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Diffuse Astrocytoma and Oligodendroglioma: An Integrated Diagnosis and Management

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Abstract

For the first time, the WHO classification of brain tumors has introduced molecular parameters in the diagnosis of brain tumors. Together with embryonal tumors, the diffuse gliomas have suffered significant changes in diagnosis, prognosis, and response to treatment. A new concept of “integrated diagnosis” comes to combine the classical diagnosis with the molecular one. While it is still impossible to disregard the histopathological component, according to the new rule (“molecular beats histology”) makes molecular parameters dominant in the final diagnosis. Currently, the diffuse gliomas (oligodendroglial or astrocytic) are nosologically closer than the astrocytomas with a diffuse growth pattern, and the astrocytomas with a more circumscribed growth pattern defined by the presence of the IDH mutation. The family tree was redefined by the presence of the IDH mutation and of the 1p/19q codeletion. The implementation of this new concept in clinical practice will improve patient management, as well as the design of clinical trials and experimental studies. This must also be seen as a model for diagnosis setting in the new molecular era.

Keywords: astrocytoma, oligodendroglioma, glioblastoma, IDH mutation, 1p/19q co-deletion, integrated diagnosis

1. Introduction

The introduction of the new classification represents the first step in the switch of paradigm in brain tumor management toward an individualized-based treatment from the, nowadays, evidence-based management. The classification of diffuse gliomas has undergone significant changes following the introduction of molecular testing, the new WHO 2016 classification introducing a new concept—integrated diagnosis [1]. The current update takes into

account both the phenotype and the genotype [2]. This was the preferred alternative, as it is currently impossible to resort only to the molecular parameters in the definition of tumoral entities [3]. The classification relies both on the morphological character (growth pattern) and on the definition of the genetic status by determining the presence of mutations in the IDH1 and IDH2 genes and of the 1p/19q codeletion [4]. According to WHO 2016, diffuse gliomas are lumped together, regardless of their histopathological aspect (astrocytomas or oligodendrogliomas) [5].

The recourse to an integrated diagnosis makes it possible to formulate a more precise diagnosis of the various entities and acknowledges the existence of new entities [6].

The introduction of molecular parameters comes to improve the clinical management of patients, defining entities which feature a similar prognosis. It also paves the way to the identification of new treatment methods aimed at the biological mechanisms common to this type of tumors [5]. The genetic information supplied by the Cancer Genome Atlas Research indicate that the supratentorial gliomas with a diffuse growth pattern can be categorized separately from the other brain tumors (**Figure 1**) [7]. They are grouped into three categories, according to the genetic profile—the presence or absence of the 1p/19q codeletion and the mutational status of the IDH gene. The first category includes the gliomas with a classical morphology of oligodendrogliomas, having both the IDH gene mutation and the 1p/19q codeletion. The second category is represented by the tumors with an astrocytic histological pattern and IDH mutation, but without the 1p/19q codeletion. In the third category, we find the tumors with an astrocytic phenotype, which display no IDH mutation or 1p/19q codeletion. The latter category usually falls under the classical wild-type glioblastoma diagnosis [8].

Figure 1. Integrated diagnosis of astrocytic and oligodendroglial tumors.

This approach separates between the astrocytomas with a circumscribed growth pattern, no IDH mutation, and with BRAF mutation (pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and subependymal giant cell astrocytoma), on the one hand, and the diffuse astrocytic and oligodendroglial tumors, on the other. According to the new classification, the diffuse astrocytic and oligodendroglial tumors are nosologically closer than the diffuse astrocytoma and the pilocytic astrocytoma [9].

The inclusion in these categories on the basis of genetic determinations also has a role in prognosis [10].

When there is a mismatch between the phenotype and the genotype, genetic tests set the final diagnosis in keeping with the rule “molecular beats histopathology [1].”

Glioblastoma with the IDH mutation show a better evolution than the wild-type ones, generally corresponding to a secondary glioblastoma. They also have a better prognosis than the wild-type anaplastic astrocytoma. The wild-type astrocytomas have the worst prognosis of all astrocytomas, their molecular profile being characteristic for glioblastomas (EGFR amplifications, PTEN mutation, and 10q, 9p loss) [11].

The introduction of molecular parameters in the definition of entities has led to the recognition of a new entity in the group of diffuse pediatric gliomas: the tumors with a midline location, diffuse growth pattern, and the K27 M mutation in the H3 histone gene. This is the first attempt to distinguish between the pediatric brain tumors and their adult counterparts, the difference in behavior between histopathologically identical tumors being long known [12].

As to the histological grading, the WHO 2016 classification keeps the three-tiered system. The shift from low to high malignancy depends upon morphological parameters that reflect the emergence of new biological processes. The *first malignancy* criterion is represented by the variations in the size, shape, and color intensity of the nucleus (*atypia with hyperchromasia*) [13]. The *proliferation* is reflected in the presence of mitoses, which must be unequivocal, with no additional specifications in terms of number and morphology [14]. A significant proliferation typical for high-grade tumors is highlighted by the advent of *necrosis* and of the attempt to compensate for this hypoxia through the emergence of *microvascular proliferation*. According to this classification, the *diffuse astrocytomas* limited to *cytological atypia* are deemed to be *grade II*, while those with *both anaplasia* and *mitotic activity* are deemed to be *anaplastic (grade III)*. The presence of mitoses must be seen in context as only one mitosis in a large section is not enough for a grade III. If we are dealing with a small biopsy, then the presence of a mitosis may be sufficient. *Grade IV* is reserved to those tumors that display *necrosis and/or vascular proliferation*. Microvascular proliferation is defined as the stratification of the endothelium, or the “glomeruloid” aspect. The necrosis can be of any type [15].

In the group of low-grade gliomas (II and III), the histopathological stratification features a significant interobserver variability, as also demonstrated by the considerable differences in terms of survival rates manifest within this group. The evaluation of molecular parameters can also be useful in the sense of defining groups that correlate better with the prognosis [16].

Gliomatosis cerebri no longer exists as an entity, being considered rather a specific growth pattern. More research is needed in order to identify the biological substrate of this unusual invasive capacity [17].

2. Low-grade gliomas

2.1. Diffuse astrocytoma IDH-mutant (DA IDH-mut)

Definition. Tumoral proliferation which, from a histopathological point of view, shows an astrocytic phenotype with a diffuse growth pattern and IDH1 or IDH2 gene mutations.

As to *grading*, diffuse astrocytoma is deemed to be *grade II* (low-grade diffuse astrocytoma).

Clinically, the emergence of symptoms is usually insidious, as they precede the diagnosis by weeks months or sometimes years. Seizures are the symptom most likely to raise suspicions. There are studies underling the presence of the seizures at the debut in up to 80% of cases, approximately 50% presenting with uncontrollable seizures at the time of surgery. Factors predisposing to a poorer response to antiepileptic drugs are: partial seizure type, temporal location, and a longer seizure duration [18]. The most frequent location of those tumors is in the frontal lobe, followed by temporal and parietal lobe. Other associated symptoms could appear related to the location. Behavioral and personality changes, visual disturbances, aphasia, or agnosia are most frequently mentioned, meanwhile the increased intracranial pressure symptoms install later in the course of disease, related to the tumoral volumes and mass effect.

Imaging. MRI is the “golden standard” imagistic tool. For DA IDH-mut, the typical aspect is a homogeneous tumor with low signal intensity on T1-weighted images and a high intensity on T2-weighted sequences. This high T2 signal is rather related to edema, demyelination, or degenerative changes than to cellular atypia. Fluid-attenuated inversion recovery (FLAIR) sequences are the most appropriate for defining the infiltrating tumor margins. Usually, astrocytomas are confined in the white matter, meanwhile the oligodendrogliomas are cortical-based tumor, but this difference is attenuated in the later stages (**Figure 2**). Cystic components are not infrequent and low contrast enhancement could be observed in 20% of cases without malignant transformation [19].

Figure 2. Axial T2W (a), FLAIR (b), and T1W + C (c) MRI examination of a patient with right insular DA IDH-mut (personal archive).

Advanced MRI techniques such as diffusion-weighted imaging (DWI) and MRI spectroscopy will complete the anatomical information, while the functional MRI and diffusion tensor imaging (DTI) will offer important data for surgical planning. On DWI, DA IDH-mut presents a decrease cellularity and non-restricted diffusion. *MRI Spectroscopy* will reveal not only decreased N-acetyl-aspartate (NAA) peak, medium choline peaks, absence of lactate peak, and increased myo-inositol [20], but is also able to detect IDH mutation through oncometabolite 2-hydroxyglutarate (2HG) present in tumor cells [21]. MRI could also serve as a *prognosis tool* if is combined with IDH status. Just recently it was suggested that minimum apparent diffusion coefficient (ADC_{MIN}) threshold of $0.9 \times 10^{-3} \text{ mm}^2/\text{s}$ or less is associated with a worst prognosis especially when it is combined with IDH wild-type grade II diffuse astrocytomas [22]. *DTI with tractography* usually reveals a displacement rather than an infiltration or destruction of fiber tracts in DA IDH-mut tumors.

CT scan reveals a homogeneous lesion, poorly defined, with no contrast enhancement. This can be associated with cystic changes and calcifications that are more specific for oligodendrogliomas.

Macroscopy. In section, the tumor does not display clearly delineated limits, on account of its infiltrative growth pattern. We can see areas of soft consistency or firmer ones, granular areas, and cystic ones. Cystic changes can include sponge-like areas, consisting of cysts of various sizes that may have a gelatinous aspect. There can be only one large cysts, filled with liquid, and this is associated with the identification of gemistocytes during microscopy. The calcifications can be focal or diffuse, and in this case, the appearance is one of grittiness.

