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IDH-mutant diffuse gliomas: tips and tricks in the era of genomic tumor classification

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Summary. According to the fifth edition of the World Health Organization (WHO) Classification, diffuse gliomas typically occurring in adults are classified as oligodendroglioma IDH-mutant and 1p/19q codeleted, astrocytoma IDH-mutant, and glioblastoma IDH-wildtype. Among these, the former has the most favorable clinical course, whereas the latter has the worst prognosis. In IDH-mutant gliomas, the *IDH1* p.R132H is the most frequent IDH mutation. Other mutations in *IDH1* are rare and predominantly found in astrocytomas, whereas *IDH2* mutations are mostly observed in oligodendrogliomas. Astrocytomas IDH-mutant display frequent immunohistochemical loss of ATRX, which is mutually exclusive with 1p/19q codeletion. They are graded based on histopathological features and the presence of *CDKN2A/B* homozygous deletion, whereas the criteria for grading oligodendrogliomas are less defined.

DNA methylation profiling has recently shown three additional distinct tumor types among diffuse IDH-mutant gliomas: infratentorial astrocytoma IDH mutant; primary mismatch repair deficient IDH-mutant astrocytoma (PMRDIA); and oligosarcoma. Infratentorial astrocytoma IDH-mutant is enriched in *IDH1* or *IDH2* mutations that differ from the *IDH1* p.R132H mutation and are detectable only by gene sequencing, displays less frequent ATRX loss and *MGMT* promoter methylation than supratentorial IDH-mutant astrocytomas, and may additionally harbor the *H3* K27M mutation, which is typically found in H3 K27-altered diffuse midline glioma. PMRDIA occurs in the context of primary mismatch repair deficiency, is characterized by frequent *MSH6* mutations, hypermutation, low frequency of *MGMT* promoter methylation, and poor clinical outcomes. Finally, oligosarcoma is a tumor featuring oligodendroglial and sarcomatous areas, and is characterized by worse outcome and frequent 1p/19q copy number loss of heterozygosity.

Key words: Astrocytoma, IDH, Oligodendroglioma, Mismatch repair deficiency, Oligosarcoma

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Introduction

Gliomas are the second most common tumors of the central nervous system (CNS) (Ostrom et al., 2022).

According to the fifth edition of the World Health Organization (WHO) Classification, they are distinguished based on their growth pattern, into “diffuse”, which diffusely infiltrate the surrounding nervous tissue, and "circumscribed”, which rather display a more contained growth (WHO Classification of Tumours Editorial Board, 2021). This distinction is clinically relevant, because circumscribed gliomas can be cured by surgical resection. However, owing to their intrinsic infiltrating nature, diffuse gliomas tend to recur after surgery and may require adjuvant treatment. The fifth WHO edition further classifies diffuse gliomas into “adult-type” and “pediatric-type”, recognizing for the first time that diffuse gliomas primarily occurring in adults and those primarily occurring in children have distinct clinical and molecular features (Louis et al., 2021; WHO Classification of Tumours Editorial Board, 2021).

Because of the significant favorable prognostic value of *IDH* mutations and 1p/19q codeletion in diffuse gliomas in adults (Eckel-Passow et al., 2015; Reuss et al., 2015; Han et al., 2020), they have been classified as: astrocytoma IDH-mutant, oligodendroglioma IDH-mutant and 1p/19q codeleted, and glioblastoma IDH-wildtype (Louis et al., 2021, WHO Classification of Tumours Editorial Board, 2021).

According to specific histopathological or molecular features (detailed in the following sections), astrocytoma IDH-mutant is graded as CNS WHO grade 2, 3, or 4, oligodendroglioma IDH-mutant and 1p/19q codeleted as CNS WHO grade 2 or 3, while glioblastoma IDH-

wildtype is classified as CNS WHO grade 4 (Louis et al., 2021; WHO Classification of Tumours Editorial Board, 2021). Therefore, differently from the previous classification (2016), the designation of "glioblastoma" is now reserved to IDH-wildtype tumors, whereas astrocytic IDH-mutant tumors with histologically worrisome features (microvascular proliferation and/or necrosis) are classified as IDH-mutant astrocytomas, CNS WHO grade 4 (WHO Classification of Tumours Editorial Board, 2021).

IDH mutations were first identified in diffuse gliomas in 2008 (Parsons et al., 2008). They represent an early event in the tumorigenesis of these neoplasms (Watanabe et al., 2009) and are maintained during tumor recurrence (Aihara et al., 2017; Barresi et al., 2022). Approximately 90% of IDH-mutant diffuse gliomas harbor the *IDH1* p.R132H mutation (Yan et al., 2009; Capper et al., 2010), which can be detected via immunohistochemistry using a specific antibody against the mutant epitope (Capper et al., 2010). Of the remaining 10%, 5% displayed other mutations in IDH1 at residue R132, and 5% displayed mutations in IDH2 at residue R172 (Hartmann et al., 2010). DNA sequencing is required to identify *IDH2* and *IDH1* mutations that differ from p.R132H (so-called "non-canonical IDH mutations) (Reuss et al., 2015). *IDH1* mutations at residue R132 and *IDH2* mutations at residue R172 are gain-of-function alterations that induce overproduction of the oncometabolite 2-hydroxyglutarate, which leads to a hypermethylation phenotype (Dang et al., 2009).

Over the last decade, a novel diagnostic approach, as an alternative to histopathology, has been used for tumors of the central nervous system. This is based on the concept that different tumor types harbor distinct DNA methylation profiles depending on their cell of origin and the molecular alterations that they acquire during tumorigenesis (Fernandez et al., 2012). In 2018, CNS tumors were distinguished in 82 methylation classes (Capper et al., 2018). The methylation class family glioma IDH-mutant included three subclasses: i) subclass astrocytoma (mainly including tumors histologically classified as CNS WHO grade 2 or 3); ii) subclass high-grade astrocytoma (mostly formed of tumors histologically classified as CNS WHO grade 3 or 4); and iii) subclass 1p/19q codeleted oligodendroglioma (Capper et al., 2018). Other subtypes with distinct DNA methylation profiles have been recently described.

The aim of this paper was to review the clinical, pathological and molecular features of IDH-mutant diffuse gliomas and discuss potential diagnostic clues and algorithms, possible pitfalls and rare subtypes.

Astrocytoma IDH-mutant

The majority of IDH-mutant astrocytomas are localized in the supratentorial compartment, and specifically in the frontal lobes, similar to oligodendrogliomas IDH-mutant and 1p/19q codeleted (Stockhammer et al., 2012). Although a subset of tumors may localize to the infratentorial compartment, they represent a molecularly distinct tumor type (Banan et al., 2020), which is discussed in a separate section of this review.

