The 2016 World Health Organization classification of tumours of the central nervous system

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Summary

The 2016 WHO classification of tumours of the central nervous system represents the new paradigm among the specialists in the brain tumours and proposes a new approach combining histopathological and molecular features into diagnosis named ‘integrated diagnosis’. The aim of this challenge is to overstep the interobserver variability of diagnosis based on previous classifications in order to ensure homogenous biological entities with a more accurate clinical significance. Over the last two decades, several molecular aberrations into gliomagenesis were highlighted and then confirmed as emerging biomarkers through prognostic stratification. In particular, IDH1/IDH2 genes mutations, 1p/19q codeletion and mutations in genes encoding histone H3 variants drastically changed the knowledge about diffuse gliomas inducing the WHO working group to consider the phenotype-genotype approach. In the present review, the historical development of the diagnosis of brain tumours from the 3D spatial configuration to the integration of multidisciplinary data up to recent molecular alterations is discussed. At the national level, the RENOCILIP network (supported by the National Cancer Institute) contributes to improve the standardization of histological diagnosis and the facilitation of access to molecular biology platforms for the detection of genetic aberrations necessary for integrated diagnosis. Importantly, the French POLA cohort allowed to test the clinical impact of the new criteria introduced by 2016 WHO classification of CNS tumours confirming the high accuracy in predicting clinical behaviour for diffuse gliomas.
Introduction

In 2014, a consensus meeting among the world's leading experts in the field of CNS (Central Nervous System) tumours took place in Haarlem, the Netherlands. The participants established a set of guidelines [1,2] for a major revision of the 2007 4th World Health Organisation (WHO) classification of CNS tumours [3,4]. This update, called "2016 WHO Classification of Tumours of the CNS", was published in 2016 by the WHO working group composed of 35 neuropathologists with the contribution of 117 experts from 20 countries and the support of neuro-oncologists and scientists from 10 countries [2]. The WHO classification is adopted in diagnostic practice:

• to introduce a common language among the several specialists in CNS tumours (neuroradiologists, neurosurgeons, neuropathologists, neuro-oncologists, radiotherapists) based on a standardized nomenclature;
• to introduce an approach integrating histopathological and molecular features into a more accurate diagnosis and improve the prediction of outcome and treatment response;
• to give the basis to improving patient management in term of treatment-decision making, clinical trials and experimental studies.

The integration of molecular and histological features to define tumours entities is the most relevant changes of this revised edition. Other important amendments include [2]:

Gliomas [4]

• they are classified according two main molecular alterations: mutations in the IDH1 and IDH2 genes and the presence of 1p/19q codeletion;
• the category of the mixed oligoastrocytomas as a distinct entity is dismissed. Gliomas which do not fit into the well-defined categories are classified as "Not Otherwise Specified" (NOS);
• new entities such as IDH-wildtype and IDH-mutant glioblastoma, diffuse midline glioma H3 K27M-mutant, RELA fusion-positive ependymoma, diffuse leptomeningeal glioneuronal tumour and anaplastic pleomorphic xanthoastrocytoma are introduced;
• gliomatosis cerebri, protoplasmic and fibrillar astrocytomas and cellular ependymoma are removed.

Embryonal tumours [4]

• medulloblastomas variants are classified according to "genetically defined" and "historically defined". Histological subtypes include: classic, desmoplastic/nodular, with extensive nodularity, large cell and anaplastic. Genetic (molecular) groups include: WNT-activated, SHH-activated, "group 3" and "group 4";
• embryonal tumour with multi-layered rosettes (ETMR), C19MC-amplified or NOS, CNS embryonal tumour with rhabdoid features and CNS embryonal tumour NOS are added;
• Primitive neuroectodermal tumour (PNET) is no longer used.

Meningiomas [4]

Brain invasion was considered as a criterion for atypical meningioma, grade II.

Nerve sheath tumours

Refinement of some entities.

Hematopoietic/lymphoid tumours [5]

Entities in line with the WHO classification of Tumours of Haematopoietic and Lymphoid Tissues were introduced.

Considering the contents of this special issue on "Neuro-oncology" and the major changes in 2016 WHO classification, the present review will focus on diffuse gliomas of adulthood. Gliomas account for 30% of all primary brain tumours and are responsible for around 13,000 cancer-related deaths in the US per year (figure 4) [6–8]. Newly diagnosed gliomas are estimated around 20,000 in the US and 2500 to 3000 in France per year [9]. Glioblastoma is the most frequent and aggressive type with a median survival of 14 months and 9.8% of 5-years overall survival after surgery, chemo and radiotherapy [61].

Glioma is not a solid tumour: spatial configuration of CNS tumours

The diagnosis of the CNS tumours, particularly for gliomas, is a "history of data integration" because these neoplasms have distinctive characteristics compared to carcinomas or sarcomas.

The latter are solid tumours defined as a relatively well-limited mass with an expansive or infiltrative growth pattern, consisting of densely packed tumour cells.