Histological diagnosis. Under the microscope, we see a diffuse tumoral proliferation consisting of atypical fibrillary astrocytes. Hypercellularity is moderately increased, the tumor imperceptibly blending with the surrounding normal structures. Cellular proliferation (star-shaped cells, with extensions) is situated on a fibrillary loose matrix which often forms microcystic structures. The main characteristic is the nuclear atypia, neoplastic astrocytes being based on the aspect of the nucleus. This is enlarged, hyperchromatic, and irregular in shape [23].

The *differential diagnosis* is performed with the help of reactive astrocytosis. The diagnosis can be done only on the basis of morphological criteria, but more often than not, this requires an extremely nuanced approach. The morphological criteria include a numerical increase, but especially the homogeneous aspect of the nuclei, as we are dealing with a clonal neoplastic proliferation. As opposed to neoplastic astrocytes, the reactive ones have a heterogeneous nuclear aspect, with nuclei of various sizes and with cytoplasm in variable quantities. The background of these nuclei is of normal or increased density in the case of tumoral proliferation, and of decreased density in the case of reactive astrocytosis. Immunohistochemistry is extremely useful in distinguishing between reactive and tumoral astrocytes. As the IDH1 mutation falls under the definition of this type of tumor, the antibody identifying the protein altered by the presence of the R132H mutation can be used. Also, the tumoral cells displaying the TP53 mutation can be identified through a recourse to the antibody [24].

Mitotic activity is low to absent, the presence of a mitosis in a large biopsy being compatible with the diagnosis. If a mitosis is present in the context of an important nuclear anaplasia within a small biopsy, then the diagnosis of anaplastic astrocytoma cannot be ruled out. The proliferation index determined by way of Ki-67 is under 4%. If there is a gemistocytic component, the proliferation rate is significantly reduced [25].

As we are dealing with low proliferation rate tumor, the changes induced by hypoxia, such as microvascular proliferation and necrosis, are absent.

Secondary structures (Sherer) such as perineuronal satellitosis, subpial infiltration, and perivascular aggregation can also be present.

Other entities that need to be factored in for the differential diagnosis are: the normal brain, the demyelinating disease, anaplastic astrocytoma, oligodendroglioma, and pilocytic astrocytoma. In what concerns the diffuse pattern, the differential diagnosis must be done with lymphomas and small-cell carcinomas [26].

Gemistocytic astrocytoma is a variant of the grade II diffuse astrocytoma, characterized by the presence of more than 20% angular neoplastic astrocytes, with abundant eosinophilic cytoplasm. The nucleus is pushed toward the periphery, showing nucleoli and a dense chromatin. Electronic microscopy reveals the presence of numerous mitochondria and glial filaments, as also confirmed by the positive GFAP. A characteristic feature is the presence of the perivascular cuffing lymphocyte [27].

The classical morphopathological aspect is astrocytic, but an “oligodendroglioma-like” component can be accepted in the absence of 1p/19q codeletion.

Immunohistochemically, the battery of antibodies that can be used includes: GFAP, vimentin, IDH R132H, p53, ATRX, Olig2, and Ki67. GFAP and vimentin are positive, but of variable expression. The existence of an antibody that makes it possible to indirectly identify the R132H mutation, present in approximately 90% of tumors, is one way of identifying the tumor cells featuring this mutation. Another important antibody is ATRX, and the presence of the mutation leads to the loss of nuclear expression in the tumor cells. P53 can be used, as an intense nuclear expression is consistent with the presence of the TP53 mutation. Olig2 is nearly always present. As already indicated, Ki-67 can be used in assessing the proliferation index [28–31].

Genetic diagnosis. Integrated genomic analysis has made it possible to identify the IDH gene mutation in glioblastomas, leading to the “IDH era” in diffuse gliomas [32]. The sequencing of a large number of brain tumors has revealed the high incidence of the IDH gene mutation in low-grade astrocytomas and oligodendrogliomas, suggesting that it might play a role in the early onset of such tumors [33]. The presence of this mutation is relevant for both diagnosis and prognosis, its absence meaning a less favorable prognosis [34]. A study conducted on a large cohort has indicated a survival rate of 10.9 years for the diffuse astrocytomas with the IDH mutation [35]. The consequence-inducing mechanism involves the excessive production and the accumulation of an oncometabolite—2 hydroxyglutarate [36]. This mutation leads to significant epigenetic changes and to changes in the regulation of the expression of differentiating factors [37]. More particularly, we witness a hypermethylation of the genome, generating tumors with the CpG island methylator phenotype (CIMP) [38]. This group of tumors shows a distinct biological behavior, with epigenetic changes in the whole genome, by remodeling the methylome and reorganizing the transcriptome. This leads to the activation of key genetic programs and to the emergence of a cellular phenotype allowing for better survival rates. Also, the IDH allows for chromosomal aberrant interactions, with the activation of the oncogene expression [39].

ATRX encodes a chromatin-binding protein. The mutations of this gene are associated with epigenetic changes [40]. It also activates the alternative telomere lengthening mechanism, necessary

Figure 3. Integrated histological and molecular diagnosis of diffuse astrocytoma IDH-mutant grade II.

in the pathogenesis of diffuse gliomas [41]. Furthermore, ATRX deficiency can create a context of generalized genetic instability which, when P53 is intact, can induce apoptosis. The occurrence of a P53 mutation alongside the ATRX one can allow tumor cells to survive [42]. This instability is reflected in the occurrence of low-level amplifications for other oncogenes, such as MYC and CCND2 [43]. The TP53 mutation is also present in nearly all IDH-mutant gemistocytic astrocytomas, in both gemistocytes and non-gemistocytes, indicating that gemistocytes are oncogenically non-reactive cells [44]. Quite interestingly, the presence of the ATRX mutation is mutually exclusive with the mutation of the gene that encodes the catalytic component of TERT telomerase. The mutations of the TERT gene are characteristic for oligodendrogliomas and wild-type glioblastomas [45].

The methylation of the MGMT gene promoter is present in more than 50% of IDH-mutant astrocytomas, but the presence of this methylation is not correlated with the status of G-CIMP [46]. By definition, 1p/19q codeletion is absent (Figure 3).

The genetic profile of diffuse astrocytomas is different in children and in adults; so, we can talk of adult-type and pediatric-type diffuse astrocytomas. The genetic profile of pediatric tumors involves amplifications and rearrangements of the MYB gene, alterations of FGFR1, and mutations of BRAF (V600) and KRAS [47].

There are two entities that increase the susceptibility to diffuse astrocytomas. Low-grade astrocytomas are usually diagnosed in patients with Ollier-type multiple enchondromatosis [48]. Also, those having the Li-Fraumeni syndrome are more likely to develop diffuse gliomas, but these are high-grade anaplastic astrocytomas and high-grade glioblastomas [49].

2.1.1. Multimodal treatment

2.1.1.1. Surgical treatment

In the last decade, a great switch was produced in the therapeutical management of DA. Since it was demonstrated that these tumors have an annual linear grow of 4 mm/year on diameter [50], they are nowadays considered “infiltrating chronic disease that invades the central nervous system, that will ineluctably become malignant” [51]. It is now well established that surgery has a great impact on both the natural history and the malignant transformation. As a consequence, radical surgery becomes the goal in the treatment of diffuse gliomas in order to prevent malignant transformation and to prolong overall survival [52]. Additionally, radical surgery significantly improves seizure control compared with subtotal resection [53].

General principles of surgery, as they were underlined by Hugh Duffau, are generally accepted by the neurosurgical community: early radical surgery, awake surgery in high eloquent areas, cortical mapping, “resection according to cortico-subcortical functional and not oncological boundaries,” and “multistage resection in critical regions” [51]. The most difficult part of the operation is at the boundaries of the tumor, where even with adjuvant intraoperative MRI, the distinction between normal brain and infiltrative brain is very difficult. Surgical experience contributes to better surgical results, but even in very experienced hands, there are cases in which a residual tumor could be identified on postoperative MRI (**Figure 4**).

Neuronavigation and intraoperative ultrasound are more and more used in order to improve the surgical resection, since they are able to reveal large residual tumors. Enhanced intraoperative ultrasound is at the beginning of experience, more studies being necessary to establish its usefulness in diffuse astrocytomas resection (**Figure 5**) [54].

2.1.1.2. Adjuvant therapy

Factors to take into consideration for immediate postoperative therapy are those considered risk factors for worse outcome namely age > 40 years, preoperative tumor diameter > 4 cm, incomplete resection, astrocytic histology, and absence of 1p/19q codeletion [55]. In this perspective, cases with DA IDH-mut completely resected, in young patients (<40 year), are candidates for close clinical imagistic observation and no adjuvant therapy is recommended in the immediate postoperative period. However, it is expected that these tumors will recur, so additional surgical and adjuvant therapy will be added at the time of progression. For tumors with subtotal removal, in patients older than 40 years of age, immediate postoperative treatment is recommended. Concerning adjuvant radiotherapy, the recommendation is in favor of lower doses (45–50.4 Gy) which are equivalent in terms of results with the high doses (59.4–64.8 Gy) but with reduced toxicity. Relative to chemotherapeutic regimen, actual data suggest that the PCV (procarbazine, CCNU, and vincristine) formula is superior to temozolomide regimen in terms of overall survival for the treatment of DA IDH-mut, mostly in cases with codeletion of 1p/19q genes [19].