IDH-mutant astrocytomas mainly affect young adults, with a median age of 37 years at diagnosis (interquartile range: 29-45 years; frequency in subjects > 60 years: 1.8%). According to the fifth edition of the WHO Classification, this is defined as a diffusely infiltrating *IDH1*- or *IDH2*-mutant glioma with frequent *ATRX* and/or *TP53* mutations and the absence of 1p/19q codeletion (which instead characterizes oligodendroglioma) (Brat et al., 2021). Therefore, the mere presence or absence of specific genetic features defines this tumor type, whereas a diffuse growth pattern represents the only histological diagnostic criterion. However, while the mutation at codon 132 of *IDH1* or codon 172 of *IDH2* is an essential diagnostic criterion, demonstrating the absence of 1p/19q codeletion is not necessary in cases showing *ATRX* mutations or the immunohistochemical loss of ATRX protein (Brat et al., 2021). Indeed, *ATRX* mutations are found in approximately 70% of IDH-mutant astrocytomas (Suzuki et al., 2015) and they are mutually exclusive with 1p/19q codeletion (Leeper et al., 2015). Because *ATRX* mutations are truncating, this results in the loss of the corresponding protein, which can be demonstrated using immunohistochemistry and a specific antibody (Reuss et al., 2015). Therefore, immunohistochemical loss of ATRX protein is highly specific to astrocytoma in the differential diagnosis of oligodendroglioma, and its association with IDH mutation is sufficient for the diagnosis of astrocytoma IDH-mutant with no need to analyze 1p/19q codeletion status (Reuss et al., 2015; Ammendola et al., 2021) (Fig. 1). It should be emphasized that the assessment of ATRX immunostaining should always consider the endothelial or other non-neoplastic cells that serve as internal positive controls for the immunoreaction. Indeed, because even mild hypoxic damage can result in ATRX degradation in tissues, approximately 18% of diffuse gliomas have a complete loss of ATRX immunostaining in all cells (Ammendola et al., 2021), which could be misinterpreted as ATRX loss related to gene mutations. In cases with no positivity in the internal controls, ATRX immunostaining should be considered non-conclusive (Ammendola et al., 2021), and other immunostainings (discussed in the following section) can be used as a surrogate for 1p/19q codeletion, if this test is not available.

TP53 mutation is an additional molecular feature that characterizes over 90% of IDH-mutant astrocytomas and is mutually exclusive with 1p/19q codeletion (Suzuki et al., 2015). Immunohistochemical staining of p53 protein is commonly used in routine practice as a surrogate for *TP53* mutation; indeed, immuno-positivity of >10% tumor nuclei is considered to predict the presence of *TP53* mutations with a high diagnostic accuracy (Takami et al., 2015). Therefore, strong

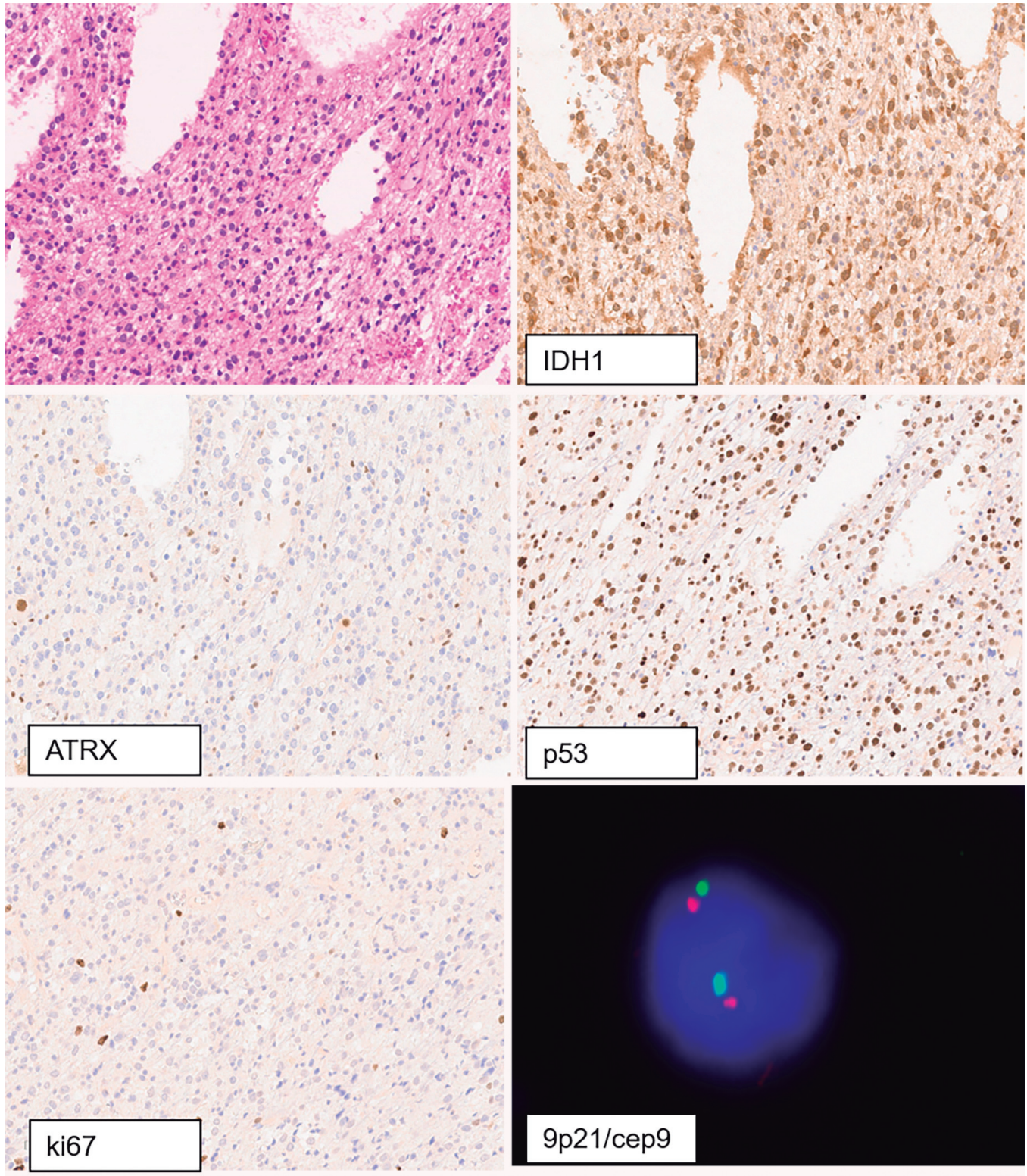

Fig. 1. Astrocytoma IDH-mutant CNS WHO grade 2. The immunohistochemical loss of ATRX with retained expression in the internal positive control is specific to astrocytoma in the differential diagnosis versus oligodendroglioma, with no need to assess 1p/19q codeletion. Extensive and strong p53 immunostaining further supports astrocytoma. FISH analysis showed the presence of two copies of 9p21 region and two copies of chromosome 9 (cep: centromeric probe for chromosome 9). Ki-67 labelling index showed low proliferation.

widespread immunostaining for p53 supports the diagnosis of astrocytoma IDH-mutant in the differential towards oligodendroglioma IDH-mutant and 1p/19q codeleted (Louis et al., 2018). However, it should be acknowledged that recurrent oligodendroglioma IDH mutant and 1p/19q codeleted may acquire *TP53* mutations after treatment with temozolomide and radiotherapy (Barresi et al., 2022) and show widespread positivity for p53, which should not be misinterpreted as a change to astrocytic genotype. In addition, truncating mutations in *TP53* may lead to complete absence of p53 immunostaining (Barresi et al., 2022). Therefore, the use of p53 immunostaining alone is discouraged as a surrogate to 1p/19q codeletion testing for the differential diagnosis between astrocytoma IDH-mutant and oligodendroglioma IDH-mutant and 1p/19q codeleted (Brat et al., 2021).

