically lower grade 2 o 3, when present concurrent gain of whole chromosome 7 and loss of whole chromosome 10 (+7/–10), EGFR amplification and/or TERT promoter mutation. However, TERT promoter mutation is the less specific parameter for GBM than the aforementioned two parameters, suggesting being cautious to perform a diagnosis of molecular glioblastoma if only this alteration is present in otherwise low grade diffuse IDH wildtype glioma [41]. To strength this concept it is important to remember that other gliomas with a diffuse and infiltrative growth pattern share histopathological similarity with IDH-wildtype diffuse glioma. These tumors usually affect children and adolescent age groups, and include diffuse hemispheric glioma, H3 G34-mutant [42], diffuse astrocytoma MYB- or MYBL1-altered or diffuse low-grade glioma MAPK pathway-altered [43]. These entities typically arise in children and adolescent but could originate also in adult patient. This important consideration should keep in mind during the diagnostic algorithm (Fig. 2). Tumor mutation burden is a potential biomarker for estimating the abundance of neoantigens, which have a relevance for immunotherapy. IDH-wildtype glioblastoma with high mutation burden (hypermutation) is associated with mutation or loss of one or more MRM (mismatch repair) genes. [44]. Microsatellite instability (MSI) appear as a 'predictive' biomarker for a good response to immunotherapy. In some cases of recurrent GBM, hypermutations are detected after alkylating chemotherapy and are associated with somatic mutations or the decreased expression of MMR genes [45].

Diagnosis

Patients > 55 years, with a non-midline tumor, retained nuclear ATRX expression and IHC negativity for IDH1 R132H, diagnosis of IDH-wildtype glioblastoma grade 4 could be made. In all other cases of diffuse gliomas, a lack of IDH1-R132H immunoreactivity should be followed by IDH1 and IDH2 DNA sequencing to exclude the presence of non-canonical mutations. IDH-wildtype diffuse gliomas without vascular proliferation or necrosis should be tested for EGFR amplification, TERT promoter mutation and a +7/–10 cytogenetic. In addition, the presence of histone H3.3 G34R/V muta-

tions should be evaluated to identify H3.3 G34-mutant diffuse hemispheric gliomas in young patients with IDH-wildtype gliomas and ATRX mutated neoplasm. Diffuse gliomas of the thalamus, brainstem or spinal cord must be tested for histone H3K27M mutations to identify H3K27M-altered diffuse midline gliomas. Finally, MYB- or MYBL1-altered or diffuse low-grade glioma MAPK pathway-altered should be considered in diffuse low grade IDH-wildtype in adults (Fig. 2).

Prognosis and treatment

Glioblastoma (GBM) represents an aggressive tumor type with a median survival of only 14 months [1]. Some subtypes of glioblastomas have a different survival, for example prognosis of giant cells glioblastoma (< 1% of all glioblastoma) is poor but slightly better than conventional glioblastoma (11 vs 8 months) [46]. Epithelioid glioblastoma usually progresses rapidly and has a particularly poor prognosis with a median survival from the time of diagnosis of 5 months in children and 6 months in adults [47]. Prognosis of gliosarcoma is poor and like classic IDH-wildtype glioblastoma [48-50]. Concomitant radiotherapy and chemotherapy with Temozolomide are the standard of care for adults with newly diagnosed glioblastoma who are in good general and neurological condition and are aged < 70 years. Temozolomide is more effective in patients with MGMT promoter-methylated tumors whereas its activity in patients with MGMT promoter-unmethylated tumors is probably marginal. IDH-wildtype glioblastoma is characterized by marked intra-and inter-tumoral molecular heterogeneity and this highlight the intrinsic limitations of standard single approaches in glioblastoma. Targeting multiple tumor cell clones with distinct therapeutic susceptibility and resistances represents a significant barrier to the treatment of glioblastoma. EGFR gene amplification and overexpression are typical of GBM. Approximately 50% of GBM showing EGFR amplification, and a specific EGFR mutant, EGFR variant III (EGFRvIII), can be detected. EGFRvIII compared to patients with wildtype EGFR GBM, have an older age at diagnosis, worse prognosis, and resistance to chemo-radiotherapy. Aberrant activation of receptor tyrosine kinases (RTKs), including platelet-derived growth factor receptor (PDGFR), are frequently observed in glioma. Accumulating evidence suggests that PDGFR plays critical roles during glioma development and progression and is a promising drug target for GBM therapy. Inhibition of FGFR kinase and oral administration of an FGFR inhibitor prolongs survival of mice harboring intracranial FGFR3-TACC3–initiated glioma. FGFR-TACC fusions could potentially identify a subset of GBM patients who would benefit from targeted FGFR kinase inhibition [39].

Conclusive remarks

In the last few decades, the identification of relevant molecular alterations and biomarkers has considerably enhanced the prognostic stratification and recognition of potentially druggable molecular pathways for adult-type diffuse glioma. The fifth edition of the WHO 2021 further strengthens the value of an integrated approach to CNS tumor classification, thereby generating more uniform nosological entities likely providing a more accurate prognostic stratification of patients. While histological parameters, such as microvascular proliferation and/or necrosis, are still of a great value in the diagnosis of glioblastoma, it has to be considered that the presence of certain molecular alterations allows the diagnosis of glioblastoma independently from the histopathological features, establishing the concept of a "CNS WHO molecular grade 4". Glioblastoma is now defined as a diffuse astrocytic glioma with no mutations in either IDH or histone H3 genes and characterized by either microvascular proliferation and necrosis or the presence of EGFR amplification, chromosome 7 gain/10 loss, and/or TERT promoter mutations (Fig. 2). This observation implies that IDH-wildtype gliomas without histological features malignancy require molecular testing for gain or loss of whole chromosome 7 and 10 respectively, TERT promoter mutation and EGFR amplification. IDH-mutated astrocytoma are now graded as CNS WHO grade 2, 3 or 4, depending on both histopathological features and/or the presence of homozygous deletion of CDKN2A/B locus, a molecular marker of WHO grade 4 (Fig. 2). These findings highlight the importance of integrating histological grading and molecular profiling for the diagnosis of IDH-mutant and IDH-wildtype gliomas and to ensure the best stratification for patient care.

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Conflicts of interest

The authors declare no conflict of interest.

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Ethical consideration

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

Authors' contributions

Both M.A. and P.L.P. contributed to conceptual design, manuscript preparation and critical revision, and approved the final version of this manuscript.

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