Figure 4. Preoperative FLAIR MR sequence (a) and immediate postoperative axial CT (b) and 3 months postoperative FLAIR examination revealing a complete removal of an insular grade II DA. (c) One year follow-up axial FLAIR MR examination reveal complete removal of tumor with no local signs of recurrence.

Figure 5. Preoperative coronal FLAIR (a), axial T2W (b) and intraoperative ultrasound examination (c) before resection of a diffuse astrocytoma subtotally removed as it is demonstrated by intraoperative ultrasound (f) and immediate postoperative CT scan (d and e).

2.2. Diffuse astrocytoma IDH wild type

Definition. This astrocytoma is a diffuse growth pattern astrocytoma without the IDH mutation. WHO 2026 lists it as a rare and provisional entity. Most tumors falling under this definition can be classified with the help of genetic testing as a variety of other entities with different clinical evolutions.

Clinically, their behavior is not different from the DA IDH-mut.

The *standard MRI* findings are generally the same, the presence of a more intense enhancement suggesting an increased microvascularization and, as a consequence, a more aggressive evolution. As it was already mentioned, the presence of 2HG peak on MR spectroscopy is suggestive for IDH1 mutation, so the absence of this peak could serve as an indicator for a wild-type tumor. 2HG was also studied as a marker for the response to adjuvant therapy [56].

2.2.1. Multimodal treatment

2.2.1.1. Surgical treatment

Surgical treatment with the goal of early radical surgery is nowadays the first step in the standard of care of low-grade gliomas. Complete tumor removal based on functional borders more than on imagistic ones with the help of neuronavigation, awake surgery, and intraoperative neurophysiological monitoring (IEM) proved to offer a longer progression-free survival (PFS) and overall survival (OS) compared with subtotal removal, with reduced neurological deficits [57, 58].

2.2.1.2. Adjuvant treatment

Knowing that the wild-type astrocytomas have a worse prognosis, a low-dose radiotherapy regimen in the immediate postoperative period is recommended instead of close clinical imagistic observation. There are arguments in favor of radiotherapy in terms of prolonged PFS but not necessarily the OS [59]. Due to the fact that the patients receiving associated radiotherapy and chemotherapy have an improved long-term survival [60], a combined radio-chemotherapy regimen seems to be recommendable. Further studies will have to determine whether temozolomide, which was historically preferred by neuro-oncologists due to its lower toxicity and oral administration, is superior or not to PCV [61].

2.3. Diffuse astrocytoma NOS

Definition. A diffuse astrocytoma for which the presence of the IDH mutation could not be determined.

2.4. Oligodendroglioma, IDH-mutant, and 1p/19q codeleted

Definition. Tumor with a diffuse growth pattern, slow growth, showing IDH mutations and the 1p/19q codeletion. Oligodendrogliomas develop many similarities with DA IDH-mut concerning clinical presentation, imagistic diagnosis, and therapeutical management.

Grading. Grade II WHO.

Clinical presentation. The classical presentation of a patient with oligodendroglioma is with seizures that precede with years and with other neurological signs like mental disturbances, neurological deficits, or signs of increased intracranial pressure (mostly headache). Even in patients with no seizures at the onset, they will develop such clinical manifestations during the course of disease. The brutal debut due to an intratumoral hemorrhage although rare, is more frequent in oligodendrogliomas than in other low-grade gliomas [62].

Imaging. *CT scan* is usually the first imagistic tool in emergency, and for more than 50% of oligodendrogliomas that present intratumoral calcification, it is a useful diagnostic method. On CT scan, oligodendrogliomas are present as well-delineated cortical-based hypodense lesion with intratumoral or peripheral calcification. Cystic component are not rare and hemorrhagic regions may also be encountered (**Figure 6**). Contrast enhancement is absent or marginal.

Figure 6. Axial and coronal CT scan examination of a case with an hypodense left frontal lobe lesion with intratumoral calcifications and discrete enhancement (a and b).

Figure 7. Axial T2W (a), axial (b) and coronal (c) FLAIR sequences of a left frontal oligodendroglioma.

Recently, it was suggested that presence of calcification is highly associated with 1p/19q codeletion and, as a consequence, has a prognosis role [63].

Standard MRI reveals an hyperintense inhomogeneous lesion on T2W and FLAIR sequences, respectively, an inhomogeneous hypointense cortical-based well-delineated lesion on T1W sequences with no or discrete enhancement. Calcifications, cysts, and hemorrhagic lesions increase the heterogeneity of the lesion (**Figure 7**).

Advanced MRI techniques. As for the diffuse astrocytomas, MRI spectroscopy reveals higher NAA (N-acetylaspartate) peak near 2 ppm. The mIns (myo-inositol) peak is relatively higher when compared with normal brain; the reduction of peak signaling is a possible malignant transformation of tumor. The NAA/Cr (creatinine) ratio is higher in low-grade gliomas, meanwhile the ratio Cho(Choline)/Cr is higher in high-grade gliomas [64]. As it was already mentioned, 2HG peaks serve as an indicator for IDH 1 mutation. Perfusion MRI, another physiologic imaging sequence, reveals that relative cerebral blood volume (rCBV) is reduced in low-grade gliomas (LGG) compared with high-grade ones. Increase in rCBV in a LGG is an indicator for rapid progression and possible malignant transformation (**Figure 8**) [65]. Diffusion tensor imaging (DTI) could also add some supplementary information in order to differentiate LGG from high-grade gliomas (HGG) [66].

Figure 8. T1W + C sequence (a), DTI (b) and perfusion MR (c) sequences in a patient with a LGG with a nodule of contrast enhancement corresponding to a region of increased rCBV; the patient presented 4 years previously for epileptic fits; MRI examination performed at that time was suggestive for a LGG, but the patient refused any treatment except the antiepileptic one; readmitted for signs of increased intracranial pressure and clear imagistic findings for progression and malignant transformation.

Macroscopy. It is a well-defined lesion at the interface between the white and the gray matter (*with an affinity for the cerebral cortex*). In cross-section, the surface is soft and frequent calcifications gives it a gritty look. Hemorrhagic and cystic degeneration areas can be seen.

Histological diagnosis. The aspect is that of an infiltrative tumor consisting of monomorphic cells. Cellularity is moderately increased, but it can vary considerably. The well-differentiated tumors can feature well-circumscribed nodules of increased cellularity. The nuclei are slightly enlarged, uniform, round (low atypia), and slightly hyperchromatic (“salt and pepper”). In hematoxylin-eosin, they are surrounded by a water clear cytoplasm with sharp borders, which gives them the artefactual aspect of fried egg or honeycomb. Typically, they show a network of branching capillaries in a chicken wire shape. Calcifications, cysts, and areas of mucoid degeneration can also be encountered. Mitoses are rare [67]. Immunohistochemically, the cells are IDH+ and ATRX+, and p53– [3]. GFAP and vimentin are variably expressed. Olig1 and Sox10 are positive but non-specific [68]. The differential diagnosis can be done with macrophage-rich lesions, diffuse astrocytoma, and clear cell ependymoma.

Genetic diagnosis. By definition, these tumors feature mutations in the IDH1 and IDH2 genes, as well as the deletion of the whole arm of the 1p and 19q chromosomes [69]. The incomplete/partial codeletion is present in glioblastomas and anaplastic astrocytomas. TERT mutation is associated with the IDH mutation and codeletion in the early onset of the tumor. *CIC* and *FUB* occur at a later stage [70]. The TP53 mutation is absent. As to the epigenetics, just like in the other cases, the IDH mutation induces a hypermethylated status—G-CIMP [38]. The methylation of the MGMT promoter is associated with a better survival rate, given the response to treatment (**Figure 9**) [71].

2.4.1. Multimodal treatment

2.4.1.1. Surgical treatment

Surgical principles are the same with those of DA IDH-mut, gross total resection (>90% volume reduction on 24–48 h postoperative MRI) being correlated with a longer PFS and OS, compared with subtotal resection [57].

Figure 9. Molecular and histopathological diagnosis of oligodendrogliomas.

The use of neuronavigation with co-registered preoperative T2W and FLAIR sequences and CT scan data in the presence of intratumoral calcification improved the grade of resection (Figure 10). As for other LGG, intraoperative electrostimulation mapping (IEM) on awake patient greatly improved not only functional outcome of the patient but also made possible to extend safely the grade of resection, with a great impact on the prolonged survival rate [72].

Figure 10. Preoperative T2W and T1W + C (a and b), respectively; 12 months postoperative T2W and T1W + C (c and d) sequences of a case with left frontal oligodendroglioma completely removed without any neurological deficit; (e) intraoperative aspect after completion of radical removal of a right temporal low-grade glioma with the preservation of Labbe vein (personal archive).

Figure 11. Staged resection of a high-grade gliomas with the aid of intraoperative contrasted ultrasound: after the dural opening (a), partial resection (b) and after complete resection (c) of the tumor.

Despite the fact that there are no randomized trials comparing grade of resection with and without intraoperative MRI, it is intuitive that having real-time data on the progression of removal is at least useful for completion of tumor excision. An alternative is the intraoperative ultrasound which can detect significant remnants in real time without interruption of surgery (**Figure 11**). The introduction of new equipment with 4D and contrast enhancement dramatically improves the quality of images, but their role in detecting fine details in low-grade gliomas is to be established in near future.