Histologically, IDH-mutant astrocytomas may range from well-differentiated, low-density and lowly proliferative tumors to anaplastic, hypercellular and highly proliferating tumors. Their histopathological features, together with genetic features consisting of the homozygous deletion (HD) of *CDKN2A/B*, are used as criteria for grading these tumors (Brat et al., 2021). Astrocytoma IDH-mutant CNS WHO grade 2 is defined as an infiltrative tumor, with a mild to moderate cellularity, lacking microvascular proliferation, necrosis, and *CDKN2A/B* HD, which is composed of glial cells with mild nuclear atypia, oval hyperchromatic nuclei (Fig. 1) and absent or uncommon mitoses (Brat et al., 2021). Compared to CNS WHO grade 2, astrocytoma IDH-mutant CNS WHO grade 3 features higher tumor density, even focal anaplasia, and significant mitotic activity (Fig. 2) (Brat et al., 2021). Although the cut-off of mitoses distinguishing CNS WHO grade 2 and 3 astrocytomas IDH-mutant has not been established, one mitosis is sufficient for the designation of grade 3 in small biopsies, whereas more are required in larger specimens (Brat et al., 2020). Finally, IDH-mutant astrocytoma is classified as CNS WHO grade 4 in the presence of microvascular proliferation and/or necrosis (Fig. 2) and/or *CDKN2A/B* HD (Brat et al., 2021). The latter has been incorporated as a criterion for grading astrocytomas IDH-mutant in the fifth WHO edition because tumors histologically defined as grade 3 and showing this genetic abnormality had a clinical course corresponding to that of grade 4 tumors (Shirahata et al., 2018; Appay et al., 2019). According to the WHO classification, an astrocytoma IDH-mutant with histological features consistent with CNS WHO grade 2 is diagnosed as CNS WHO grade 4 if *CDKN2A/B* HD is present. Therefore, the analysis of *CDKN2A/B* HD is mandatory for the proper grading of these tumors, according to the WHO.

CDKN2A/B HD can be identified using fluorescence in situ hybridization (FISH), methylation profiling, or next-generation sequencing (Nikiforova et al., 2016; Marker and Pearce, 2020; Tirro et al., 2022). All these techniques have several limitations and require expert personnel to interpret the results. FISH analysis is carried out using two probes: one that hybridizes to chromosome 9 centromeric sequences, and another that covers a region of 9p that includes *CDKN2A, CDKN2B,* and *MTAP* genes (Marker and Pearce, 2020). Therefore, FISH may give false-positive results in cases with deletions smaller than the region covered by the 9p probe. In addition, there is currently no consensus on the cut-off for nuclei with *CDKN2A/B* HD required to define the presence of this genetic alteration.

In a study of 121 IDH-mutant astrocytomas using FISH and a cut-off of 30% deleted nuclei, *CDKN2A/B* HD was found in 1.8%, 3.2% and 27% of cases

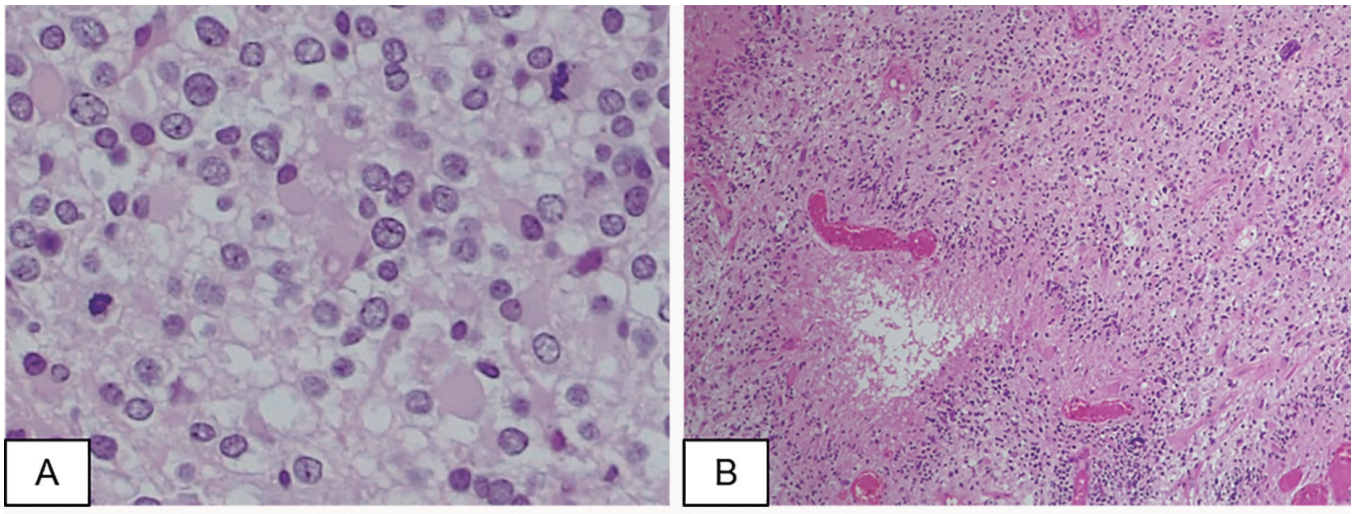

Fig. 2. Histopathological features of astrocytoma IDH-mutant CNS WHO grade 3 **(A)** and grade 4 **(B)**. Brisk mitotic activity was consistent with histological CNS WHO grade 3 **(A)**, while microvascular proliferation and palisading necrosis were consistent with CNS WHO grade 4.

histologically classified as grade 2, 3 or 4 (Marker and Pearce, 2020). Notably, this genetic alteration was significantly associated with a worse prognosis only in tumors histologically classified as grade 4 (i.e. with microvascular proliferation and/or necrosis) and this result was invariable using a cut-off value of 10% or 20% nuclei with *CDKN2A/B* HD (Marker and Pearce, 2020). Another study, using FISH and a cut-off of 10% nuclei, did not find *CDKN2A/B* in any of the 15 analyzed IDH-mutant astrocytomas histologically classified as grade 2 (Satomi et al., 2021). Moreover, using DNA methylation array, Shiharata et al. did not evidence *CDKN2A/B* HD in any of the 54 IDH-mutant astrocytomas analyzed and histologically classified as grade 2, and this genetic alteration was prognostically relevant only in tumors histologically classified as grade 3 or 4 (Shirahata et al., 2018). Finally, in the Tumor Cancer Genome Atlas (TCGA) merged cohort of low-grade gliomas and glioblastomas (accessible at www.cbioportal.org), we found *CDKN2A/B* HD in 4/110 (3.6) and in 7/102 (7%) IDH-mutant astrocytomas histologically defined as grade 2 or 3 (unpublished data). *CDKN2A/B* HD was significantly associated with shorter patient overall survival in the latter (P=0.0229), but not in the former (P=0.161). Therefore, *CDKN2A/B* seems to be rare in IDH-mutant astrocytomas histologically classified as grade 2 and its prognostic value in this setting probably needs further confirmation.

