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IDH-wildtype lower-grade diffuse gliomas: the importance of histological grade and molecular assessment for prognostic stratification

Giulia Berzero, Anna Luisa Di Stefano, Susanna Ronchi, Franck Bielle, Chiara Villa, Erell Guillerm, Laurent Capelle, Bertrand Mathon, Alice Laurenge, Marine Giry, Yohann Schmitt, Yannick Marie, Ahmed Idbaih, Khe Hoang-Xuan, Jean-Yves Delattre, Karima Mokhtari, and Marc Sanson

Sorbonne University, Brain and Spinal Cord Institute, Paris, France (G.B., A.L.D.S., A.L., M.G., Y.S., Y.M., A.I., K.H.X., J-Y.D., K.M., M.S.); University Hospitals of Pitié Salpêtrière, Charles Foix, Department of Neurology 2 Mazarin, Paris, France (G.B., A.L.D.S., A.L., A.I., K.H.X., J-Y.D., M.S.); Department of Brain and Behavioral Sciences, University of Pavia, Italy (G.B.); Department of Neurology, Foch Hospital, Suresnes, France (A.L.D.S.); University Hospitals of Pitié Salpêtrière, Charles Foix, R Escourolle Laboratory, Paris, France (S.R., F.B., K.M.); Department of Pathology, Foch Hospital, Suresnes, France (C.V.); University Hospitals of La Pitié Salpêtrière, Charles Foix, Functional Unit of Oncogenetics and Molecular Angiogenetics, Department of Genetics, Paris, France (E.G.); University Hospitals of La Pitié Salpêtrière, Charles Foix, Department of Neurology 2, Paris, France (L.C., B.M.); Onconeurotek Tumor Bank, University Hospitals of Pitié Salpêtrière, Charles Foix, Paris, France (Y.M., J-Y.D., K.M., M.S.).

Corresponding Author: Marc Sanson, MD PhD, Service de Neurologie 2, GH Pitié-Salpêtrière, 47 bd de l'Hôpital, 75013 Paris, France (marc.sanson@aphp.fr).

Abstract

Background. Isocitrate dehydrogenase (*IDH*) wildtype (wt) grade II gliomas are a rare and heterogeneous entity. Survival and prognostic factors are poorly defined.

Methods. We searched retrospectively all patients diagnosed with diffuse World Health Organization (WHO) grades II and III gliomas at our center (1989–2020).

Results. Out of 517 grade II gliomas, 47 were "diffuse astrocytomas, *IDH*wt." Tumors frequently had fronto-temporo-insular location (28/47, 60%) and infiltrative behavior. We found telomerase reverse transcriptase (*TERT*) promoter mutations (23/45, 51%), whole chromosome 7 gains (10/37, 27%), whole chromosome 10 losses (10/41, 24%), and *EGFR* amplifications (4/43, 9%), but no *TP53* mutations (0/22, 0%). Median overall survival (OS) was 59 months (vs 19 mo for *IDH*wt grade III gliomas) ($P < 0.0001$). Twenty-nine patients (29/43, 67%) met the definition of molecular glioblastoma according to cIMPACT-NOW update 3. Median OS in this subset was 42 months, which was shorter compared with patients with *IDH*wt grade II gliomas not meeting this definition (median OS: 57 mo), but substantially longer compared with *IDH*wt grade III gliomas meeting the definition for molecular glioblastoma (median OS: 17 mo, $P < 0.0001$). Most patients with *IDH*wt grade II gliomas met cIMPACT criteria because of isolated *TERT* promoter mutations (16/26, 62%), which were not predictive of poor outcome (median OS: 88 mo). Actionable targets, including 5 gene fusions involving *FGFR3*, were found in 7 patients (24%).

Conclusions. Our findings highlight the importance of histological grading and molecular profiling for the prognostic stratification of *IDH*wt gliomas and suggest some caution when assimilating *IDH*wt grade II gliomas to molecular glioblastomas, especially those with isolated *TERT* promoter mutation.