2.4.1.2. Adjuvant treatment

Immediate postoperative radiotherapy of completely removed oligodendroglioma IDH-mut 1p/19q codeletion is still under debate. Based on the results of EORTC 22845 trial which revealed that there is no significant difference in terms of OS between patients receiving immediate

Figure 12. Serial follow-up MRI examination of a patient with oligodendroglioma completely removed in September 2009, presenting with imagistic signs of progression 1 year later, but without any additional clinical manifestation; 15 months later the patient was readmitted for signs of increased intracranial pressure and clear findings of malignant transformation; re-operated in emergency, patient followed the standard radio-chemotherapy regimen.

postoperative radiotherapy and those receiving radiotherapy as a salvage therapy, the actual recommendation is to delay radiotherapy until signs of progression are evident. In cases of incompletely removed tumors or in the presence of any imagistic or clinical signs of progression, combined radiotherapy and PCV chemotherapy is superior to radiotherapy alone (**Figure 12**) [73].

Some authors argued that even in cases with foci of anaplasia imbedded in low-grade glioma, the total resection is sufficient for a long-term PFS, and no adjuvant treatment is needed; but this is not the standard of care as the authors already mentioned at the conclusions [74].

2.5. Oligodendroglioma, NOS

Definition. Infiltrative tumor with the histopathological aspect of oligodendroglioma, for which the genetic determinations relevant for the diagnosis (IDH mutation and 1p/19q codeletion) cannot be determined.

3. High-grade gliomas

3.1. Anaplastic astrocytoma, IDH-mutant

Definition. Tumoral proliferation which, from a histopathological point of view, displays an astrocytic phenotype, a diffuse growth pattern and proliferative activity, and which, genetically speaking, features mutations in the IDH1 or IDH2 genes.

Figure 13. Upper row: left frontal lesion hyperintense in T2W (a) and FLAIR (b) sequences with no contrast and T1W sequence (c) MRI in a 24 years old men diagnosed with grade III astrocytoma; lower row: left frontal lesion having similar characteristics in T2W (d) and FLAIR (e) sequences but with a discrete enhancement in T1W (f) sequences revealed in the young men's father MRI examinations, operated 5 years before with an anaplastic astrocytoma (personal archive) suggesting a hereditary determinism in this particular case.

Grading. Diffuse astrocytoma is deemed grade III WHO.

Clinically, the patients experience seizures, headache, neurocognitive disturbances, and neurological deficits in more advanced stages.

Imaging. MRI features are quite variable, with isointense mixed with hypointense signaling on T2 W sequences and heterogeneous hyperintensity in T2W and FLAIR sequences. The contrast enhancement is subtle or is lacking in majority of cases [75] (**Figures 13 and 14**).

Macroscopy. Anaplastic astrocytoma appears as an expansion of the tissue tending to infiltrate the surrounding nervous structures, but without destroying them. It is difficult to distinguish from the grade II astrocytoma, but the increased cellularity makes it easier to identify the edges of the tumor. Also, in cross section, we see areas of low consistency, granular, or opaque. Cysts are rarely encountered.

Histological diagnosis. The aspect is that of a tumoral proliferation with astrocytic phenotype and a diffuse growth pattern. As compared to grade II astrocytomas, it shows increased cellularity, anaplasia, and mitotic activity. These three parameters can vary between grade II astrocytoma and glioblastoma and must therefore be assessed in context. A heightened mitotic activity is sufficient for a diagnosis, even if the cellularity is low or normal. Also, just one mitosis in a stereotactic biopsy can be sufficient for a diagnosis. There are also atypical mitoses. In a large biopsy,

Figure 14. The immunohistochemical analysis (Clone H09) of the IDH1 R132H mutation performed on a patient with anaplastic astrocytoma. Infiltrating tumor cells positives at cytoplasmic level are observed in a background of negative normal astrocytes (left). In vivo single-voxel localized PRESS spectra were performed for the same patient. Black line represents the sum of spectra obtained during MRS. Pink line represent the spectra of 2-hydroxyglutarate identified at TE 35 ms (right).

the presence of several mitoses is not sufficient for a diagnosis. The nuclei are larger, hyperchromatic, and their shapes vary more than in the case of grade II astrocytomas, while the nucleoli are more visible. Multinucleated cells, gemistocytes, small cells, and perivascular lymphocytes can also be seen. There are no calcifications, necrosis, or microvascular proliferation [76].

The immunohistochemical profile is the same as in the grade II IDH-mutant diffuse astrocytoma: Olig2+, IDH1 R132H+, ATRX–, and p53+ in some cases. The Ki-67 proliferation index is generally high (5–10%), but it can vary considerably [77]. The differential diagnosis is performed with reactive gliosis, demyelinating diseases, progressive multifocal leukoencephalopathy, grade II astrocytoma, glioblastoma, and anaplastic oligodendroglioma.

Genetic diagnosis. Generally, it matches that of grade II astrocytomas. The IDH 1/2 mutation is present by default, in association with TP53 and ATRX. Chromosome arm 9p and 19q losses are more frequent in grade III tumors. As with grade II astrocytomas, the presence of the IDH mutation means a better prognosis, with the median survival rate for these patients being 9.3 years. The presence of EGFR, 10q loss, and 7q gain means a less favorable prognosis, and may suggest a molecular diagnosis of glioblastoma, even if there is no indication of it in the histology (Figure 14) [35]. By definition, the 1p/19q codeletion is absent.

3.2. Anaplastic astrocytoma, IDH wild-type

Definition. Tumoral proliferation which, from a histopathological point of view, displays an astrocytic phenotype with a diffuse growth pattern, proliferative activity, and increased anaplasia, and which, genetically speaking, does not feature mutations in the IDH gene.

Grading. Grade III tumor.

Genetic diagnosis. One in five anaplastic astrocytomas does not feature the IDH mutation, the genetic profile and the clinical evolution of these tumors being that of wild-type glioblastoma.

3.3. Anaplastic astrocytoma NOS

Definition. Diffuse anaplastic astrocytoma for which the presence of the mutation in the IDH gene could not be determined.

Grading. Grade III tumor.

3.3.1. Multimodal treatment

3.3.1.1. Surgical treatment

Being malignant tumors, early radical surgery represents the first step of the multimodal treatment of anaplastic astrocytomas. Due to the diffuse infiltration of the brain, radical excision is rarely achieved, meaning that if 1% of the initial volume remains in place, a local recurrence is almost certain. Additionally, the location of tumor in high eloquent areas prevents a radical excision in order not to produce severe neurological deficits. The main goals of surgery in high-grade gliomas (HGG) are to reduce the mass effect, to obtain relevant pathological tissue, to reduce the tumor burden, and to prolong survival with improved quality of life. There are many factors influencing OS in high-grade gliomas such as preoperative Karnofsky score, age, general and neurological status at the preoperative and postoperative period, pathology and genetics of the tumor, grade of resection, response and tolerance to the adjuvant therapy. Among all these factors only those related to surgery could be influenced, namely the grade of resection and the neurological status after the operation [78]. There is a very delicate balance between the radical surgery and preservation of neurological function. The more aggressive the surgery, the higher the risk for neurological deficiencies stands (**Figure 15**). After the publication of the trial conducted by Stupp and in 2005 [79], in which the role of surgery was minimized, a large amount of studies were published underlying the importance of gross total resection (GTR) compared not only with biopsy but also with near total resection (NTR) and subtotal resection (STR) as a factor that independently influences OS in HGG. For example, in anaplastic astrocytomas, there is a difference of 12 months in median of survival in favor of GTR compared with STR [80].

A retrospective study performed by us on 266 cases of HGG reveals the fact that gross total removal (GTR) greatly influences survival compared with STR. At the three periods of monitoring (12, 18, and 24 months), the difference regarding survival mean between GTR vs. STR ranged from 2.8 months (at 12 months monitoring) to 4.4 months (at 18 months monitoring) up to 5.1 months (at 24 months monitoring) including all types of malignant gliomas. We also found that the type of surgery and the age are prognostic factors that significantly influenced in all three periods of monitoring, while the histopathology was a prognostic factor for survival only at the 24 months monitoring (**Table 1**) [81].

A more detailed discussion regarding the role of surgery will be presented later in the chapter, along with the types of glioblastomas.

3.4. Anaplastic oligodendroglioma, IDH-mutant, 1p/19q-codeleted

Definition. Infiltrative tumor with the histopathological aspect of oligodendroglioma showing a significant proliferative activity and microvascular proliferations.

Figure 15. Integrated histological and molecular diagnosis of anaplastic astrocytoma IDH-mutant.

Grading. The tumor is grade III WHO.

Clinically, the most frequent presenting symptom is seizures, less frequent than in the lower-grade tumors, accompanied with signs of increased intracranial pressure and cognitive deficits. Other signs are related to the location of tumor [82].

Imaging. On *MRI* studies, they are typically heterogeneous, predominantly hypointense in T1W, and respectively hyperintense in T2W sequences. The frontal lobe cortical based location, the presence of calcification, cystic degeneration and hemorrhage, and intense enhancement along with minimal perilesional edema are more suggestive for anaplastic oligodendroglioma than for other gliomas. For follow-up imagistic studies, the T2W and FLAIR sequences are more sensitive in identification the local progression [83]. *CT scans* usually performed in emergency may clearly define calcification and hemorrhagic changes (Figure 16).

Macroscopy. As compared to grade II oligodendroglial tumors, they show additional necrotic areas.