CDKN2A encodes for the p16 protein; however, whether the immunohistochemical loss of p16 correlates with *CDKN2A* homozygous deletion is controversial (Satomi et al., 2021). Therefore, the p16 immunohistochemical assessment is not recommended as a surrogate for *CDKN2A* homozygous deletion. Since *MTAP* is located on chromosome 9p21, only 165 kb telomeric to *CDKN2A*, and it is often deleted simultaneously with *CDKN2A* in tumors, immunohistochemical loss of MTAP protein has been recently proposed as a surrogate for *CDKN2A/B* homozygous deletion (Satomi et al., 2021). Although further studies are needed to validate its use in clinical practice, the loss of *MTAP* seems to predict *CDKN2A/B* with a high sensitivity (88%) and specificity (98%) (Satomi et al., 2021).

Although the morphology of histologically defined astrocytomas IDH-mutant CNS WHO grade 4 was traditionally considered overlapping with that of glioblastoma IDH-wildtype, some differences exist between these tumor types. Indeed, zonal and/or palisading necrosis is considerably more frequent in IDH-wild type glioblastoma than in IDH-mutant astrocytoma CNS WHO grade 4 (90% vs 50%) (Nobusawa et al., 2009). In addition, the latter features significantly more *TP53* (96.2% vs 27%) and *ATRX* (76.9% vs 4.6%) mutations and less frequent *EGFR* amplification (0% vs 46.3%), *CDKN2A/B* HD (16.7% vs 57.7%) and *PTEN* alterations (4.2% vs 33.9%) (Barresi et al., 2021).

Oligodendroglioma IDH-mutant and 1p/19q codeleted

Establishing the precise epidemiology of molecularly defined oligodendrogliomas is not easy; most reported data are, indeed, related to histologically defined, and not molecularly confirmed, oligodendrogliomas. A recent study of 93 patients reports a median age of onset of 43 years (interquartile range: 37-52 years), a frequency of 11% in patients older than 60 years and no sex predilection (Wijnenga et al., 2018). Therefore, oligodendroglioma IDH-mutant and 1p/19q codeleted seems to affect slightly older individuals and have a higher frequency in the elderly compared to IDH-mutant astrocytoma (Wijnenga et al., 2018). On imaging, these tumors often show calcifications, though this is not a diagnostic criterion (Smits and van den Bent, 2017).

According to the fifth edition of WHO Classification, oligodendroglioma is defined as a diffusely infiltrating glioma characterized by the co-occurrence of *IDH1* or *IDH2* mutations and codeletion of the entire chromosome arms 1p and 19q (Reifenberger et al., 2021). Therefore, similarly to IDH-mutant astrocytoma, this tumor type is defined only by the presence of genetic alterations, and not by histological features. However, its typical morphology (Fig. 3), consisting of oligodendrocyte-like cells with uniformly round nuclei and a perinuclear halo (a fixation artifact that confers a characteristic "fried-egg" appearance), and chicken-wire vessels (Reifenberger et al., 2021), is highly helpful in recognizing oligodendroglioma IDH-mutant and 1/19q codeleted before genetic testing.

According to the WHO Classification, oligodendroglioma IDH-mutant and 1p/19q codeleted is graded as CNS WHO grade 2 or 3. This latter is characterized by high cellularity, nuclear atypia, microvascular proliferation, necrosis and brisk mitotic activity (Fig. 4) (Reifenberger et al., 2021). It has been suggested that the presence of $\geq$ 6 mitoses/ 10 high power fields of 0.55 mm in diameter is associated with worse prognosis (Giannini et al., 2001), although, this cut-off value has not been established in genetically defined oligodendroglioma IDH-mutant and 1p/19q codeleted. The grading of oligodendrogliomas IDH-mutant and 1p/19q codeleted tumors may have relevant clinical implications. Indeed, according to the current guidelines of the American Society of Clinical Oncology and Society of Neuro-Oncology, patients with CNS WHO grade 3 tumors are treated with radiation therapy (RT) combined with chemotherapy, while patients with CNS WHO grade 2 tumors undergo a watch-and-wait approach until they show any signs of tumor progression (Mohile et al., 2022). *CDKN2A/B* HD was found in approximately 10% of WHO CNS grade 3 tumors, in association with shorter overall and progression-free survival, and in none of the WHO grade 2 oligodendrogliomas (Alentorn et al., 2015; Appay et al., 2019). Therefore, in cases with borderline histological