During the 1980s, Sainte-Anne Hospital School of neurosurgeons and neuropathologists respectively led by J. Talairach, G. Szikla [10] and C. Daumas-Dupont [11,12] described the spatial development of CNS tumours using serial stereotaxic cerebral biopsies technique matched with CT scan and MRI. Using intra-operative smear preparations, the 3D configuration was extensively analysed comparing the histological aspect of several staged biopsies (mapping) to the areas of contrast-enhancement, pre-contrast hyperdensity and hypodensity on CT scan [11,12]. Following this method, three main patterns of growth were recognized: Type I-solitary tumour tissue without peripheral isolated tumour cells; Type II-solid tumour tissue with significant peripheral scattered isolated tumour cells; Type III-isolated tumour cells with variable density within intact brain parenchyma (without solid tumour tissue). In Type I, the contrast-enhancement coincided spatially with solid tumour tissue and the peripheral hypodense areas on CT scan corresponded to oedematous non-infiltrated brain. In the Type II, the contrast-enhancement coincided with solid tumour tissue and the peripheral hypodense areas on CT scan corresponded to infiltrated brain with isolated tumour cells. In Type III, hypodense areas correspond to isolated tumour cells in oedematous brain parenchyma [11,12]. In the late 1980s, the input of MRI (Magnetic resonance imaging)
improved these results by adding more detailed information on the density and extension of isolated tumour cells, in particular through the T1, T2 and T2-Flair weighted sequences [13]. The impact of the correlation between histological and radiological data in the field of brain tumours was an epochal discovery conditioning the nowadays practice. For example, it's currently an evident fact that:

- a diffuse glioma is made up of infiltrating tumour cells at variable density (from scattered single cells to dense infiltration) that are able to spread very far into normal parenchyma. The infiltrating component may be associated or not to a solid tumour tissue formed by densely packed tumour cells with micro-vascular proliferation. In the latter case, the brain parenchyma is completely substituted by the tumour;
- type I model may match to the so-called “circumscribed gliomas” such as pilocytic astrocytoma, pleomorphic xanthoastrocytoma, ependymomas and some glioneuronal tumours such as ganglioglioma;
- type II model approximately corresponds to diffuse astrocytic or oligodendroglial tumours especially grade III and IV of the WHO classification;
- type III model suggests a low-grade diffuse gliomas such as grade II astrocytomas and oligodendrogliomas of the WHO classification.

**Origins of "integrated diagnosis": clinical data and radiological findings in CNS tumour pathology**

Diagnostic practice in surgical pathology consists of gross (i.e., macroscopic) and histologic (i.e., microscopic) examination of specimens. In neuropathology, the size of specimens ranges from small biopsies measuring a few millimetres (for instance, stereotaxic biopsies) to large samples. Tumour “debulking” depends on two main factors: the functional role of the region in which the tumour is located and the suspected tumour type. Examination of surgical specimens representing the whole tumour mass is not frequent. For this reason, review of MRI and in some instances CT scans is mandatory as neuroimaging allows for the study of the entire lesion. MRI with perfusion-weighted imaging is the gold standard for pre-operative assessment of gliomas and primary CNS lymphomas. Moreover, clinical information such as age, types and duration of the symptoms,
tumour location and treatment provide important clues for narrowing down the differential diagnosis in patients with a CNS tumour [14]. For example, low-grade diffuse gliomas are hyperintense on T2 weighted MRI scans but generally do not enhance following administration of the contrast-agent Gadolinium-DTPA. The absence of contrast-enhancement in these tumours can be explained by incorporation (co-option) of normal microvessels without noticeable changes to the blood–brain barrier (BBB) and lack of neovascularization. Contrast-enhancement in high-grade gliomas conversely indicates disruption of the BBB and neoangiogenesis. Many glioblastomas present radiologically with a non-enhancing, necrotic core surrounded by a contrast-enhancing ring of viable, highly cellular and angiogenic tumour tissue. Correlation of histological sections of glioblastomas with radiology revealed that tumour cells of diffuse gliomas are often present several centimetres away from the enhancing component and even outside the hyperintense areas on FLAIR MRI images. As previously described, diffuse gliomas often show marked phenotypical heterogeneity. Correlation with the imaging features is therefore crucial for an accurate diagnosis [14]. In the daily clinical practice, the neuropathologists must integrate clinical, radiological, morphological and molecular features to make a correct diagnosis of CNS tumours. For this reason, attendance at multidisciplinary neuro-oncological meetings is critical to 'fine-tune' the diagnosis and check the concordance of findings (among the neuroradiologists, neurosurgeons, neuro-oncologists and molecular biologist/geneticists). Such a multidisciplinary approach in the diagnosis of CNS tumours is the key for optimal management of patients [14].

Diagnostic pitfalls of 2000 and 2007 WHO CNS tumours classifications: grading and cell differentiation

The international classification of human tumours published by World Health Organisation (WHO) was initiated through a resolution of the WHO Executive Board in 1956 and the World Health Assembly in 1957 [15]. The first edition covering histological typing of the tumours of nervous system was edited by Zülch and published in 1979 [16]. The second edition was edited by Kleihues et al. in 1993 and integrated the contribution of immunohistochemistry into diagnostic pathology [17]. The third edition was published in 2000 [18] and the fourth in 2007 [3] incorporating genetic profiles, epidemiology, clinical signs, symptoms, imaging and prognosis and predictive factors [15]. The aforementioned classifications were almost entirely built on the morphological features of tumours and on the assumption that the cytological features likely reflected the cell origin and their levels of differentiation. Histological grading aims at predicting the natural behaviour of a neoplasm. WHO grading is a 4-tier "malignancy scale": Grade I applies to lesion with low proliferative potential and the possibility of cure following surgical resection. Grade II are generally infiltrative and despite low-level proliferative activity, often recur after many years or progress to higher grades of malignancy. Grade III defines CNS tumours with evident histological signs of malignancy, including nuclear atypia, brisk mitotic activity and neoangiogenesis (only for some histotypes). Frequently, patients with grade III tumours need adjuvant radiation and/or chemotherapy. The designation WHO grade IV is assigned to cytologically malignant, mitotically active, necrosis-prone and neo-vascular forming tumours. They grow rapidly with a fatal short-term outcome such as glioblastoma and embryonal tumours. [15,19].