Microscopic diagnosis. Increased cellularity, in a pattern that is infiltrative or compact with little intervening parenchyma. The cells have the characteristic fried egg aspect, but a significant pleomorphism or giant multinucleated cells can also occur. Significant mitotic activity is

Disease free interval	Log rank (Mantel-Cox) factor: age (<65 years/≥65 years)	Log rank (Mantel-Cox) factor: type of surgery (gross total removal/subtotal removal)	Log rank (Mantel-Cox) factor: histopathological diagnosis	Log rank (Mantel-Cox) factor: gender
12 months	0.000	0.000	0.090	0.296
18 months	0.000	0.000	0.122	0.836
24 months	0.000	0.000	0.031	0.756

Table 1. Disease free interval regarding the age, type of surgery, and histopathology.

Figure 16. Preoperative T2W and T1W+C (a and b) sequences of a case with left frontal anaplastic astrocytoma completely removed; follow-up MRI performed at 4 months postoperatively and after concomitant radio- and chemotherapy with temozolomide showed no signs of local recurrence (c and d).

also present [84]. Sarcomatoid areas can be seen. The minigemistocytes are more numerous than in the case of grade II oligodendrogliomas [85]. As a characteristic, the chicken wire aspect is accompanied by microvascular proliferation. As opposed to grade II oligodendrogliomas, they can involve palisading necrosis. Secondary structures can also be encountered. Immunohistochemically, the profile is that of grade II tumors, with a higher proliferation index evinced by way of Ki-67 (generally >5%). The differential diagnosis can be done using clear cell ependymoma, glioblastoma, and anaplastic astrocytoma.

Genetic diagnosis. Apart from the IDH mutation and the codeletion of the whole arm of the 1p and 19q chromosomes, the concurrent polysomy of 1p and 19q is more frequently encountered in anaplastic oligodendrogliomas [86]. Other chromosomal aberrations can occur: gain of 7, 8q, and 15q, and losses of 4q, 18, and 22q. Just like in the case of low-grade tumors, TERT, CIC, and FUBP1 mutations are present. Epigenetically, the IDH mutation induces G-CIMP, while in most cases MGMT is hypermethylated [87].

3.5. Anaplastic oligodendroglioma, NOS

Definition. The diagnosis concerns the anaplastic oligodendrogliomas whose genetic profile (IDH mutation and 1p/19q codeletion) cannot be determined.

The diagnosis of *oligoastrocytoma* has been discontinued following the latest WHO classification.

3.5.1. Multimodal treatment

As for the other types of gliomas, the grade of surgical resection is an independent factor for PFS and OS. As a consequence, GTR is recommendable wherever is possible without neurological damage (**Figure 17**).

Figure 17. Axial (a) and coronal (b) contrasted CT scan demonstrating the presence of a voluminous expansive lesion with large cystic components and peripheral calcification with a reduced perilesional edema, aspect highly suggestive for an anaplastic oligodendroglioma; after GTR the axial T2W (c) and coronal FLAIR (d) sequences at 2 years follow-up do not indicate a local progression.

Due to the good response of anaplastic oligodendrogliomas to chemotherapy, the subtotal resection with preservation of neurological function is also an acceptable surgical strategy [88].

3.5.1.1. Adjuvant treatment

Adjuvant treatment is recommended in anaplastic oligodendrogliomas on a regular basis. Recent studies failed to demonstrate a benefit of temozolomide over the classical PCV regimen, which remains the main tool for first-line chemotherapy in anaplastic oligodendrogliomas. Radiotherapy is reserved for cases of clear imagistic evidence of tumor progress. Fractions of 1.8–2 Gy for a total dose of 54–60 Gy are the actual recommended radiotherapy regimen based on clinical evidence [89].

3.6. Glioblastoma IDH wild-type

Definition. High-grade tumor with a diffuse growth pattern and a dominantly astrocytic differentiation showing cellular pleomorphism, nuclear atypia, and brisk mitotic activity. Glioblastomas are characterized by the presence of necrosis and microvascular proliferations.

Grading. Glioblastomas are grade IV WHO.

Clinically, the evolution is usually short due to the high growth rate of the tumor [90]. Almost 40% of the patients are admitted for surgical treatment in the first month from the clinical debut marked by progressive headache (almost 80% of cases). Mental disturbances, changes in personality, and neurological deficits are subsequent manifestations of the evolution of the disease. Seizures are less common (18.9% in our study on 276 cases) than in the LGG [81].

Imaging. *Native CT scan* is usually performed in emergency and reveals a heterogeneous expansive process with significant mass effect. Addition of contrast enhancement clearly defines a variable-enhanced lesion with perilesional edema, the classical aspect of a ring enhancement circumscribing an necrotic area which is frequently present (**Figure 18**).

Once the suspicion brain tumor is raised, an *MRI examination* will more accurately define the lesion. T1W and T2W sequences are able to reveal the tumor and also other pathological changes within or around it: edema, necrosis, hemorrhage, and calcifications. In addition, the FLAIR sequences will demonstrate a hyperintense perilesional halo, which is in fact a combination of tumoral infiltration and edema. On enhanced T1W sequences, the classical appearance of “ring enhancement” delineating a central necrosis is the most eloquent aspect of glioblastoma (**Figure 19**).

Nevertheless, *Advanced MRI* techniques will offer functional elements in favor of a malignant lesion. On MR spectroscopy, glioblastoma presents higher Cho (Choline) peaks along with a higher Cho/Cr (creatine) ratio. This ratio is additionally elevated owing to the decrease of Cr compared with the normal level. Lactate and lipid peaks that indicate necrosis and disruption of myelin sheaths could also be detected in glioblastomas [91]. On DWI, high-grade gliomas typically present a restricted diffusion. DTI studies may help in differentiating HGG from LGG through fractional anisotropy (FA), which is higher in the former [92]. On tractography, HGG will show a disruption of tracts. MRI perfusion studies reveal a heterogeneous relative cerebral volume (rCBV), demonstrating areas of increased cellularity and mitotic activity in glioblastomas (**Figure 20**) [93].

Figure 18. Axial T2W (a), axial FLAIR (b) and coronal T1W + C (c) sequences of an extremely rare intraventricular anaplastic oligodendroglioma completely removed, as it is demonstrated by the 6 months follow-up MRI (d–f).

Advanced MRI techniques are also useful for differentiating between local recurrence and radionecrosis (pseudoprogression). Recently, it was showed that rCBV as a single examination modality is superior to volume transfer constant (Ktrans) or apparent diffusion coefficient (ADC) for the prediction of local recurrence. The combination of rCBV and Ktrans improves the accuracy of the recurrence diagnosis [94].

Macroscopy. Poorly delineated lesions, with yellow, white, or granular areas caused by the necrosis, surrounded by grayish tumoral areas. In glioblastomas, cysts filled with a turbid liquid are formed through the liquefaction of the necrotic tissue. Typically, in cross section, we see purple-red or brown-red areas caused by the hemorrhage occurred during the progression of the tumor. The hemorrhages can be massive. Also in cross section, we can see thrombotic vessels with the aspect of “dark veins.”

Microscopic diagnosis. The histology of the tumor is extremely variable and the cellular composition is highly heterogeneous. The astrocytic nature of the tumor can be identified in the well-differentiated tumors. In the poorly-differentiated ones, the astrocytic phenotype is recognized with considerable difficulty [95].

There are a number of cellular morphologies common to glioblastomas: fibrillary astrocytes, gemistocytes, smalls cells (highly infiltrative, bipolar, and mitotically active), epithelioid,

Figure 19. Coronal (a) and axial (b) preoperative contrasted CT scan examination of a case with left temporal inhomogeneous expansive lesion with a “ring enhancement” highly suggestive for a glioblastoma; (c) and (d) immediate postoperative contrasted CT scan examination demonstrating gross total removal of the tumor.

Figure 20. T2W (a), FLAIR (b) and T1W + C MRI (c) sequences of a left frontal expansive lesion, aspects that are highly suggestive for a glioblastoma.

rhabdoid, granular, or lipidized cells. The areas that contain astrocytes whose phenotype is more easily identified can be more or less clearly separated from the areas of high pleomorphism. The cells are poorly differentiated, with pleomorphism, significant atypia, and brisk mitotic activity. The presence of a cellular population having a different phenotype indicates the emergence of a new tumoral clone through new genetic events [96].

The dominance of a specific cell type in the tumor generates a pattern that is useful in the diagnosis of small cells, with a neuroectodermal component, with an oligodendroglial component, with granular cells, with gemistocytes, and with lipidized cells. This is an indicative for the diagnosis but insufficient for a distinct variant.

The small cell glioblastoma pattern consists of small, round, or slightly oblong monomorphic cells, faint atypia, and hyperchromatic nuclei surrounded by a small amount of cytoplasm. One characteristic is the heightened proliferative activity. The GFAP is variably expressed, and when present, it can be seen at the level of delicate processes. Several morphological elements found in this pattern, such as the monomorphic nuclei surrounded by a clear halo, chicken wire vasculature, or calcifications, can cause problems in the sense of a differential diagnosis with oligodendrogliomas [97]. The difference is made here by molecular tests, which evidence a molecular profile typical for glioblastomas: EGFR amplifications, Ch 10 losses, IDH wild-type, no 1p/19q codeletion [98].

The primitive neuronal component glioblastoma is a glioblastoma featuring nodules that show neuronal differentiation. These nodules are clearly distinguishable from the rest of the tumor. Cellularity is increased, and so is the proliferation index in these areas. The cells are similar to those in other CNS embryonal neoplasms or they form Homer-Wright rosettes. The immunohistochemical profile is closer to that of neuronal precursors, being positive for Synaptophysin and with a low GFAP expression [99].