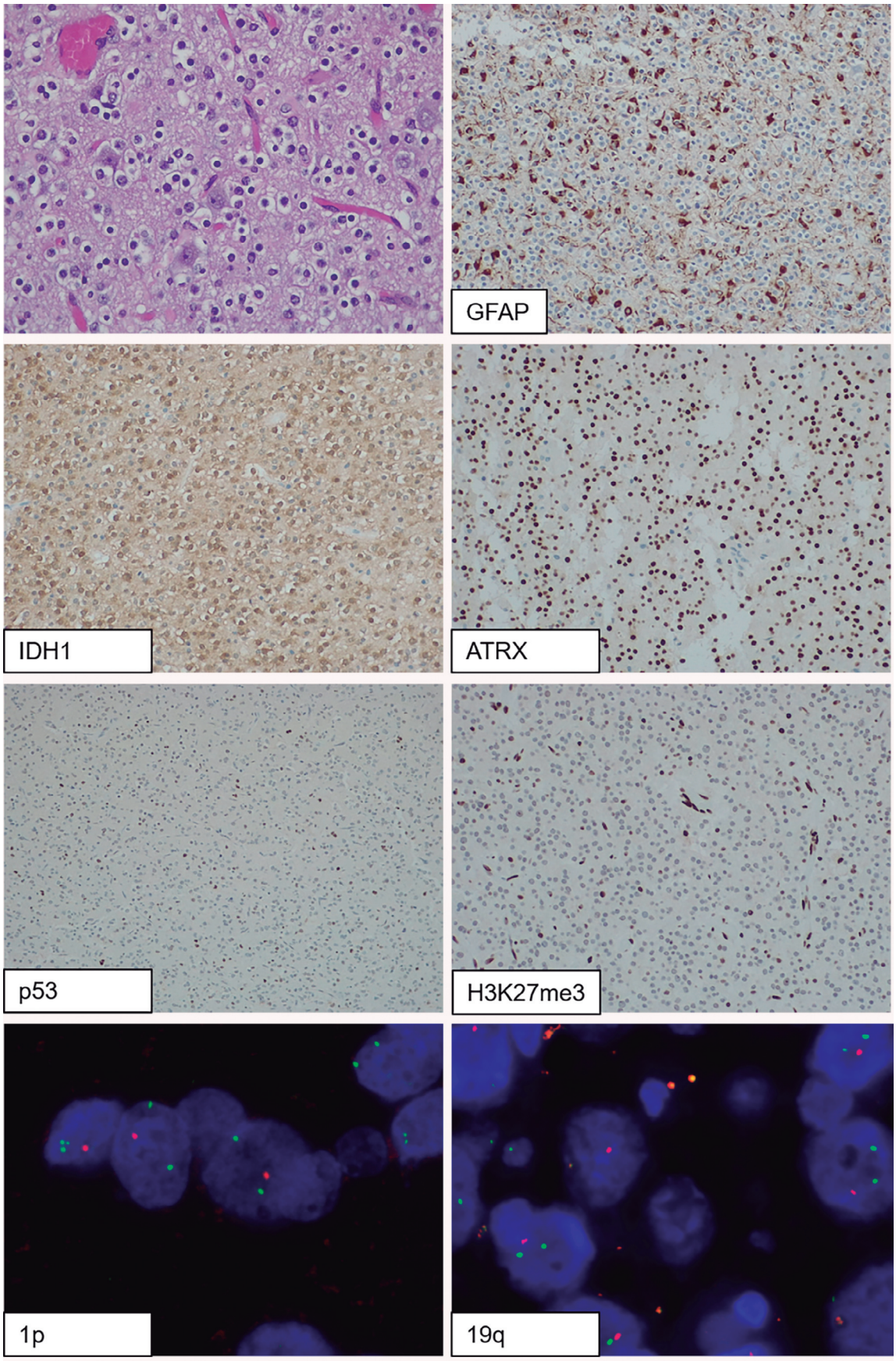

Fig. 3. Oligodendroglioma IDH-mutant and 1p/19q codeleted, CNS WHO grade 2. The tumor cells had uniform rounded nuclei and a peri-nuclear clear halo, stained positive for IDH1 p.R132H, retained ATRX immunostaining and featured p53 in <10%. H3 K27me3 immunostaining was lost consistent with an oligodendroglioma. FISH analysis showing 1p/19q codeletion confirmed this hypothesis.

features, *CDKN2A/B* homozygous deletion can be used as a diagnostic marker for CNS WHO grade 3 tumors (Reifenberger et al., 2021).

Histologically, oligodendroglioma should be differentiated from non-neoplastic entities, such as demyelinating diseases; the presence of a high number of macrophages in the latter is a useful diagnostic clue. In addition, several tumor types, including astrocytoma IDH-mutant, dysembryoplastic neuroepithelial tumor (DNET), and pediatric-type diffuse low-grade gliomas, enter the histological differential diagnosis of oligodendroglioma IDH-mutant and 1p/19q codeleted. The demonstration of IDH mutation allows the exclusion of DNET or pediatric-type diffuse gliomas, which are IDH-wildtype tumors. However, distinguishing between oligodendroglioma and astrocytoma IDH-mutant can be challenging in some cases. As specified in the previous section, cases showing the immunohistochemical loss of ATRX can be diagnosed as astrocytomas IDH-mutant without testing 1p/19q codeletion, while the latter analysis is required to differentiate astrocytoma IDH-mutant and oligodendroglioma IDH-mutant and 1p/19q codeleted when ATRX immuno-expression is retained.

1p/19q codeletion results from the balanced whole-arm translocation of chromosomes 1 and 19, followed by the loss of one of the two derivative chromosomes composed of 1p and 19q (Jenkins et al., 2006; Woehrer and Hainfellner, 2015). This genetic alteration remains stable during tumor recurrence (Barresi et al., 2022).

FISH is one of the most commonly used methods to detect 1p/19q codeletion in routine practice (Fig. 4) (Woehrer and Hainfellner, 2015). However, it should be acknowledged that this technique may lead to false positive results in in 3.6% of cases (Ball et al., 2020). Indeed, the probes bind to a small part of 1p (1p36) and 19q (19q13); therefore, FISH cannot distinguish between the complete loss (which is required for the diagnosis of oligodendroglioma IDH-mutant and 1p/19q codeleted) and the partial loss of chromosomes1p and 19q (Horbinski et al., 2011; Woehrer and Hainfellner, 2015) (which is also found in a percentage of astrocytomas). In addition, FISH analysis can give false negative results in the context of copy neutral loss of heterozygosity (LOH), where 1p deletion is followed by duplication of the retained 1p chromosome (Barresi et al., 2022). In doubtful cases, re-testing using other methods, such as Comparative Genomic Hybridization Array (CGH-A), which can differentiate between the partial and complete loss of 1p and 19q, is warranted. Next-generation sequencing can also be applied to allow the simultaneous detection of gene mutations and 1p/19q codeletion with a high diagnostic accuracy (Pallavajjala et al., 2022).

In a recent study, our group demonstrated that the immunohistochemical loss of histone 3 trimethylated in lysine 27 (H3K27me3) (Fig. 4), combined with the retention of ATRX expression, is 100% specific for oligodendroglioma IDH-mutant and 1p/19q codeleted in the differential diagnosis of IDH-mutant diffuse gliomas with an ambiguous (oligoastrocytic) morphology (Ammendola et al., 2021). H3K27me3 immuno-staining can be helpful in the differential diagnosis between astrocytoma IDH-mutant and oligodendroglioma IDH-mutant and 1p/19q codeleted, when 1p/19q codeletion testing has equivocal results or is not available, although this latter analysis cannot currently be amended to diagnose oligodendroglioma according to WHO criteria.

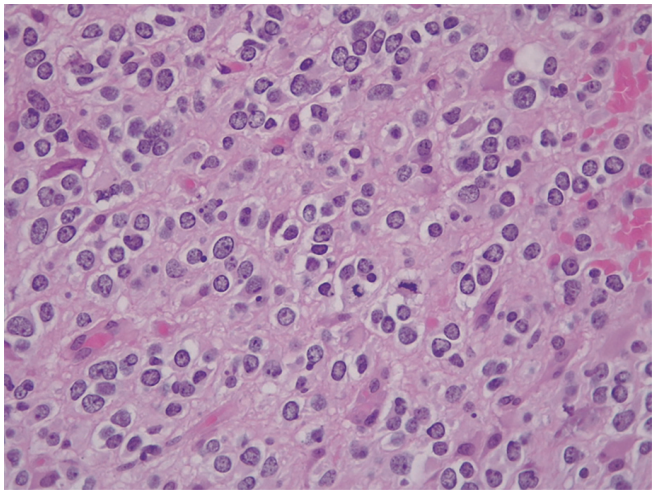

Fig. 4. Histological features of oligodendroglioma IDH-mutant and 1p/19q codeleted, CNS WHO grade 3. Brisk mitotic activity indicated WHO CNS grade 3.

Dual-genotype oligoastrocytoma, not elsewhere classified

The term "oligoastrocytoma" traditionally referred to diffuse gliomas showing an ambiguous morphology between astrocytoma and oligodendroglioma. The diagnosis of oligoastrocytoma was strongly discouraged already by the 2016 WHO classification of CNS tumors (Louis et al., 2016). Indeed, it has been shown that these tumors can be genetically classified as oligodendrogliomas or astrocytomas. However, rare tumors displaying morphologically distinct oligodendroglial areas, with 1p/19q codeletion and ATRX retention, and astrocytic areas, with ATRX loss and p53 accumulation, have been described (Barresi et al., 2017). These are referred to as oligoastrocytoma dual-genotype. The fifth edition of WHO classification does not consider this latter as a tumor type or subtype, but suggests a tentative classification as oligoastrocytoma, not elsewhere classified.