Key Points

1. *IDH*-wildtype diffuse grade II gliomas should be distinguished from grade III because of a lower burden of genetic alterations (including *EGFR* amplifications, whole chromosome 7 gain/whole chromosome 10 loss, *TERT* promoter mutations, *TP53* mutations, deletions of cyclin-dependent kinase inhibitor 2A, and chromosome 9p loss) and a much better outcome.
2. With a median overall survival of 88 months, *IDH*-wildtype grade II gliomas with isolated *TERT* promoter mutations should not be assimilated to molecular glioblastomas.

Importance of the Study

The cIMPACT-NOW update 3 has recently established that *IDH*wt histological grade II and III diffuse gliomas with *EGFR* amplifications, and/or combined whole chromosome 7 gain and whole chromosome 10 loss, and/or *TERT* promoter mutations should be considered as bona fide glioblastomas. Our data suggest that, while true for histological grade III gliomas, these considerations do not fit a subset of grade II gliomas, and namely those with isolated *TERT* promoter mutations (median overall survival: 88 mo). These findings highlight the importance of histological grade, in parallel to molecular profile, for the prognostic stratification of *IDH*wt lower-grade gliomas and suggest that *IDH*wt gliomas with grade II histology (<2 mitosis per 10 high power fields) and isolated *TERT* promoter mutations should not be assimilated to molecular glioblastomas.

Lower grade (ie, World Health Organization [WHO] grades II and III) diffuse gliomas form a heterogeneous group of tumors including entities characterized by different malignant behavior. The *IDH* mutation and the chromosome 1p/19q codeletion represent the main diagnostic and prognostic markers in this group.[1,2] The *IDH* mutation is an independent predictor of prolonged survival and its prevalence is inversely correlated with tumor grade.[2]

Isocitrate dehydrogenase wildtype (*IDH*wt) grade II diffuse gliomas correspond to a rare subgroup of low-grade tumors associated with dismal prognosis and poor response to treatments.[3] Due to the lack of large prospective studies, there is still conflicting evidence regarding the clinical and molecular profile associated with these tumors, and survival estimates widely range from 4.7 to 8.4 years across studies.[3–6] Given the rarity of *IDH*wt grade II diffuse gliomas, most of the studies analyzed grade II and III gliomas altogether[7,8] to generate more solid data. However, evidence suggests that *IDH*wt grade II and grade III tumors significantly differ in terms of prognosis and biological behavior: while *IDH*wt grade III gliomas strikingly resemble primary glioblastomas,[7,9] *IDH*wt grade II neoplasms display less malignant features.[4,6]

A recent consensus from the cIMPACT-NOW consortium has proposed that grades II and III *IDH*wt astrocytomas harboring epidermal growth factor receptor (*EGFR*) amplification, and/or combined whole chromosome 7 gain and whole chromosome 10 loss (+7/−10), and/or telomerase reverse transcriptase (*TERT*) promoter mutation should be considered as bona fide glioblastomas, given their poor outcome,[10,11] though these recommendations are not yet part of the WHO classification.

The aim of this study was to better define the outcome of *IDH*wt grade II diffuse gliomas compared with *IDH*-mutant (*IDH*mut) grade II and *IDH*wt grade III diffuse gliomas, highlighting the main prognostic factors in this cohort.

Materials and Methods

We performed retrospective research in the OncoNeuroTek database for all patients with diagnoses of WHO grades II and III diffuse gliomas between January 1989 and February 2020. After dividing WHO grades II and III gliomas in molecular subgroups based on their *IDH1/2* and chromosome 1p/19q codeletion status,[7,12] we focused on the subgroup of patients with *IDH*wt grade II gliomas. The clinical, radiological, histological, and molecular features of the patients in this subgroup were thoroughly reviewed to ensure an accurate patient selection. All available histological specimens were independently reviewed by 2 expert neuropathologists (K.M., F.B.), who assigned an integrated diagnosis according to the 2016 WHO Classification of Tumors of the Central Nervous System.[1] Discordant assessments were resolved after collective discussion and additional immunohistochemical and molecular studies. Immunohistochemical staining for *IDH1* R132H, which was performed systematically in all cases, allowed identification and exclusion of *IDH1*-mutant patients who were falsely negative on Sanger sequencing because of massive contamination of the tumor specimen with normal tissue. Immunostainings for alpha thalassemia/mental retardation syndrome X-linked (ATRX), p53, H3K27M,