According to the 2007 WHO classification, diffuse gliomas are graded as WHO grade II (low-grade), WHO grade III (anaplastic) or WHO grade IV (glioblastoma) [3]. For the grading of diffuse gliomas, nuclear atypia, mitotic activity, necrosis, and florid microvascular proliferation (MVP) are used but the approach is different for astrocytic or oligodendrogial tumours. For example, a diffuse astrocytic glioma without significant mitotic activity, necrosis or florid MVP is diagnosed as grade II astrocytoma, irrespective of the degree of nuclear atypia. In general, when the tumour shows a mitotic activity the diagnosis is grade III astrocytoma. The presence of necrosis and/or florid MVP leads to a diagnosis of glioblastoma. In oligodendrogliomas the grade relies on the same criteria but in a somewhat different way. Necrosis and florid MVP do not have the same unfavourable value as in diffuse astrocytic neoplasms. Oligodendrogliomas with MVP or necrosis are still considered as WHO grade III lesions [14].

The WHO grading system requires the definition of the histotype but the tumour typing can be difficult leading to diagnostic pitfalls [2]. Different factors can contribute to such pitfalls in typing and grading of gliomas:

- sampling of the tumour tissue can be partial, resulting in an inaccurate grading. Correlation with neuroimaging features often help in resolving possible discrepancies;
- the reproducibility of the 2007 WHO criteria for typing and grading of diffuse gliomas is not ideal. Evaluation of mitotic activity has to consider the size of the surgical samples. While a single mitosis in a large resection specimen is not sufficient for a grade III, a single mitosis in a small biopsy fragment may indicate high proliferative activity [3,14]. Interobserver variability in the classification of diffuse gliomas even among experienced neuropathologists may affect diagnostic accuracy [14].

Studies over the past of two decades have clarified some of the genetic basis of tumorigenesis in the common and some rare CNS tumours. An integrated genotypic and phenotypic diagnosis is therefore now possible for several entities [2]. Diffuse gliomas result from inactivation of tumour suppressor genes or activation of oncogenes and which accumulate with tumour progression. Low-grade diffuse astrocytomas frequently
show mutation of the tumour suppressor gene *IP53* located at 17p13.1, loss of heterozygosity (LOH) on chromosome arm 17p, and gains on the long arm of chromosome 7 (7q). In contrast, combined and complete loss of the short arm of chromosome 1 (1p) and of the long arm of chromosome 19 (19q) is present in the vast majority of typical oligodendrogial tumours. The complete 1p/19q codeletion results from a translocation between chromosome 1 and 19. Some gliomas histopathologically diagnosed as oligoastrocytic tumour genetically resemble either pure astrocytic or oligodendrogial tumours. Mutation of the isocitrate dehydrogenase 1 (*IDH1*) gene (less commonly of the *IDH2* gene) was identified as a common and early event in the oncogenesis of low-grade and anaplastic diffuse gliomas. These latter often carry additional, progression-associated genetic changes such as loss of the tumour suppressor genes *CDKN2A* (coding for p14ARF and p16INK4A), *CDKN2B* on 9p21, deletions on some chromosomes. The vast majority of glioblastomas present de novo in elderly patients with a short clinical history (‘primary glioblastomas’). In contrast, ‘secondary glioblastomas’ develop by progression from pre-existing lower grade gliomas. While primary glioblastomas frequently show *EGFR* amplification and *PTEN* mutation and lack *IDH1* mutation, the ‘secondary glioblastomas’ are characterized by frequent mutations in the *TP53* and *IDH1* genes while lacking *EGFR* amplification [20]. At the chromosomal level, primary glioblastomas are distinct from secondary glioblastomas by the frequent occurrence of trisomy of chromosome 7, monosomy of chromosome 10, 13 and 14. Despite these differences and the slightly better prognosis for patients with secondary glioblastomas, most of the genetic alterations in primary and secondary glioblastomas can be assigned to a common set of functional pathways [21,22].

Some molecular changes could be used as additional diagnostic, prognostic and/or predictive biomarkers to supplement and at time override the histological assessment. These markers could therefore be used to improve tailored management of glioma patients [23].

**Integrated diagnosis: why including molecular findings into histological diagnosis?**

For the past century, the classification of brain tumours was built on the morphological appearance of the tumour cells and their resemblance to normal glial cells [24]. However, research into glioma biology led to the discovery of molecular alterations which are distinctive of each glioma and can predict clinical aggressiveness. As a result of this stratification according to genetic and molecular features, *IDH1* and *IDH2* mutations, *ATRX* mutations and 1p/19q codeletion had introduced in the diagnostic practice irrespective of similarities of tumour cells to putative progenitors [2]. They allow a reliable distinction between astrocytomas and oligodendrogliomas. The revised WHO 2016 classification does not specify or recommended what techniques should be applied to assess molecular alterations given the rapid evolution of molecular platforms. With high-throughput techniques becoming rapidly more affordable, next-generation sequencing panels tailored for glioma diagnostics are increasingly being used for routine diagnostics, including assessment of copy number alterations (CNAs). Nevertheless, the routine use of screening for the 50 most frequent cancer genes or whole exome in glioma has not been proven to be clinically relevant. Previous studies have shown the clinical usefulness of gene expression analysis and genome-wide methylation analysis. In particular, the latter approach has been shown to be very informative, allowing the classification of tumours without knowledge of specific mutations [24].