The glioblastoma with an oligodendroglioma component contains foci (often minor) of classic oligodendroglioma, associated with necrosis. The presence of the two components in the same tumor, in association with necrosis, raises the possibility of a grade IV oligoastrocytoma. While the presence and the size of the necrosis means a less favorable prognosis, this type of tumor, nevertheless, has a better prognosis than the standard glioblastomas [100]. From a molecular point of view, this histopathological aspect includes several molecular groups, lacking a distinct molecular signature. The tumor may or may not feature IDH1 mutations or 1p/19q codeletion, and also TP53 mutations, Chr 7 gain, Chr 10 loss, EGFR, and PDGFRA amplifications [101].

Granular cells glioblastomas are tumors with a distinct histological aspect, characterized by an aggressive behavior, despite a morphological character indicative of a grade II or III tumor. The cells are large, similar to macrophages. They are positive for CD163 and CD68 due to the high lysosome content. They are generally negative for GFAP, even if a certain positivity can be occasionally seen in the peripheral cellular areas. They are little understood from a molecular point of view, but a study involving array-based comparative genomic hybridization has indicated a genetic profile very similar to the conventional glioblastomas (gain of Chr 7, loss of Chr. 1p, 8p, 9p, 10p, 13q, and 22q, EGFR amplification, and homozygous deletion of CDKN2A). There are no IDH mutations and no 1p/19q codeletion [102].

The *lipidized glioblastoma* occurs rarely, a significant number of large cells with a lipidized (foamy) aspect being present against a histological background typical for a standard glioblastoma. In such cases, the diagnosis of pleomorphic xanthoastrocytoma cannot be ruled out. From a genetic point of view, they are little studied [103]. Phenotypically, they are similar to adipocytes, but the fact that they are positive for GFAP comes to confirm their glial origin.

Three histological variants are known: giant cell glioblastoma, gliosarcoma, and epithelioid glioblastoma.

Giant cell glioblastomas are characterized by the occurrence of giant multinucleated cells (up to 20 nuclei), with prominent nucleoli. Also noticeable are the small syncytial fusiform cells. The infiltration patterns typical for the diffuse gliomas are less visible in this variant. It features a significant component of connective tissue in the shape of a reticulin network [104]. Atypical mitoses are frequent. Another characteristic feature of this variant is the perivascular positioning of tumor cells (pseudorosette-like pattern). There is a significant ischemic necrosis. Microvascular proliferation and the pseudopalisading necrosis are rarely encountered. Perivascular lymphocytes may be present [105]. The neuronal immunohistochemical markers are negative. GFAP has variable expression. The nuclear expression of p53 shows a high incidence of TP53 gene mutation [106]. The presence of the connective component, together with their circumscribed nature, makes it more difficult to distinguish macroscopically between these tumors and meningiomas and metastases.

From a molecular point of view, they showed a high incidence of PTEN and TP53 mutations; but, no EGFR amplifications and no homozygous deletion of CDK2A were shown. As a variant of the wild-type glioblastoma, it features no IDH mutations. The prognosis is slightly better than in the case of usual glioblastomas [107].

The *epithelioid glioblastoma* is characterized by the presence of a significant population of cells with an epithelioid aspect, eosinophilic cytoplasm, peripheral nucleus, and sharp border. Rhabdoid cells with discrete borders and eccentric oval nuclei can be present, as well as necrosis and vascular proliferations. Immunohistochemically, the GFAP has a variable expression [108]. The epithelial markers (EMA and CK AE1/AE5), as well as S100 and vimentin, are positive. The antibody that indicates the BRAF V600 mutation is positive in 50% of cases [109]. From a molecular point of view, the epithelioid glioblastoma occasionally shows genetic events characteristic of glioblastomas (EGFR amplifications, CDK2A deletion, and PTEN mutation). As a variant of the wild-type glioblastoma, it features no IDH mutations. The prognosis is worse than in the case of other glioblastomas [110].

Gliosarcoma is characterized by a monoclonal proliferation with a highly malignant biphasic histological pattern, astrocytic and mesenchymal. The astrocytic component displays the typical aspect of a glioblastoma. The mesenchymal component consists of long bundles of spindle cells, closely packed, showing malignancy (atypia, necrosis, and mitosis), similar to fibrosarcoma or the malignant fibrous histiocytoma [111]. The confirmation of malignancy in the mesenchymal component is required in order to differentiate it from desmoplasia, which can occur in the glioblastomas with meningeal invasion. Elements of differentiation can appear (cartilage, bone, muscle, and osteoclastic giant cells) [112]. The presence of connective tissue

structures in the mesenchymal component (but not in the astrocytic one) can be demonstrated using staining for reticulin and trichrome. As with the giant cell glioblastomas, the mesenchymal component makes the tumor macroscopically similar to a metastasis or meningioma. This entity can also appear in ependymomas and oligodendrogliomas, *de novo* or following treatment [111]. Immunohistochemically, the astrocytic component is positive for GFAP, while the sarcomatoid one is negative. P53 is positive in both components. IDHR132H is negative. Genetically, this variant shows PTEN and TP53 mutations, as well as CDK2A deletions. There are no EGFR amplifications [113]. The epithelial-mesenchymal transition in the sarcomatoid component is highlighted by the SNAIL and TWIST expression.

Necrosis and microvascular proliferations are required for a diagnosis.

The microvascular proliferations are in direct relation with the necrosis, hypoxia being a significant factor that stimulates the formation of vessels by way of HIF1A and VEGFA [114]. Another characteristic is the thrombosis of small vessels. Although, in glioblastomas, vascularization is very well represented, the vascular structures are immature and largely incapable of compensating for the hypoxia caused by the exuberant proliferation [115].

Necrosis, of the coagulative type, is typical for glioblastoma. It affects both the cells and the vascular structures and is indicative of the extremely high proliferation rate associated with this type of tumor. It is considered to be an aggressive factor in astrocytic tumors, its size being associated with a lower survival rate. In the necrotic areas, we can see “shadows” of the dilated vessels surrounded by tumor cells in various stages of decay [116]. We can also see vessels that still contain oxygenated blood surrounded by viable cells. Undoubtedly, the high proliferation rate of glioblastomas plays an important part in the onset of necrosis, as there is a mismatch between the need for oxygen and the number of functioning vessels. As in the vicinity of the necrotic areas, we notice venal occlusions and vascular thrombosis, and it has been hypothesized that this is the mechanism that could trigger or spread the necrosis. Lesions of the endothelium trigger the release of procoagulants, which generate microscopic or macroscopic vascular thrombosis. Vascular thrombosis is usually accompanied by the so-called pseudopalisading [25]. This is caused by the migration of cells from the central necrotic area to the more oxygenated exterior ones, in a “moving away wave” toward the vessels unaffected by thrombosis and capable of maintaining a level of oxygenation sufficient for their survival. In an attempt to somewhat restore the level of oxygenation, these cells also secrete proangiogenic factors (VEGFs), causing the vascular alterations mentioned above [117].

A perivascular positioning of lymphocytes can occur in the areas with gemistocytes. In glioblastomas, the number of lymphocytes can vary, and they are mainly LT CD8. Quite interestingly, the presence of an extensive LT CD8 infiltration has been identified with the long-term survivors. LT CD4 and LB are also present, but in small numbers. Microglia and histiocytes can also be seen [118].

Immunohistochemically, GFAP has a positive expression, its variability reflecting the heterogeneous nature of the tumor. Sarcomatous and primitive cellular components are negative, while the gemistocytic component is strongly positive. It is also positive in the lipidized variant. S100 and Olig2 are positive in glioblastomas and are quite useful in the diagnosis of poorly differentiated tumors [119]. Nestin is of particular importance in the differential diagnosis in

regard to other high-grade gliomas, as it is positive in glioblastomas [120]. P53 is positive in the glioblastomas with a missense mutation of TP53 [121]. Together with WT1 (which can be positive in low-grade gliomas), it makes the distinction between tumor cells and the reactive post-treatment cells [122]. EGFR indicates the relative amplification of the gene, being expressed in 45–95% of cases. EGFRvIII is present in one third of all cases [123]. The expression of Ki-67 varies. A positive IDH R132H is incompatible with the diagnosis of IDH wild-type glioblastomas.

Genetic diagnosis. Glioblastomas were the first tumors investigated by The Cancer Genome Atlas (TCGA), which highlighted alterations in the signaling pathways of EGFR, PDGFR, PI3K, NF1, TP53, and *Rb* [124]. The genetic profile of wild-type glioblastomas differs from that of IDH mutated glioblastomas, which are considered secondary and which have a genetic profile very similar to that of grade II and III astrocytomas. The characteristic genetic alteration in glioblastomas is 7p gain combined with 10q loss [125].

In what concerns the *tyrosine kinase receptors* and their signaling pathways (PI3K/PTEN/AKT/mTOR and EGFR/RAS/NF1/PTEN/P13K), EGFR is the amplified gene most frequently present in primary glioblastomas, but it is more rarely encountered in the secondary ones [126]. The amplification is accompanied by different truncations in the same tumor. The best known one is EGFRvIII, present in nearly half of the glioblastomas with amplified EGFR. The structure of the receptor is similar to v-erb, and it activates independently of the ligand. Other possible amplifications accompanied by truncation are PDGFRA and MET [127].

The PTEN gene suffers changes almost exclusively in the primary glioblastomas, either by the way of a missense mutation in the area homologous to tensin/auxilin, or following truncation at various sites caused by the loss of the chromosomal region [128].