FGFR3, EGFR, CD34, and neurofilaments allowed to better characterize and classify the tumor and to exclude circumscribed gliomas. Grading was assigned using widely accepted criteria[13] that have recently been embraced by the cIMPACT consortium for lower-grade *IDH*mut diffuse astrocytomas[14]: tumors with high cellularity, marked nuclear atypia, and ≥2 mitoses (per 10 high power fields for biopsy specimens and per 30 high power fields Ki67 hotspots for resection specimens) were attributed grades III–IV histology.[1,14] Patients with midline tumors were excluded from the study if positive for the *H3F3A* K27M mutation on immmunohistochemistry and/or DNA sequencing, as they are assigned grade IV according to the 2016 WHO classification. MRI scans acquired at diagnosis were systematically reviewed to verify that imaging features were compatible with diffuse glioma and to exclude the presence of gross nodules of contrast enhancement that would suggest that it was sampled the periphery of a higher-grade neoplasm. Patients who, upon the revision of histological, molecular, and MRI features, had a confirmed diagnosis of "diffuse astrocytoma, IDH-wildtype (grade II)" were included in subsequent analyses.

The clinical and paraclinical characteristics in patients with *IDH*wt grade II gliomas were analyzed and compared with patients with *IDH*wt grade III gliomas. We separately assessed how many patients in the group of *IDH*wt grade II and *IDH*wt III gliomas met the definition of "diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, grade IV" according to cIMPACT-NOW update 3[10] and compared their clinicomolecular features to patients with *IDH*wt tumors of the same grade not meeting this definition.

All tumor samples and clinical data were collected upon written informed consent in accordance with the tenets of the Declaration of Helsinki. The study was approved by the ethical committee CPP "Ile-de-France VI."

Molecular Analyses

Tumor DNA was extracted from formalin-fixed paraffin-embedded (FFPE) and/or from snap frozen tumor samples (–80°C). DNA was extracted using commercial kits (GeneJET FFPE DNA purification kit, Thermo Scientific [FFPE tissue]; QIAamp DNA mini kit, Qiagen [frozen tissue]) or by automated DNA extraction (Maxwell, Promega). The mutational status of *IDH1* (codon 132), *IDH2* (codon 172), *H3F3A* (codon 27 and 34), *FGFR1* (codons 546 and 656), *BRAF* (codon 600), and *TERT* promoter (-250 and -228) was obtained by Sanger sequencing following standard PCR amplification, using previously reported primers[15–17] or from next-generation sequencing (NGS). Information on copy number was acquired by comparative genomic hybridization (CGH-array)[18] or by copy number analysis from NGS data. *FGFR3-TACC3* fusions were assessed by real-time (RT)-PCR amplification followed by Sanger sequencing from RNA extracted from snap-frozen tumor tissue.[19] A subset of patients who tested negative for *FGFR3-TACC3* fusions on RT-PCR but had suggestive cell morphology underwent a wider research for fusion genes using Illumina NGS panels: the Archer Comprehensive Thyroid Lung Fusion Plex (MiniSeq), which identifies rearrangements in *ALK, AKT1, BRAF, CALCA, CCND1, CTNNB1, DDR2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FOXL4, GNAS, HRAS, IDH1, IDH2, KRAS, KRT20, KRT7, MAP2K1, MET, NRAS, NRG1, NTRK1, NTKR2, NTRK2, PIK3CA, PPARG, PTH, RAF1, RET, ROS1, SLC5A5, THADA, TTF*, or the AmpliSeq FOCUS (Miseq), which identifies rearrangements in *ABL1*, *ALK*, *AKT3*, *AXL*, *BRAF EGFR*, *ERBB2*, *ERG*, *ETV1*, *ETV4*, *ETV5*, *FGFR1*, *FGFR2*, *FGFR3*, *MET*, *NTRK1*, *NTRK2*, *NTRK3*, *PDGFRA*, *PPARG*, *RAF1*, *RET*, and *ROS1*.