**IDH1 and IDH2 genes mutations**

Isocitrate dehydrogenase is an enzyme part of the Krebs cycle. The three isoforms, i.e., *IDH1*, *IDH2*, and *IDH3* [25–27]. In intracellular compartment, it catalyses the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) [26]. *IDH* mutations occur in gliomas (70–90%) of adult gliomas grade II and III and 5–20% of glioblastoma, in particular in patients below 50 years of age, in haematological malignancies (~20% of acute myeloid leukaemia), in intrahepatic cholangiocarcinoma, chordosarcoma and melanoma [27–31]. The fact that the vast majority of high-grade gliomas in the paediatric age group lacks *IDH1* mutation corroborates the notion that paediatric neoplasms are fundamentally different from their adult counterparts [14]. Glioma-specific mutations always affect the amino acid arginine in position 132 in *IDH1* and arginine at position 172 in *IDH2* [32]. They are one of the earliest known genetic events in low-grade gliomas and they are thought to be a “driver” mutations for tumorigenesis probably by accumulation of the onco-metabolite 2-hydroxylutarate (2HG) [33,34]. At a prognostic level, *IDH* mutations have revealed to have a major impact on morphological stratification based on the WHO’s 2007 glioma grades, depicting a more favourable prognosis in *IDH*-mutant compared to tumours with *IDH*-wildtype in all glioma grades [35] and recognizing a worse outcome common to the group of *IDH*-wildtype gliomas independent of their grading [2]. Testing for mutations in *IDH1* or *IDH2* genes can now be performed using various molecular methods (Sanger sequencing or integrated NGS [Next Generation Sequencing]) but it seems reasonable to limit routine testing to an age range of 15 to 55 years, for example, and test beyond that only on clinical indications (e.g., in all adult grades II and III glioma, in the presence of oligodendrogial features). Additionally, specific monoclonal antibodies were established that allow for immunohistochemical analysis of the product of the most frequently
occurring IDH1 mutation (R132H; representing >90% of the IDH mutations in gliomas) [14].

1p/19q Codeletion

Chromosome 1p/19q codeletion is strongly associated with classical oligodendrogliarial features and is detected in over 80% of low-grade oligodendrogliomas and approximately 60% of anaplastic oligodendrogliomas. It results from an unbalanced translocation between the entire arm of 1p and 1q [36]. At the genomic level, it corresponds to a complete loss of the 1p and 19q arms, which is important to distinguish from 1p partial distal deletions (typically 1p36) that occur in astrocytic tumours and are associated with a poor prognosis [37-39]. 1p/19q codeletion is a strong favourable prognostic factor and since 1998 it has been associated with response and benefit to adjuvant chemotherapy with procarbazine, CCNU, vincristine, (PCV) after or before radiotherapy in anaplastic oligodendrogliomas [24,31]. 1p/19q codeleted gliomas almost invariably bear IDH1 or IDH2 mutations [40] and combinations of these molecular subgroups IDH mutated/IDH wild type, 1p/19q codeleted/non codeleted, IDH-wildtype gliomas correlate with marked differences in survival rates. It is important to use a technique for molecular diagnosis that allows for robust recognition of complete loss of these chromosome arms such as CGH-array (Comparative Genomic Hybridization-array) or integrated NGS (Next Generation Sequencing) panel [14,41]. However, the expression of Alpha-Internexin (INA, a neurofilament interacting protein) by immunohistochemistry represents a fast, cheap and reliable marker, useful as surrogate marker for 1p/19q complete codeletion. INA expression was analysed on 409 gliomas and correlated with histology, progression free survival (PFS), overall survival (OS), genomic profile assessed by CGH-array, IDH1/IDH2 mutation and p53 expression. INA expression was detected in 85% of gliomas with complete 1p/19q codeletion (‘true 1p/19q signature’) (n = 85) versus 15% of gliomas without 1p/19q codeletion (n = 245), including 14% of gliomas with variable/partial 1p/19q deletion (‘false 1p/19q signature’) (n = 72) (P < 0.0001). In oligodendrogial gliomas (n = 240), INA expression specificity for 1p/19q codeletion was 80%, sensitivity 85%, positive predictive value 70%, and negative predictive value was 91% [42-45].

Other molecular aberrations in gliomas

Alpha-Thalassemia Syndrome (ATRX) Gene

Mutations in the alpha-thalassemia/mental retardation syndrome X-linked (ATRX) gene occur in 70% of IDH mutated gliomas without 1p/19q codeletion, the astrocytic type of glial tumour. They are mutually exclusive with TERTp (Telomerase Reverse Transcriptase Promoter) mutations. There are no hot spot regions for ATRX mutations, and they can be subclonal with different ATRX mutations in different parts of the tumour and with different ATRX mutations from initial tumour versus relapses. If present, they suggest an IDH mutated/TP53 mutated astrocytoma. ATRX mutations also occur in histone H3 mutated tumours. ATRX mutations can be assessed by immunohistochemistry and by sequencing. Loss of ATRX immunostaining is a rapid method to detect ATRX mutations, and it has been suggested that it may obviate the need for 1p/19q testing. While some neuropathologists use ATRX immunostaining as a criterion to select which gliomas are to be tested for 1p/19q status, further experience is needed to test whether it can substitute for a 1p/19q test; but for now, the WHO 2016 classification explicitly does not accept positive staining for ATRX in IDH mutated tumours as an alternative to diagnose 1p/19q codeleted/IDH mutated oligodendroglioma [24,46-49].