The *TP53/MDM2/MDM4/p14ARF signaling pathway* is affected in both primary and secondary glioblastomas, especially in the secondary ones, and it is also present in grade II and III astrocytomas. MDM2 amplification is a mechanism whereby the proapoptotic and antiproliferative control of p53 is eluded and encountered in the glioblastomas that do not present TP53 mutations [129].

The CDKN2A locus generates several CDKN2 and p14ARF proteins that act as tumor suppressors. The loss of p14ARF expression is encountered in glioblastomas, being correlated with the methylation of the promoter or the deletion of the CDKN2 gene.

The *CDKN2A/CD4/RB1 signaling pathway* is altered in most glioblastomas and it occurs in both primary and secondary glioblastomas. The mutations of the RB1 gene are rare, and the methylation of the promoter followed by the loss of protein expression is more frequent in secondary glioblastomas than in the primary ones [7].

TERT can show mutations at the level of the promoter, especially in wild-type glioblastomas, being mutually exclusive with TP53. The occurrence of the mutation (in one of the two hot spots) is followed by the accumulation of the GABP transcription factor at the level of the promoter, leading to the aberrant expression of the gene [130].

The IDH gene is not mutated by definition in wild-type glioblastomas, and the evaluation of the mutational status of this gene can make the distinction between primary and secondary glioblastomas [131].

Epigenetics. A study conducted in cooperation with TCGA and based on the determination of the genomic profile has defined four subtypes of glioblastomas: pro-neural, neural, classic, and mesenchymal. These subtypes are characterized by a different mutational and epigenetic profile, reflected in the response to treatment. These results have paved the way toward the assessment of the epigenetic profile of glioblastomas through a determination of the whole methylation genome profile in the glioblastomas with artificially induced mutations (IDH H3F3A). These experiments have led to the identification of six different subtypes, with a different clinical evolution [132].

MicroRNA and long non-coding RNA have also been studied in glioblastomas. miR10b controls the cycle of stem and tumoral cells in GBM and is associated with a poor prognosis. The role of the interaction between microRNA and the oncogenetic pathways known as drivers in GBM, such as for instance PI3K, has also been demonstrated [133].

The currently defined histological entities have different genetic expression profiles, which are correlated with the grade and are a better predictor of patient outcome [37].

The methylation profile of the MGMT gene promoter is predictive for the response to the treatment with alkylants such as temozolomide or methylants. It is variable, being higher in the IDH-mutant tumors, having a G-CIMP profile [134].

3.7. Glioblastoma IDH-mutant

Definition. High-grade tumor with a dominantly astrocytic differentiation and a diffuse growth pattern, involving the mutation of the IDH gene.

Grading. It is ranked as a grade IV tumor.

Clinically speaking, as it is mostly located in the frontal lobe, it is often accompanied by behavioral and neurocognitive changes. As the growth is more sluggish than in the case of wild-type tumors, the signs indicating an increase in intracranial pressure are also milder.

Imaging. The areas of central necrosis typically seen in wild-type GBM are usually absent here. They are larger in size; the cystic structures are more frequent; and they show no enhancing zones on MRI.

Macroscopy. The aspect is that of a tumor infiltrating the adjacent cerebral parenchyma. The purple hemorrhagic areas and the yellow-white necrotic ones are absent.

Histological diagnosis. The morphological aspect of IDH-mutant glioblastomas is very similar to that of wild-type GBM. The areas of necrosis (ischemic or palisading) are more rarely encountered. On the other hand, the oligodendroglioma-like component is more frequent and has been associated with the presence of the IDH1 mutation [135].

Immunohistochemically, GFAP is positive and shows a certain variability. The presence of genetic events is reflected by the positivity for IDH1 and p53, and the negativity for ATRX. The overexpression of EGFR is unusual, with amplification being a characteristic of the wild-type GBM [136].

Genetic diagnosis. The presence of the IDH mutation falls under the definition of the tumor, and its identification makes it possible to diagnose the two types of glioblastoma. It is an early

	Astrocytoma	Oligodendroglioma	GBM-wt
IDH	+	+	-
ATRX	+	-	-
TERT	-	+	+
p53	+	-	+
1p/19q	-	+	-

Table 2. Synthesis of the mutational status in the main categories of diffuse gliomas.

event in tumorigenesis and remains present during the progression toward glioblastoma. The most frequent mutation of the IDH1 gene (90% of all astrocytic or oligodendroglial tumors) is R132H, when a guanine is replaced by an adenine (CGT->CAT). Other mutations, such as R132C, R132S, or R132L, occur much more rarely [137].

The ATRX gene is also mutated, the same mutation being present in the grade II and III precursor astrocytic lesions. Alongside the ATRX and IDH mutation, TP53 is more frequently mutated in secondary glioblastomas [31]. EGFR amplifications are rare, as opposed to the GBM wild-type, indicating that the genetic onset and progression pathways are different.

The genetic expression of GBM IDH-mutant is relatively homogeneous, most of them falling under the proneural profile [126]. Epigenetically, the occurrence of the IDH mutation induces an extensive hypermethylation of the DNA, all IDH-mutant tumors belonging to a hypermethylated phenotype [138]. The prognosis for GBM IDH-mutant is better than that for GBM wild-type, the survival rate being 2.4 times higher (Table 2) [139].

3.8. Glioblastoma, NOS

Definition. High-grade tumor with dominantly astrocytic differentiation and diffuse growth pattern, in which the mutational status of the IDH gene cannot be determined. They are grade IV tumors.

3.8.1. Multimodal treatment

3.8.1.1. Surgical treatment

Sir Rickman Godlee was the first surgeon who reported a resection of a glioma in 1884. More than one century later, the results of the treatment in malignant gliomas remained unsatisfactory. Improvements of surgical techniques and technology developed in this period, including microsurgery, neuronavigation, intraoperative MRI, intraoperative neuromonitoring, and 5-ALA merely improved the grade of resection while increasing the postoperative quality of life of the patient; however, they were unable to change the inexorable course of malignant gliomas to recurrence and death. Perhaps the most important adjuvant of surgery was the introduction of radiotherapy in the middle of the last century, adding a median survival of at least 7 months, as it was highlighted by Ley and coworkers in 1962. In their reported series of

Figure 21. MRI examination of a patient with left paraventricular glioblastoma: (a) T2W sequence reveals an inhomogeneous lesion adjacent to occipital horn of left lateral ventricle surrounded by a hyperintense region of edema; (b) T1W + C sequence expose classical aspect of “ring enhancement”; (c) DWI study demonstrating a restricted diffusion corresponding to the region of tumor; MRI perfusion reveals a heterogeneous rCBV (d) and increased cerebral blood flow (CBF) corresponding to the enhanced lesion (e) ; (f) Tractography shows a disruption of fibers compared to the contralateral side.

glioma, the largest at that time, the median of survival through surgery alone was 7 months, whereas in the group receiving additional radiotherapy the median survival rate was at 15 months [140]. The addition of chemotherapy further improved the survival, but despite the large advancements in research within the last decade, the median survival rate barely increased by no more than 2 months, inexorably close to the data reported 20 years ago [141].

Regarding the role of surgery, there is an increasing body of literature underlining the importance of GTR comparing with STR or biopsy. A meta-analysis covering 41,117 unique patients in 37 retrospective studies revealed a significant improvement of OS in GTR cases compared with STR [142]. Another prospective study comparing grade of resection with the aid of immunofluorescence (5-ALA) versus white light also demonstrated a significant median of survival in favor of those with GTR (16.7 months vs. 11.7 months) [143]. Currently, there is sufficient data favoring GTR to encourage surgeons to increase the grade of resection, while also striving not to cause additional neurological deficits (**Figure 21**).

New tools were introduced in the last two decades in order to facilitate GTR, simultaneously with the permanent improvement of the operative microscope. Contemporary neuronavigation

Figure 22. Preoperative (a and b) and postoperative (c and d) contrasted CT scan images demonstrating the GTR of a left gliosarcoma.

equipment offers a more precise localization with real-time correction adapted to the brain shift. Functional and anatomical data are merged in order to facilitate surgical intervention, while also avoiding damage to eloquent areas and tracts. Intraoperative tools, such as intraoperative MRI, are now available in many centers, allowing surgeons to achieve a more complete tumor removal and, as a direct consequence, prolong survival [144]. Intraoperative ultrasonography, having been introduced in practice two decades prior, is at present more accurate in defining intracerebral lesions, especially in cases of HGG, facilitating a real-time control of resection (**Figure 22**) [145].

Contrasted intraoperative ultrasound guidance apparently adds more detailed information concerning the grade of resection [146].

We may conclude that surgery, and radical surgery especially, remains the first and most important step of the multimodal treatment in prolonging the survival of patients with malignant gliomas.

3.8.1.2. Adjuvant treatment

Whole brain radiation therapy stood as the most important adjuvant to surgery up until the randomized trial conducted by Walker et al. in 1980. In this trial, the authors compared the efficacy of radiotherapy alone to the addition of nitrosourea chemotherapy. They demonstrated a

Figure 23. Ultrasonography-guided resection (a) of a diffuse astrocytoma with foci of glioblastoma (arrow) demonstrated on T1W + C image (b).

benefit of 2–3 months in the group treated with combined radio-chemotherapy and this combination has been the standard of care for almost 25 years [147]. In time, whole brain radiotherapy was replaced by a more focal radiotherapy, in order to prevent secondary effects of radionecrosis. The current standard of radiotherapy is a fractionated conformational dose of 2Gy fractions/day, 5 days/week for a period of 6 weeks. The radiotherapy regimen must be initiated in three to no more than 6 weeks after the surgery [148].