Statistical Analyses

Categorical variables were compared using the chi-square or the Fisher's exact test. Quantitative variables were compared using Student's *t*-test or the Mann–Whitney test. Overall survival (OS) was estimated by the Kaplan–Meier method and survival curves were compared using the log-rank test. The Cox model was used for continuous variables survival analyses. Hierarchical clustering and multidimensional association tables were used to explore associations between variables. The Pearson or the Spearman correlation test was used to assess statistically significant correlations between variables. For all analyses, the established threshold for statistical significance was $P = 0.05$. All statistical analyses were performed using "R" software packages.

Results

IDHwt Grade II Gliomas

The process of case selection is illustrated in Fig. 1. The clinical and molecular characteristics of the 47 patients who were assigned a diagnosis of "diffuse astrocytoma, *IDH*-wildtype (grade II)" upon centralized histological review are summarized in Table 1. The median age at diagnosis was 55.0 years (range, 19.6–82.1). Thirty-6 patients were male (36/47, 77%). Median preoperative Karnofsky performance status (KPS) was 90 (range, 70–100). Tumors commonly had their epicenter in the temporal lobe, extending to the fronto-basal lobe and the insula (28/47, 60%) (Supplementary Figure 1, panel A–C). Infiltration was commonly extensive, involving the ipsilateral deep gray matter (10/32, 57%) (Supplementary Figure 1, panel D–E), the cortex of adjacent lobes (5/32, 16%) (Supplementary Figure 1, panel C), the brainstem (3/32, 9%), and the contralateral hemisphere (5/32, 16%) (Supplementary Figure 1, panel F). As a consequence of tumor location, size, and highly infiltrative behavior, surgery commonly consisted of biopsy (27/44, 61%). Strikingly, in this population of *IDH*wt, H3K27 and G34-wildtype gliomas, immunohistochemical studies showed loss of ATRX nuclear expression in 6 cases (6/38, 16%). Seventeen patients were studied by NGS and 30 by Sanger sequencing plus CGH-array. The most common molecular alterations included *TERT* promoter mutations (23/45, 51%), whole chromosome 7 gain (10/37, 27%), and whole chromosome 10 loss (10/41, 24%). Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) deletions