Histone H3F3A and HIST1H3B genes mutations

The 2016 WHO classification introduces the “H3 K27M-mutant diffuse midline glioma”. This tumour occurs predominantly in childhood and adolescent. The mutation is part of a larger family of histone mutations with similar clinical presentation. Frequently, paediatric and young adult gliomas show mutations in genes encoding H3 variants which, through histone modification, alter gene expression [50]. Driver mutations occur in the H3F3A gene (positions K27 and G34) encoding the histone H3.3 genes, and in the HIST1H3B histone H3.1 gene (K27 position). K27-mutated tumours typically are located in the brainstem and midline structures, mostly in children and young adults. Thus, diffuse intrinsic pontine glioma frequently harbours K27 mutations in histone H3.3 genes as well as in H3.1 genes [51]. Childhood and young adult supratentorial glioma may show mutations in histone H3.3, with K27M mutations occurring in midline tumours. In contrast, pG34R/V histone H3.3 mutations are restricted to paediatric and young adult high-grade gliomas of the cerebral cortex and are almost invariably associated with ATRX and TP53 mutations [51,52]. K27 mutations are associated with a poor outcome; G34 mutations appear to have better survival. Intrinsic pontine glioma harboring a K27M mutation in H3.3 are less responsive to radiotherapy, with earlier relapses and more metastatic recurrences than those in H3.1. [53]. Although the K27M mutation was frequently observed in adult brainstem and thalamic gliomas, this mutation tended to be associated with a poorer prognosis in brainstem gliomas but not in thalamic gliomas [54]. By immunohistochemistry, antibody against the K27M mutation may prove useful to facilitate detection of this mutation. Testing for H3F3A mutations is insightful in paediatric and young adult cases with midline tumours [24].

Telomerase Reverse Transcriptase Promoter (TERTp) gene mutations

Somatic hot spot mutations in the promoter region of TERT occur in IDH-wildtype glioblastoma and in 1p/19q codeleted IDH mutated oligodendroglioma. TERTp and IDH mutations are powerful classifiers for diffuse gliomas [55-57]. In some tumours, only TERTp mutations are found, without other typical glioma alterations; these patients tend to have a poor outcome. TERTp
mutations are mutually exclusive with ATRX mutations. Patients with IDH-wildtype grades II and III tumours but without a TERTp mutation appear to have a better prognosis compared with patients with TERTp mutations. Assessment of TERTp mutational status can be useful for IDH-wildtype diffuse glioma [24].

The Gain of 7 and Loss of 10q Genotype
The combination of tri/polysomy of chromosome 7 and LOH of 10q is the most frequent genetic alteration in glioblastoma combined with EGFR amplification. They probably represent an early event [4]. Many IDH-wildtype astrocytomas and anaplastic astrocytomas and more often in patient older than 45 year, show a gain of chromosome 7 and a loss of chromosome 10q pattern (7 and/or 10q) and typically have a clinically aggressive course. Testing for this combination in patients over 45-50 years of age with grade II or III IDH-wildtype may predict a poor outcome. The 2016 WHO classification recommends that a diagnosis of glioblastoma should be considered in 7 and/or 10q gliomas. The term “molecular glioblastoma” for all 7 and/or 10q tumours (grade II or III gliomas) was not adopted by the present classification, therefore the diagnosis of glioblastoma is not allowed when only these abnormalities are present without a concordant morphology. Clinical data support the concept that they should be treated as glioblastoma [4,24].

**BRAF-KIAA1549 Fusion Genes and BRAF gene mutations**
Abnormalities in the oncogene B-Raf gene (BRAF) are characteristic of several subgroups of gliomas. Pilocytic astrocytoma in the fossa posterior typically have a tandem duplication at 7q34 resulting in a transforming fusion gene between KIAA1549 and BRAF (BRAF duplication or BRAF-KIAA1549 fusion gene), but not the BRAFv600e mutation. BRAF-KIAA1549 fusions are age specific, rare in pilocytic astrocytoma patients over 40 years of age (7%). BRAFv600e mutations are mutually exclusive with the BRAF-KIAA549 fusion gene; these are observed in 33% of non-posterior fossa pilocytic astrocytoma. They are also relatively common in Pleomorphic Xanthoastrocytoma (PXA; 43–66%), anaplastic PXA (65%), and ganglioglioma (18–43%), especially if located in the brainstem; they are rare in adult glioma (glioblastoma: 2%, adult low-grade glioma: 0–3%). Since BRAF-mutated tumours may be treated with targeted agents aiming at the BRAFv600e mutations, the finding of this abnormality may have therapeutic implications. Responses to these agents have been described, and this appears to be very promising. BRAF mutations and the BRAF-KIAA fusion routine testing must be considered in relevant cases [4,24].