Concerning chemotherapy, the actual standard consists of concomitant radiotherapy and temozolomide (TMZ) administration, followed by six cycles of TMZ at every 28 days. This was established in 2005 based on the results of the trial conducted by Stupp et al. (Figure 23) [79].

The response of the patients to the TMZ regimen is highly variable, with one of the factors influencing the positive response being the methylation of the MGMT promoter, which is present in less than 50% of glioblastoma cases. It was showed that only 65–70% of new cases of glioblastoma respond to TMZ; although in spite of these evidences, TMZ is still the main agent in multimodal treatment of glioblastoma (Figure 24) [149].

A large number of researches were made in order to decrypt the intimate mechanism of apparition and development of glioblastomas, as well as the response of tumor cells to different chemical, physical, or biological agents. Among these agents, only few met the clinical criteria to be introduced in practice. Gliadel (carmustine-impregnated biodegradable wafers) on local application proved to add a median of survival of 2.3 months compared to the standard treatment, yet was accompanied by an increased incidence of local complications (infection and CSF fistula) [150]. We also add our research effort on glioblastoma stem cell cultures in order to improve the response to TMZ by addition of new drugs like arsenic trioxide or metformin as sensitizers [151, 152]. New ways to deliver TMZ have been proposed [153]. Antiangiogenic agents (Bevacizumab) was also tested on glioblastoma stem cells with controversial response (in some cultures, they increased angiogenesis) (Figure 25).

The clinical trials testing Bevacizumab in newly diagnosed glioblastoma failed to demonstrate a benefit on survival [154, 155]. Immunotherapy is a highly experimental approach of present days, as are other therapies. A special interest was raised in the use of an electric field delivered by noninvasive transducers placed on a headpiece, the so-called Novocure

Figure 24. Preoperative axial (a), coronal (b) and sagittal (c) T1W + C sequences of a patient with glioblastoma completely removed and treated with the standard radio-chemotherapy regimen; postoperative enhanced T1W images (d–f) reveal the removal of the tumor with the persistence of a small contrasted nodule, which is stable at 5 years after surgery.

Figure 25. Preoperative T1W + C sequence (a) of a glioblastoma, almost completely removed as it was revealed by immediate postoperative CT scan (b); the patient was not responsive to the standard adjuvant treatment as it is demonstrated by the postoperative enhanced MRI at 4 months (c).

treatment. The addition of this new modality to the standard radio-chemotherapy regimen improved the PFS and OS in glioblastoma-treated patients [156].

We can conclude that despite the huge efforts and investments made in research and therapies, the results still disappoint. This suggests the fact that we are possibly on the correct course, but against the tide. The future multimodality treatment may consist of patient-tailored treatments owing to the multiple individual and tumor-related factors that influence the response to therapy.

3.9. Diffuse midline glioma, H3 K27 M-mutant

Definition. High-grade infiltrative astrocytic tumor, with midline location and showing the K27 M mutation in the H3F3A or HIST1H3B/C genes. They are grade IV tumors.

Clinically, it is usually encountered in children and more rarely in adults. The symptoms may be either general (usually produced by the obstructive hydrocephalus) or focal generated by the involvement of different cranial nerves: facial paresis, diplopia, hearing loss, dysphonia, and dysphagia. The extension of the tumor in cerebellar peduncles produces ataxia. The involvement of long tracts determines motor weakness. Particularly in diffuse pontine glioma, behavioral changes such as pathological laughter and anxiety were described before the onset of other more common clinical signs [157].

Imaging. On MRI, these lesions have a diffuse homogenous appearance of hypointense in T1W sequences and hyperintense in T2W and FLAIR ones. The contrast is discrete or absent, but some focal enhancement into an infiltrative hypointense mass on T1W + C sequences could be detected in few cases (**Figure 26**).

MRI spectroscopy will differentiate these tumors from other inflammatory, vascular, or infectious lesions. The Cho/Cr and NAA/Cr ratios are typically increased. An elevation of these ratios after radiotherapy poses the significance of progression [158].

Macroscopy. The infiltrative pattern is reflected in the size and the changes in the shape of the nervous structures affected by the tumor. In cross section, we can see purple hemorrhagic areas or yellowish-white necrotic ones.

Histological diagnosis. The aspect is that of an intensely infiltrative tumor consisting of small-size monomorphic, astrocytic cells. While most are accompanied by necrosis, microvascular proliferations, and an enhanced mitotic index, these elements are absent in a small number of tumors. According to the new rule introduced by the latest WHO classification, whereby “molecular beats histology,” even if histologically they are grade II, they shall nevertheless be deemed grade IV. *Immunohistochemistry.* GFAP shows a heterogeneous expression in these tumors. On the other hand, they are positive for S100, OLIG2, and NCAM1. Mutations of ATRX and TP53 can occur, but more rarely. An antibody targeting the protein modified by the presence of the H3.3 K27 M mutation can be used in the diagnosis [159].

Figure 26. In vitro 3D model of glioblastoma stem cells culture demonstrating an increase angiogenesis in contact with TMZ and resistance to the addition of Bevacizumab compared with the control (ctrl) (personal archive).

Genetic diagnosis. This entity is defined by the presence of the K27 M mutation in the H3F3A (80%), HIST1H3B (20%), and HIST1H3C genes. The genetic profile is completed by mutations in the TP53 and ATRX genes, homozygous CDKN2A deletion, and MYC, CDK4/6, and PDGFRA amplifications [160].

3.9.1. Multimodal treatment

This type of tumor is not amenable for surgical treatment. The single debate is related to the role of biopsy. The actual accepted strategy is that biopsy (open or stereotactic) is indicated only in atypical tumors. The term of atypical is somehow unclear, but generally it is accepted that the unilateral extension of tumor and the presence of cystic components or focal hemorrhages offer a rationale for surgery.

In centers with experience in stereotactic biopsy, this could be the method of choice. Otherwise, open surgery is performed, guided by the most superficial part of lesion or throughout the so-called “safe entry zone.” In selected cases, surgical interventions can offer the opportunity of cytoreductive surgery, thus creating proper conditions for radiotherapy (**Figure 27**) [161].

Radiotherapy is the single recommendable therapy for this kind of lesions (**Figure 28**). A total dose between 54 and 60 Gy, administered in fractions of 1.8–2 Gy, is the actual recommendation of oncologists. No chemotherapy regimen was able to demonstrate a benefit in terms of survival. Corticosteroids are also administered during radiotherapy. After a period of symptom amelioration, the tumor will inevitably progress [162].

3.10. Pediatric diffuse astrocytic tumors

Despite having a particular clinical evolution, pediatric tumors have been lumped together with the adult ones, according to the histopathological similarities [163]. We now know that their onset and progression genetic mechanisms are different. A well-defined entity that occurs predominantly in children is the one described above. As opposed to adult tumors,

Figure 27. T2W (a), FLAIR (b) and DWI (c) sequences of an infiltrative tumor into the brainstem and left cerebellar peduncles.

Figure 28. (a) T2W sequence of a diffuse infiltrative brainstem astrocytoma, partially resected; the 18 months follow-up MRI (b) demonstrates a stable lesion after radiotherapy.

the pediatric ones show changes in the genes regulating chromatin structure and the genetic expression profile. Apart from the diffuse midline glioma, H3 K27 M-mutant, another mutation present in the same gene, but in the G34 rather than the K27 position, defines another entity usually encountered in youths. The location differs, as it is no longer situated in the midline area, but in the brain hemispheres. High-grade astrocytic pediatric tumors with a telencephalic location show the TP53, CDK2A, ATRX, and SETD2 mutations [164]. The genetic syndromes that favor the onset of brain tumors during childhood are type 1 neurofibromatosis and Li-Fraumeni syndrome.

4. Conclusions

The 2016 brain tumor classification represents an important step forward in the introduction of molecular diagnosis in the daily current practice. This is also an important step toward a personalized patient-tailored multimodal management of brain tumors. Neurosurgeons, as part of a multidisciplinary team, need to be familiarized with all the aspects regarding specific brain tumors in order to offer the best chance to their patients and to prolong survival with a good quality of life.

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Conflict of interest

The authors declare that there is no conflict of interest.

Abbreviations

1p/19q-codel	1p/19q-codeleted
ATRX	alpha thalassemia/mental retardation syndrome X-linked
CK	cytokeratin
DA	diffuse astrocytoma
DTI	diffusion tensor images
EGFR	epidermal growth factor receptor
EMA	epithelial membrane antigen
FGFR1	fibroblast growth factor receptor 1
GBM	glioblastoma
GFAP	glial fibrillary acidic protein
HIF1A	hypoxia-inducible factor 1-alpha
IDH	isocitrate dehydrogenase
IDH-mut	IDH-mutant
LT	lymphocyte B
LT	lymphocyte T
MDM2	mouse double minute 2 homolog
MDM4	mouse double minute 4
MGMT	O ⁶ -methylguanine DNA methyltransferase
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
ms	milliseconds
NCAM1	neural cell adhesion molecule
NF1	neurofibromatosis 1
NOS	not other specified
PI3K	phosphatidylinositol-3 kinase

PRESS spectra	point-resolved spectroscopy
PTEN	phosphatase and tensin homolog
Rb	retinoblastoma
TE	echo time
TERT	telomerase reverse transcriptase
TP53	tumor protein
VEGFA	vascular endothelial growth factor A
wt	wild type

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