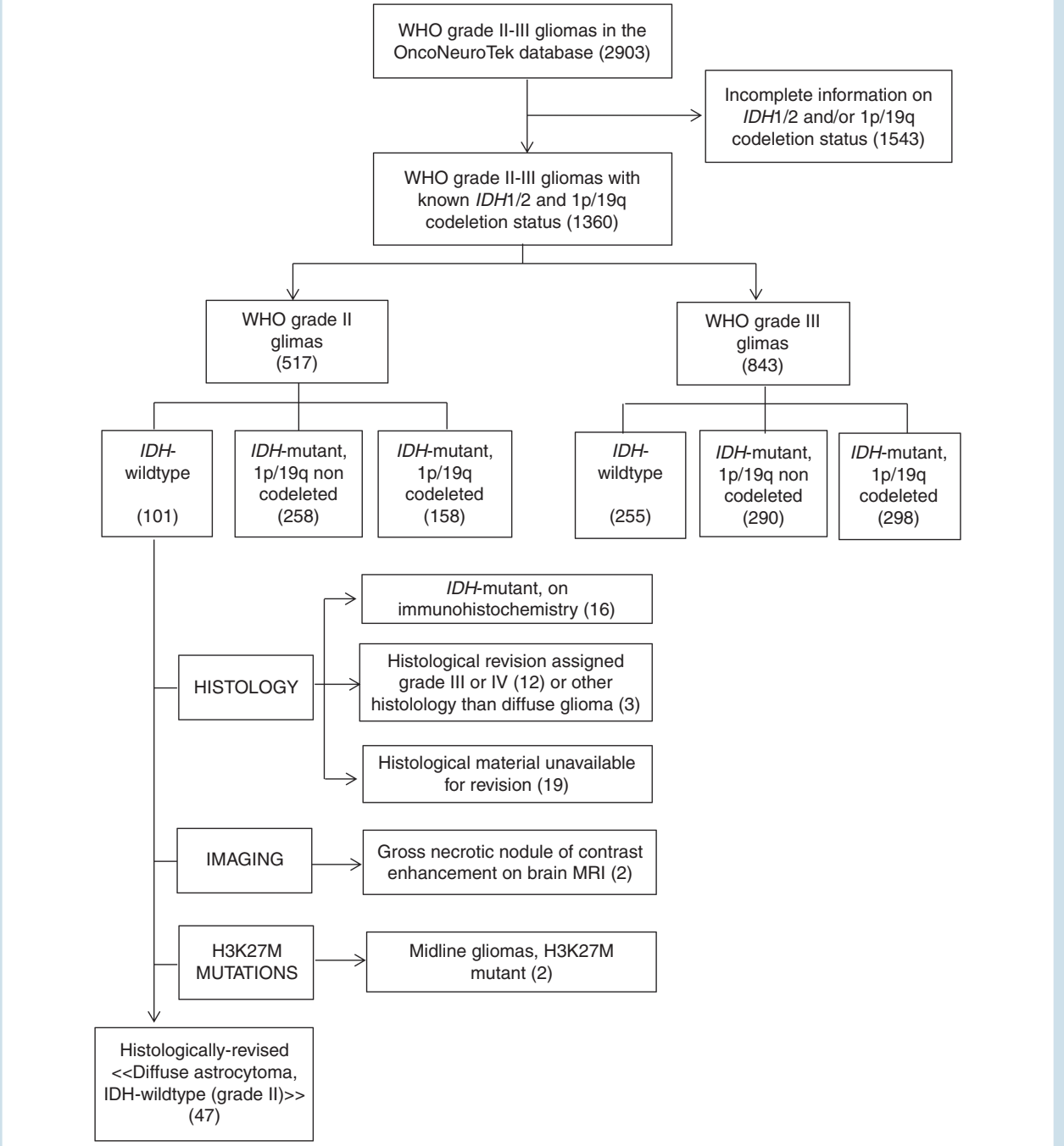

Fig. 1 Algorithm for patient selection.

(5/43, 12%), *EGFR* amplifications (4/43, 9%), and chromosome 9p loss (3/42, 7%) were far less common. One patient with a temporal tumor harbored a *FGFR1* mutation (1/26, 4%), and one patient with a bithalamic tumor harbored a *BRAF* V600G mutation (1/35, 3%). None of the tumors harbored *TP53* mutations (0/22), *PTEN* deletions (0/18), *PDGFR*α amplifications (0/17), or the chromosome 1p/19q codeletion (0/42). Twenty-nine patients underwent the research for fusion genes, with the detection of gene fusions in 5 (5/29, 17%), including 4 fusions *FGFR3* (exon 17 or 18)–*TACC3* (exon 5, 8, 11 or 23) and one fusion *FGFR3* (exon 17)–*MYH14* (exon 23).

Initial treatment included concomitant radiochemotherapy with temozolomide followed by adjuvant temozolomide according to the Stupp protocol (12/38, 32%), sequential radiochemotherapy with temozolomide or procarbazine/lomustine/vincristine (PCV) (7/38, 18%), chemotherapy alone (13/38, 34%), or radiotherapy alone

Table 1 Clinical and molecular features in the whole cohort of IDHwt grade II gliomas ($n = 47$), in the subgroup of patients meeting the definition of "diffuse astrocytic glioma, *IDH*-wildtype, with molecular features of glioblastoma (grade IV)" (molecular GBM) ($n = 29$) and in the subgroup of patients not meeting this definition ($n = 14$)