**2016 WHO gliomas classification: new entities, challenges and clinical impact**

The figure 2 and figure 3 summarize the 2016 WHO classification of CNS tumours and the figure 4 shows the simplified algorithm of the new integrated classification of gliomas based on IDH and 1p/19q status [2,4].

New entities for diffuse gliomas include: diffuse astrocytoma, IDH-mutant; diffuse astrocytoma, IDH-wildtype; anaplastic astrocytoma IDH-mutant; anaplastic astrocytoma IDH-wildtype; glioblastoma IDH-mutant, glioblastoma IDH-wildtype; oligodendroglioma IDH-mutant 1p/19q-codeleted; anaplastic oligodendroglioma IDH-mutant 1p/19q-codeleted; diffuse midline glioma, H3 K27M-mutant; anaplastic pleomorphic xanthoastrocytoma [2,4].

When gliomas do not fit in the categories reported above or in the absence of diagnostic molecular testing, they have to be defined on histological phenotype and designated as NOS (Not Otherwise specified) [2,4].

Discrepancies between histological features and genotype can exist: for example, a diffuse glioma with astrocytomatic morphology IDH-mutant and 1p/19q-codeleted or an oligodendroglioma 1p/19q intact [2]. The latter example leads to the question of whether classification can proceed on the basis of genotype alone without histology. Furthermore, the accuracy of molecular genetic testing depends on the histological selection of tissue samples. The most representative tumour samples are selected to optimize the quantity and quality of DNA and/or RNA extracted from the tissue. This stage is completely ensured by the pathologist and is crucial for the following genetic results [58].

**Diffuse midline glioma, H3 K27M-mutant**
One narrowly defined group of tumours primarily occurring in children (but sometimes in adults too) is characterized by K27M mutations in the histone H3 gene H3F3A, or less commonly in the related HIST1H3B gene, a diffuse growth pattern, and a midline location (e.g., thalamus, brain stem, and spinal cord). This newly defined entity is termed diffuse midline glioma, H3 K27M-mutant and includes tumours previously referred to as diffuse intrinsic pontine glioma (DIPG) [2].

**Anaplastic Pleomorphic Xanthoastrocytoma**
WHO grade III, has been added as a distinct entity, as opposed to the descriptive title of Pleomorphic Xanthoastrocytoma with anaplastic features. Grading of a pleomorphic xanthoastrocytoma as anaplastic requires 5 or more mitoses per 10 high-power fields; necrosis may be present, but the significance of necrosis in the absence of elevated mitotic activity is unclear [59].

The clinical impact of the present classification was recently tested in the series of the POLA French network (https://www.reseau-pola.org). All cases of diffuse anaplastic gliomas were reclassified following the 2016 WHO classification criteria, which proved to be highly accurate in predicting survival, confirming the value of adding molecular characteristics. The best prognosis is observed in anaplastic oligodendroglioma IDH-mutant 1p/19q-codeleted (median survival 211.2 months), the worst prognosis is observed in IDH-wildtype gliomas (median survival 20 months), and an intermediate prognosis is observed in
## WHO classification of tumours of the central nervous system

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<td>Diffuse midline glioma, H3 K27M-mutant</td>
<td>Cerebellar liponeurocytoma</td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma, IDH-mutant</td>
<td>Paranglioma</td>
</tr>
<tr>
<td>and 1p/19q-codeleted</td>
<td></td>
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<tr>
<td>Anaplastic oligodendroglioma, NOS</td>
<td></td>
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<tr>
<td>Oligodendroglioma, IDH-mutant and 1p/19q-codeleted</td>
<td>9450/3</td>
</tr>
<tr>
<td>Oligodendroglioma, NOS</td>
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<tr>
<td>Anaplastic oligodendroglioma, IDH-mutant</td>
<td>Embryonal tumours</td>
</tr>
<tr>
<td>and 1p/19q-codeleted</td>
<td>Medulloblastoma, WNT-activated</td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma, NOS</td>
<td>Medulloblastoma, SHH-activated and TP53-mutant</td>
</tr>
<tr>
<td>Oligoastrocytoma, NOS</td>
<td>Medulloblastoma, SHH-activated and TP53-mutant</td>
</tr>
<tr>
<td>Anaplastic oligoastrocytoma, NOS</td>
<td>Medulloblastoma, SHH-activated and TP53-mutant</td>
</tr>
<tr>
<td>Other astrocytic tumours</td>
<td>Medulloblastoma, non-WNT/non-SH</td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>Medulloblastoma, group 3</td>
</tr>
<tr>
<td>Pilomyxoid astrocytoma</td>
<td>Medulloblastoma, group 4</td>
</tr>
<tr>
<td>Subependymal giant cell astrocytoma</td>
<td>Medulloblastomas, histologically defined</td>
</tr>
<tr>
<td>Pleomorphic xanthoastrocytoma</td>
<td>Medulloblastoma, classic</td>
</tr>
<tr>
<td>Anaplastic pleomorphic xanthoastrocytoma</td>
<td>Medulloblastoma, desmoplastic/nodular</td>
</tr>
<tr>
<td>Ependymal tumours</td>
<td>Medulloblastoma, large cell / anaplastic</td>
</tr>
<tr>
<td>Subependymoma</td>
<td>Medulloblastoma, NOS</td>
</tr>
<tr>
<td>Myxopapillary ependymoma</td>
<td>Embryonal tumour with multilayered rosettes, C19MC-altered</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>Embryonal tumour with multilayered rosettes, NOS</td>
</tr>
<tr>
<td>Papillary ependymoma</td>
<td>Meduloblastoma, PNET</td>
</tr>
<tr>
<td>Clear cell ependymoma</td>
<td>CNS neuroblastoma</td>
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<tr>
<td>Tanyctic ependymoma</td>
<td>CNS ganglioneuroblastoma</td>
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<tr>
<td>Ependymoma, RELA fusion-positive</td>
<td>CNS embryonal tumour, NOS</td>
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<tr>
<td>Anaplastic ependymoma</td>
<td>Atypical teratoid/ rhabdoid tumour</td>
</tr>
<tr>
<td>Other gliomas</td>
<td>CNS embryonal tumour with rhabdoid features</td>
</tr>
<tr>
<td>Choroid glioma of the third ventricle</td>
<td>9508/3</td>
</tr>
<tr>
<td>Angiocentric glioma</td>
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<tr>
<td>Astroblastoma</td>
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<tr>
<td>Choroid plexus tumours</td>
<td>Tumours of the cranial and paraspinal nerves</td>
</tr>
<tr>
<td>Choroid plexus papilloma</td>
<td>Schwannoma</td>
</tr>
<tr>
<td>Atypical choroid plexus papilloma</td>
<td>Cellular schwannoma</td>
</tr>
<tr>
<td>Choroid plexus carcinoma</td>
<td>Plexiform schwannoma</td>
</tr>
</tbody>
</table>