IDH-mutant diffuse gliomas with non-canonical IDH mutations

Few studies have analyzed the clinical-pathological features of diffuse gliomas harboring non-canonical IDH mutations (Hartmann et al., 2009; Gravendeel et al.,

2010; Poetsch et al., 2021). These are characterized by median age at onset (38 years vs 43 years), overall survival of the patients, and preferential location in the frontal lobe similar to those of diffuse gliomas with *IDH1* p.R132H mutation (Poetsch et al., 2021). However, they were more frequently localized in the infra-tentorial region (5.5% vs 0%), multicentric (4.8% vs 0.9%), and associated with a family history of cancer (22.2% vs 5.1%) (Poetsch et al., 2021). Notably, *IDH1* non-canonical mutations are prevalent in astrocytomas, while *IDH2* mutations are predominantly seen in oligodendrogliomas (Hartmann et al., 2009; Poetsch et al., 2021). This association was confirmed in 14 diffuse gliomas with non-canonical IDH mutations diagnosed in the Unit of Pathology of Verona University Hospital between 2014 and 2018; indeed, all eight cases with *IDH1* non-canonical mutations were astrocytomas (8/8 cases), while five of six cases with *IDH2* mutations were oligodendrogliomas (unpublished data).

In the analysis of the merged cohort of low-grade gliomas and glioblastoma from the Tumor Genome Cancer Atlas, accessed at www.cbioportal.org, we found 740 diffuse gliomas with available information on IDH mutational status and 1p/19q codeletion (mean: 50 years; median: 51 years; range: 18-89 years), and 51 (7%) of these had non-canonical IDH mutations. Non-canonical *IDH1* mutations (33/33) were observed exclusively in astrocytomas (14 histologically classified as grade 2, 17 as grade 3 and 2 as grade 4), whereas *IDH2* mutations were predominant in oligodendrogliomas (14/18; 3 histologically grade 2 and 1 grade 3 astrocytomas had *IDH2* mutations) (P<0.0001). Diffuse astrocytomas with IDH non-canonical mutations were distributed across CNS WHO grades (P=0.322) and had a frequency of *ATRX* mutation (30/37 vs 52/189) (P=0.313) analogous to those of astrocytomas with IDH1 p.R132H mutation.

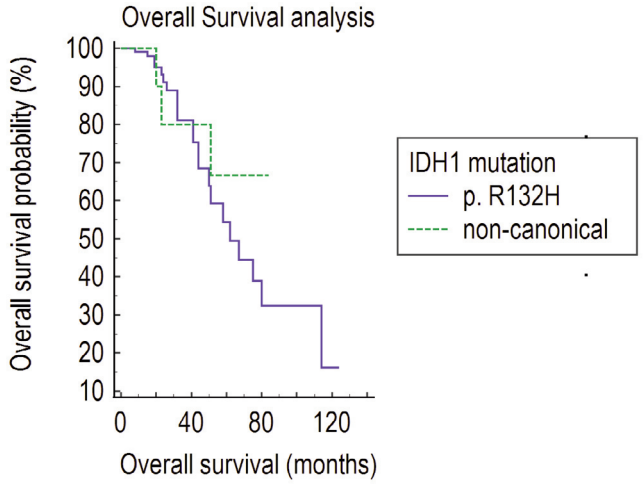

Fig. 5. In TCGA diffuse astrocytomas IDH-mutant, the patients overall survival is similar in cases with *IDH1* p.R132 H or *IDH1* non-canonical mutations

However, diffuse gliomas with non-canonical IDH mutations were more frequent in patients aged <55 years (49 vs 2 in older patients) (P=0.004). The overall survival length was similar in patients with diffuse astrocytomas harboring *IDH1* p.R132H or *IDH1* non-conventional mutations (P=0.442) (Fig. 5).

Infratentorial astrocytoma, IDH mutant

IDH-mutant astrocytomas are rarely localized in the brainstem or in cerebellum. To the best of our knowledge, fewer than 40 cases have been reported at these sites (Ellezam et al., 2012; Ida et al., 2012; Reyes-Botero et al., 2014; Javadi et al., 2018; Picca et al., 2018; Porkholm et al., 2018; Reinhardt et al., 2019; Banan et al., 2020; Chang et al., 2021). However, the rarity of these tumors may depend on the difficulty in their identification, because only 24% display the *IDH1* p.R132H mutation, which is detectable by immunohistochemistry, whereas most harbor non-conventional IDH mutations, with *IDH1* p.R132C (33%) and *IDH1* p.R132G (28%) being the most frequent (Banan et al., 2020).

DNA methylation profiling demonstrated that infratentorial IDH-mutant astrocytoma is epigenetically different from supratentorial IDH-mutant astrocytoma. Therefore, it was proposed as a distinct tumor type characterized by a higher frequency of IDH non-conventional mutations, less frequent immunohistochemical ATRX loss and *MGMT* promoter methylation (56% of cases) and common *TP53* mutations (Banan et al., 2020). The prognosis of infratentorial IDH-mutant astrocytomas is intermediate between that of diffuse midline glioma *H3* K27 altered and supratentorial IDH-mutant astrocytomas (Banan et al., 2020). However, some of these tumors may additionally harbor the H3 K27M mutation (Banan et al., 2020), thus posing doubts as to whether they should be diagnosed as astrocytomas IDH mutant or rather as diffuse midline glioma *H3* K27 altered. Although their DNA methylation profiling was consistent with "IDH glioma, subclass astrocytoma", both patients with infratentorial glioma IDH- and *H3*-mutant had an aggressive clinical course and died within two years of diagnosis (Banan et al., 2020), which suggests that this mutational pattern is associated with a worse outcome.

Oligosarcoma

Rubinstein first coined the term oligosarcoma, to refer to an oligodendroglioma with foci of sarcomatous differentiation (Rubinstein, 1972). In 2007, Rodriguez et al. published a series of seven cases of oligosarcomas with immunohistochemical expression of smooth muscle actin (Rodriguez et al., 2007). The oligodendroglial areas had 1p/19q codeletion, but as the paper was published before the "IDH-era", the mutational status of these genes was not assessed (Rodriguez et al., 2007). Based on a recent analysis of 24 cases, which