**Figure 2**

2016 WHO classification of CNS tumours: global nomenclature
The 2016 World Health Organization classification of tumours of the central nervous system

### Mesenchymal, non-meningothelial tumours

#### Solitary fibrous tumour / haemangiopericytoma**
- **Grade 1**
  - 8815/0
- **Grade 2**
  - 8815/1
- **Grade 3**
  - 8815/3
- Haemangioblastoma
  - 9161/1
- Haemangioendothelioma
  - 9120/0
- Epithelioid haemangioendothelioma
  - 9133/3
- Angiosarcoma
  - 9120/3
- Kaposi sarcoma
  - 9140/3
- Ewing sarcoma / PNET
  - 9364/3
- Lipoma
  - 8850/0
- Angiolipoma
  - 8861/0
- Fibroma
  - 8880/0
- Liposarcoma
  - 8850/3
- Desmoid-type fibromatosis
  - 8821/1
- Myofibroblastoma
  - 8825/0
- Inflammatory myofibroblastic tumour
  - 8825/1
- Benign fibrous histiocytoma
  - 8830/0
- Fibrosarcoma
  - 8810/3
- Undifferentiated pleomorphic sarcoma /
  - malignant fibrous histiocytoma
  - 8802/3
- Leiomyoma
  - 8890/0
- Leiomyosarcoma
  - 8890/3
- Rhabdomyoma
  - 8900/0
- Rhabdomyosarcoma
  - 8900/3
- Chondroma
  - 9220/0
- Chondrosarcoma
  - 9220/3
- Osteoma
  - 9180/0

### Melanocytic tumours
- Meningeal melanocytosis
  - 8728/0
- Meningeal melanocytoma
  - 8728/1
- Meningeal melanoma
  - 8720/3
- Meningeal melanomatosis
  - 8728/3

### Lymphomas
- Diffuse large B-cell lymphoma of the CNS
  - 9680/3
- Immunodeficiency-associated CNS lymphomas
- AIDS-related diffuse large B-cell lymphoma
- EBV-positive diffuse large B-cell lymphoma, NOS
- Lymphomatoid granulomatosis
  - 9766/1
- Intravascular large B-cell lymphoma
  - 9712/3
- Low-grade B-cell lymphomas of the CNS
- T-cell and NK/T-cell lymphomas of the CNS
- Anaplastic large cell lymphoma, ALK-positive
  - 9714/3
- Anaplastic large cell lymphoma, ALK-negative
  - 9702/3
- MALT lymphoma of the dura
  - 9699/3

### Histiocytic tumours
- Langerhans cell histiocytosis
  - 9751/3
- Erdheim–Chester disease
  - 9750/1
- Rosai–Dorfman disease
- Juvenile xanthogranuloma
- Histiocytic sarcoma
  - 9755/3

### Germ cell tumours
- Germinoma
  - 9064/3
- Embryonal carcinoma
  - 9070/3
- Yolk sac tumour
  - 9071/3
- Choriocarcinoma
  - 9100/3
- Teratoma
  - 9080/1
- Mature teratoma
  - 9080/0
- Immature teratoma
  - 9080/3
- Teratoma with malignant transformation
  - 9084/3
- Mixed germ cell tumour
  - 9085/3

### Tumours of the sellar region
- Craniopharyngioma
  - 9350/1
- Adamantinomatous craniopharyngioma
  - 9351/1
- Papillary craniopharyngioma
  - 9352/1
- Granulat cell tumour of the sellar region
  - 9582/0
- Pituitary adenoma
  - 9432/1
- Spindle cell oncocytoma
  - 8290/0