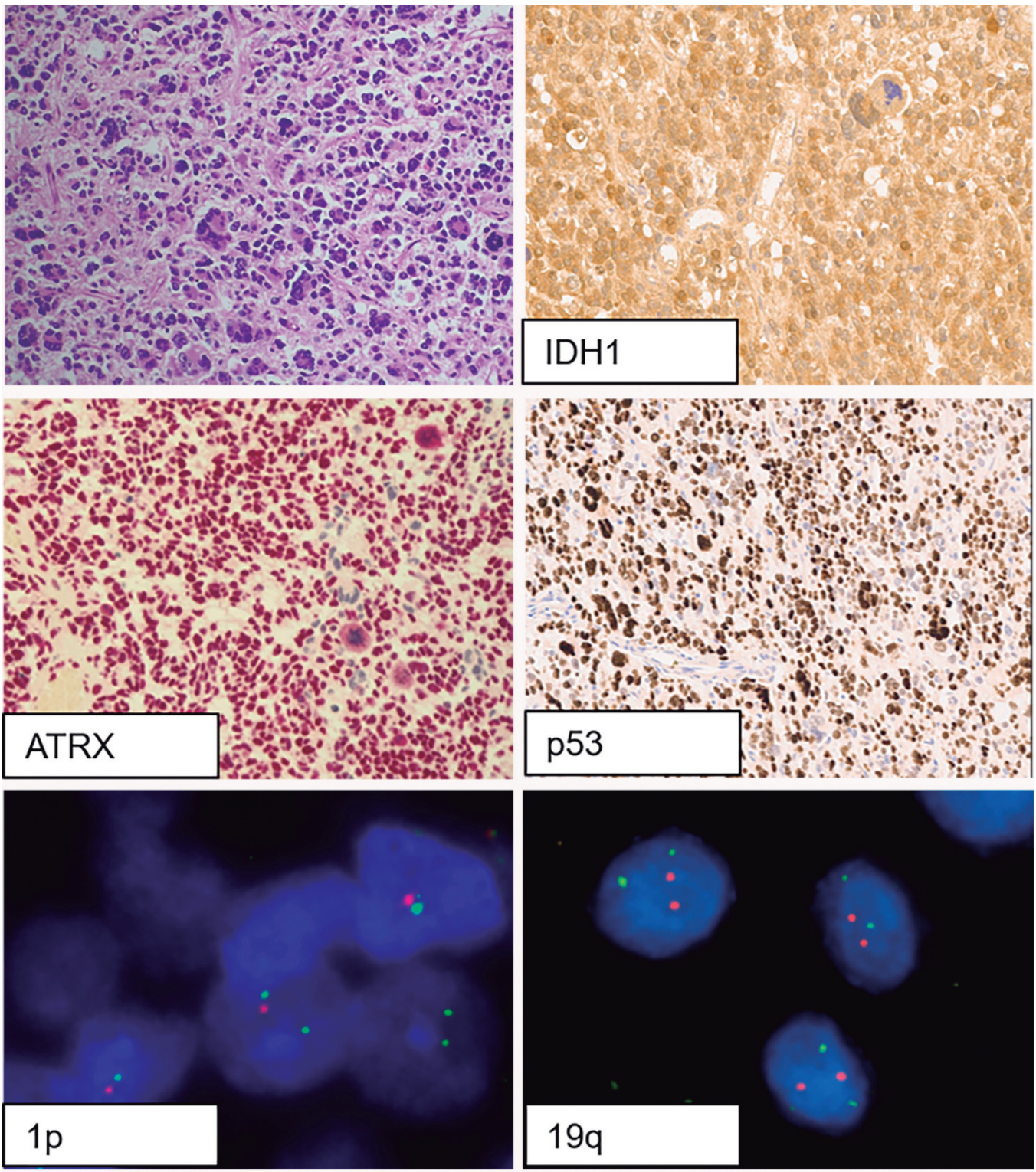

Fig. 6. Recurrent oligodendroglioma IDH-mutant and 1p/19q codeleted. The tumor showed significant nuclear atypia and multinucleated cells. At immunohistochemistry, tumor cells were positive for IDH1 p.R132H, retained ATRX expression and featured extensive p53 immunostaining. FISH analysis showed 1p deletion and disomic 19q. Next generation sequencing analysis demonstrated 1p LOH, 19q copy neutral LOH, consistent with oligodendroglioma genotype, and *TP53* mutation. Recurrent oligodendrogliomas showing these histopathological and genetic features were suggested to represent an intermediate state between oligodendroglioma and oligosarcoma.

demonstrated a DNA methylation profile different from that of oligodendroglioma IDH-mutant and 1p/19q codeleted, oligosarcoma has been proposed as a distinct tumor type (Suwala et al., 2022).

In approximately 50% of cases, oligosarcoma develops as a recurrence of an oligodendroglioma IDH-mutant and 1p/19q codeleted, following adjuvant treatment or not (Hiniker et al., 2013; Suwala et al., 2021). Notably, the corresponding primary tumor shows a DNA methylation profile coherent with oligodendroglioma IDH-mutant, suggesting that oligosarcoma is not just a recurrent oligodendroglioma, but its development follows the acquisition of genetic and epigenetic alterations that result in a distinct DNA methylation profile (Suwala et al., 2021). However, a subset of oligosarcomas may develop de novo (Suwala et al., 2021). Both the sarcomatous and oligodendroglial components of oligosarcoma harbor IDH mutations, mostly consisting of *IDH1* p.R132H mutation (Hiniker et al., 2013; Suwala et al., 2021), although *IDH2* mutations have also been reported (Suwala et al., 2021). Compared to oligodendroglioma IDH-mutant, oligosarcoma frequently (25%) shows 1p/19q copy neutral LOH (Hiniker et al., 2013; Suwala et al., 2021), which may give rise to false negative results if FISH analysis is used to detect the codeletion. In addition, in contrast to oligodendrogliomas, p53 nuclear accumulation (91.7%) and H3K27me3 nuclear retention (90.7%) occur in most cases (Suwala et al., 2021). Moreover, oligosarcoma features increased copy number variations and a high frequency of *CDKN2A/B* HD (63%) (Suwala et al., 2021).

Recurrent oligodendrogliomas showing an imbalanced 1p/19q codeletion, 1p/19q copy neutral LOH, and *TP53* mutation in the absence of sarcomatous transformation have been reported (Ono et al., 2020; Barresi et al., 2022) (Fig. 5). DNA methylation assessed in one case showed a profile consistent with that of oligodendroglioma IDH-mutant (Ono et al., 2020). Therefore, it was speculated that these cases represent an intermediate state between oligodendroglioma and oligosarcoma.

Patients with oligosarcoma as the first recurrence seem to have a shorter overall survival than patients with oligodendroglioma grade 3 as the first recurrence (Ono et al., 2020); however, further studies are needed to establish whether oligosarcoma should be classified as CNS WHO grade 4.

Diffuse gliomas IDH-mutant in children and adolescents

In spite of their classification as "adult-type", diffuse gliomas IDH-mutant are rarely observed in children and adolescents, accounting for 0-17% of all gliomas diagnosed in this age group (Balss et al., 2008; Hartmann et al., 2009; Yan et al., 2009; Pollack et al., 2011). Recently, a large multi-institutional study (Yeo et al., 2022) found 9.2% IDH-mutant gliomas among 851 gliomas diagnosed in patients aged between 0 and 21 years (Yeo et al., 2022). The frequency of IDH-mutant gliomas progressively increases with age, from 0.5% in children of 0-9 years to 16.1% in patients of 10-21 years (Yeo et al., 2022). Most tumors are supra-tentorial (95%), have astrocytic histology (80%) and are histologically low-grade (76.3%) (Yeo et al., 2022). In addition, similar to that found in adults, the IDH1 R132H mutation was the most frequent alteration (89.7%), whereas two of the four infra-tentorial cases had non-canonical *IDH1* mutations (Yeo et al., 2022).

Pediatric IDH-mutant gliomas appear to follow the same clinical course as their adult counterpart, with oligodendrogliomas having the best prognosis (Ryall et al., 2020). However, a group of IDH-mutant astrocytomas in children and adolescents may be associated with primary mismatch repair deficiency and poor prognosis. Primary mismatch repair deficient IDH-mutant astrocytoma (PMRDIA) represents a distinct type of IDH-mutant glioma, showing a unique DNA methylation profile and a median age of 14 years at diagnosis (Suwala et al., 2021). The word "primary" means that this tumor arises in the context of a constitutional defect of mismatch repair, in association with a genetic syndrome. Although mismatch repair deficiency may also result secondarily to temozolomide therapy, these tumors have a DNA-methylation profile different from that of PMRDIA (Suwala et al., 2021).

In a study of 32 cases, PMRDIA had a predominant supratentorial location (all but one case), high-grade histology with brisk mitotic activity, necrosis and microvascular proliferation, and a lower frequency of *MGMT* promoter methylation (37%) and ATRX immunohistochemical loss (69%) than that observed in all IDH-mutant gliomas (Suwala et al., 2021). All tumors had an *IDH1* mutation, and 90% had *IDH1* p.R132H mutation (Suwala et al., 2021). Most of the analyzed cases had germinal mutations in mismatch repair genes, primarily in *MSH6* (53%), which led to immunohistochemical loss of the corresponding proteins (Suwala et al., 2021). Most cases had *TP53* impairment (94%), and 24% had *RB1* alterations or *CDKN2A* HD (Suwala et al., 2021). Considering a cut-off of 10 mutations/MB, approximately 60% of cases were hypermutant, suggesting that these tumors may respond to immune checkpoint inhibitors (Suwala et al., 2021). However, the prognosis of PMRDIA is significantly worse than that of IDH-mutant astrocytomas and comparable to that of IDH-wildtype glioblastomas (Suwala et al., 2021). The low level of *MGMT* promoter methylation may account for their resistance to alkylating agents (Suwala et al., 2021).

IDH-mutant diffuse gliomas in the elderly

Diffuse gliomas IDH-mutant rarely affect patients aged ≥ 55 years and account for approximately 2% of all diffuse gliomas diagnosed in this age group (Barresi et al., 2020). Less than 1% harbor IDH non-canonical

mutations (Robinson and Kleinschmidt-DeMasters, 2017) and mainly in tumors histologically classified as CNS WHO grade 2 or 3 (Barresi et al., 2020). IDH-mutant astrocytomas in the elderly show less frequent ATRX immunohistochemical loss than those in younger patients (50% vs 70%) (Barresi et al., 2020).

Given the low frequency of IDH non-canonical mutations in histological CNS WHO grade 4 astrocytomas in the elderly, IDH mutational testing is not mandatory to classify these tumors as IDH-wildtype if IDH1 p. R132H immunohistochemistry is negative (Brat et al., 2021).

Conclusions

Although the fifth edition of the WHO Classification of CNS tumors recognizes only two types of diffuse IDH-mutant gliomas, namely oligodendroglioma IDH-mutant and 1p/19q codeleted and astrocytoma IDH-mutant, DNA methylation profiling has shown that these tumors can be further classified. Indeed, three novel and distinct methylation subclasses have recently been identified: i) infratentorial IDH-mutant astrocytoma; ii) PMRDIA; and iii) oligosarcoma (Fig. 6).

All three subtypes are associated with worse clinical outcomes compared to both astrocytoma IDH-mutant and oligodendroglioma IDH-mutant and 1p/19q codeleted; therefore, their recognition is clinically relevant.

Mutational testing should be performed in all infratentorial diffuse gliomas, because IDH non-canonical mutations are significantly more frequent in these tumors than in supratentorial ones and the presence of H3 mutations does not exclude an infratentorial IDH-mutant astrocytoma.

PRMDIA should always be suspected when IDH-mutant astrocytoma is diagnosed in children or adolescents and the family history of these patients

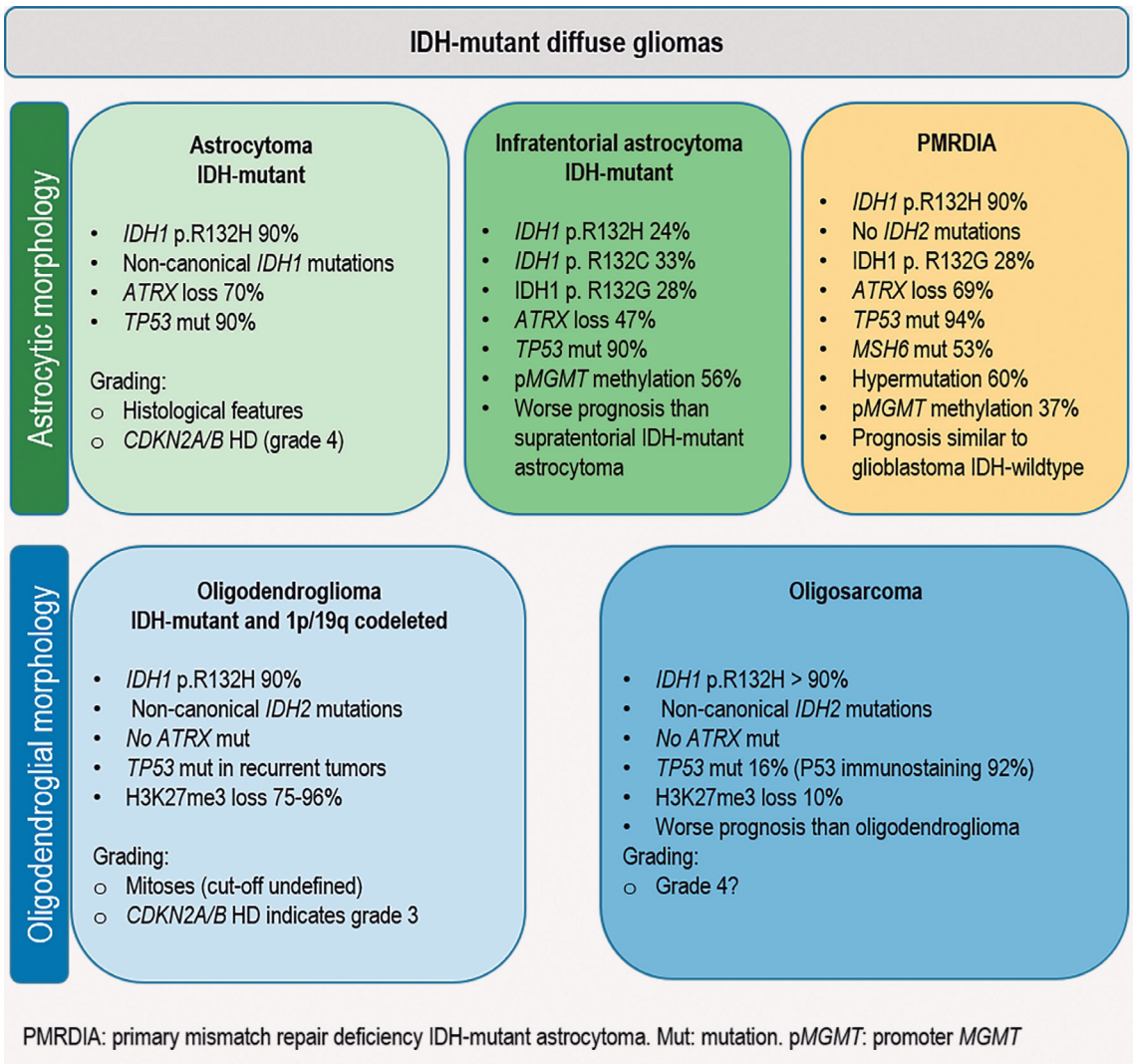

Fig. 7. Main features of IDH-mutant diffuse gliomas.

should be carefully investigated to rule out a genetic syndrome associated with mismatch repair deficiency. Finally, IDH-mutant gliomas with oligodendroglial and sarcomatous areas should be carefully tested for 1p/19q codeletion using techniques different from FISH analysis, which is not able to detect copy neutral LOH frequently found in these tumors.

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