### Metastatic tumours
- The morphology codes are from the International Classification of Diseases for Oncology (ICD-O) (T42A). Behaviour is coded 0 for benign tumours, 1 for unspecified, borderline, or uncertain behaviour, 2 for carcinoma in situ and grade III intraepithelial neoplasia, and 3 for malignant tumours. The classification is modified from the previous WHO classification, taking into account changes in our understanding of these lesions.
- *These new codes were approved by the IARC/WHO Committee for ICD-O."** Provisional tumour entities. **Grading according to the 2013 WHO Classification of Tumours of Soft Tissue and Bone.*
**IDH**-mutant 1p/19q intact gliomas (median survival 103.9 months). Interestingly, among the groups of **IDH**-wildtype gliomas and **IDH**-mutant 1p/19q intact gliomas, the grade does not impact survival and no difference is observed between grade III and grade IV [60]. According to this last updated classification, adult patients with a good Karnofsky Performance Status score (KPS ≥ 70) and younger than 65 years old should be treated according to the two following standard: Stupp concomitant radio-chemotherapy followed by adjuvant chemotherapy with temozolomide for newly diagnosed glioblastoma [62] and RT followed by adjuvant PCV chemotherapy for newly diagnosed anaplastic oligodendroglioma [63]. Regarding **IDH**-mutant anaplastic astrocytoma and high risk low-grade gliomas, those ones have been showed to benefit from procarbazine, lomustine, and vincristine following RT in two recent phase III randomized trials so far [64,65]. In elderly patients (> 65) with glioblastoma and good performance status (KPS ≥ 70), the addition of temozolomide to short-course radiotherapy 40 Gy in 15 fractions proved to result in longer survival than short-course radiotherapy alone in a recent phase III trial [66]. Finally for patients with a poor performance status, specifically if elderly and with KPS > 70, temozolomide in monotherapy could be also proposed since it was proved to be associated with improvement of functional status and increased survival compared with supportive care alone, especially in patients with methylated MGMT promoter [67]. At recurrence, a number of options can be discussed including second line chemotherapy with anti-angiogenic drugs, nitrosoureas, carboplatine and target therapies.

**Clinico-pathological network in France for diagnosis and therapy**

In France, the implementation of the 2016 WHO classification of CNS tumours in clinical and pathological daily practice represented a major multidisciplinary undertaking that has been achieved under de guidance of the French National Institute of Cancer (INCA), the French National Network for Rare Cancer, the French Neuro- oncology Society (ANOCF, Association des Neuro-oncologues d’Expression Française) and the French Neuropathology Society (SFNP, Société Française de Neuropathologie). Over the past decade, several French national
The algorithm of the new integrated classification of gliomas based on IDH mutations and 1p/19q codeletion

The 2016 World Health Organization classification of tumours of the central nervous system

clinicopathological networks were set up on different critical topics such as paediatric brain tumours (GENOP), high-grade oligodendrogial tumours (POLA), rare brain tumours of adult (TUCERA-RENOP) and glioblastomas (BCB-glioblastome). Recently, the INCA has required the merger of all French clinicopathological networks into a single comprehensive network called RENOCLIP (Réseau de Neuro-oncologie Clinico-Pathologique) under the coordination of Pr D. Figarella-Branger (Marseille) and Pr JY Delattre (Pitié-Salpêtrière Hospital, Paris). The most important goals of RENOCLIP focus on:

• to develop a network of care that ensures high quality multidisciplinary treatment of the patients with CNS tumours on French soil. This point involves the access to the second opinion (clinical or histological and molecular double reading) as well as the standardization through specific guidelines;

• to create several thematic groups centered on a tumour type or a group of specific CNS tumours, requiring different expertise. Each thematic group provides hyper-specialized periodic multidisciplinary recourse meeting, guidelines, data recording or national register, annual report, training of professionals, translational research and clinical trials;

• to facilitate the access to the molecular biology platforms for detection of the most important genetic abnormalities needed for the “integrated diagnosis” following the recommendations of 2016 WHO classification of CNS tumours;

• to harmonise the histological diagnosis of CNS tumours through a mandatory expert double reading, the standardization of the histological reports integrating the molecular data and routine immunohistochemical techniques;

• to organise regular meetings with histological expert review by multi-heads microscope providing a diagnostic adjustment tool for complex cases;

• to open a space for the exchange and training of the pathologists due to the shortage of experts in this field.

The organisation of National and Regional Reference Centres and the commitment of the multidisciplinary task force of French experts in CNS tumours have made it possible to create a very well-structured network in relation to some other European countries. In particular, the network was able to answer to the most critical challenge introduced by 2016 WHO classification of CNS tumours concerning the rapid availability of genetic tests for histo-molecular integrated diagnosis.

Conclusion

A multidisciplinary approach that results in an integrated clinical, pathological and molecular diagnosis is mandatory in the assessment of CNS tumours to avoid diagnostic inaccuracy leading to incorrect treatments. Molecular and genetic studies have provided biomarkers for a more robust classification of several tumour entities and a combined phenotypic-genotypic approach.
represents a substantial step forward of the revised version of the WHO classification of CNS tumours. However, classifications of tumours are never definitive. New molecular insights into brain tumours will further refine the present classification update.

Acknowledgement: we thank Pr C. Daumas-Dupont, Dr Karima Mokhtari and Pr D. Figarella-Branger for teaching much of what we know in neuro-oncology pathology.

Disclosure of interest: the authors declare that they have no competing interest.

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The 2016 World Health Organization classification of tumors of the central nervous system


http://dx.doi.org/10.1111/j.1365-2559.2010.03711.x.