	*IDH*wt Grade II Gliomas, Whole Cohort ($n = 47$)	*IDH*wt Grade II Gliomas Meeting the Definition of Molecular GBM ($n = 29$)	*IDH*wt Grade II Gliomas NOT Meeting the Definition of Molecular GBM ($n = 14$)	*P*-value*
Age, y, median (range)	55.0 (19.6–82.1)	58.6 (20.8–82.1)	34.5 (19.6–65.4)	**0.00057**
Male	36/47 (77%)	24/29 (83%)	10/14 (71%)	0.44
Preoperative KPS, median (range)	90 (70–100)	90 (70–100)	90 (70–100)	0.83
Tumor location				
Fronto-temporo-insular	28/47 (60%)	19/29 (66%)	5/14 (36%)	**0.0055**
Fronto-callosal or parieto-callosal	4/47 (9%)	4/29 (14%)	0/14 (0%)	
Other	11/47 (23%)	6/29 (21%)	5/14 (36%)	
Thalamo-mesencephalic	4/47 (9%)	0/29 (0%)	4/14 (29%)	
Extent of resection				
Biopsy	27/44 (61%)	19/28 (68%)	7/12 (58%)	0.88
Partial resection	9/44 (20%)	5/28 (18%)	2/12 (17%)	
Gross total resection	8/44 (18%)	4/28 (14%)	3/12 (25%)	
Initial treatment				
Chemotherapy alone	13/38 (34%)	10/25 (40%)	3/9 (33%)	0.74
Sequential radiochemotherapy	7/38 (18%)	5/25 (20%)	1/9 (11%)	
Stupp protocol	12/38 (32%)	7/25 (28%)	3/9 (33%)	
Radiotherapy alone	1/38 (3%)	0/25 (0%)	0/9 (0%)	
Surveillance	5/38 (13%)	3/25 (12%)	2/9 (22%)	
Radiological progression				
Infiltrative	13/18 (72%)	11/15 (73%)	2/3 (67%)	0.45
Nodular enhancing	5/18 (28%)	4/15 (27%)	1/3 (33%)	
Molecular profile				
TERT promoter mutation	23/45 (51%)	23/28 (82%)	0/14 (0%)	**<0.0001**
EGFR amplification	4/43 (9%)	4/26 (15%)	0/14 (0%)	0.28
7+	10/37 (27%)	10/26 (38%)	0/10 (0%)	**0.016**
10-	10/41 (24%)	10/26 (38%)	0/14 (0%)	**0.011**
7+/–10	7/41 (17%)	7/26 (27%)	0/14 (0%)	0.075
9p loss	3/42 (7%)	3/26 (12%)	0/14 (0%)	0.54
CDKN2A deletion	5/43 (12%)	3/26 (12%)	1/14 (7%)	1
Median OS, mo	59.1	42.2	56.7	0.2

7+ = whole chromosome 7 gain; 9p = chromosome 9p; –10 = whole chromosome 10 loss; 7+/–10 = whole chromosome 7 gain and whole chromosome 10 loss; FU = follow-up. **P*-values refer to the comparison between patients meeting the definition for molecular GBM and patients not meeting this definition; in bold statistically significant results.

(1/38, 3%). Tumor progression occurred either through an infiltrative pattern (Fig. 2, panel C-D) or through the appearance of gross nodules of contrast enhancement (Fig. 2, panel G-H). Patients with nodular progression showed poorer outcomes compared with patients evolving through an infiltrative pattern (median OS: 22 vs 88 mo, $P = 0.03$) (Supplementary Figure 2, panel A). Three patients underwent surgery at progression: in 1 patient, a *TERT* promoter mutation appeared 12 years after initial surgery, while an *EGFR* amplification appeared 3 and 5 years after initial surgery in 2 patients with known *TERT* promoter mutations.

The median OS for *IDH*wt grade II gliomas was 59 months (vs 101 mo for *IDH*mut 1p/19q non-codeleted

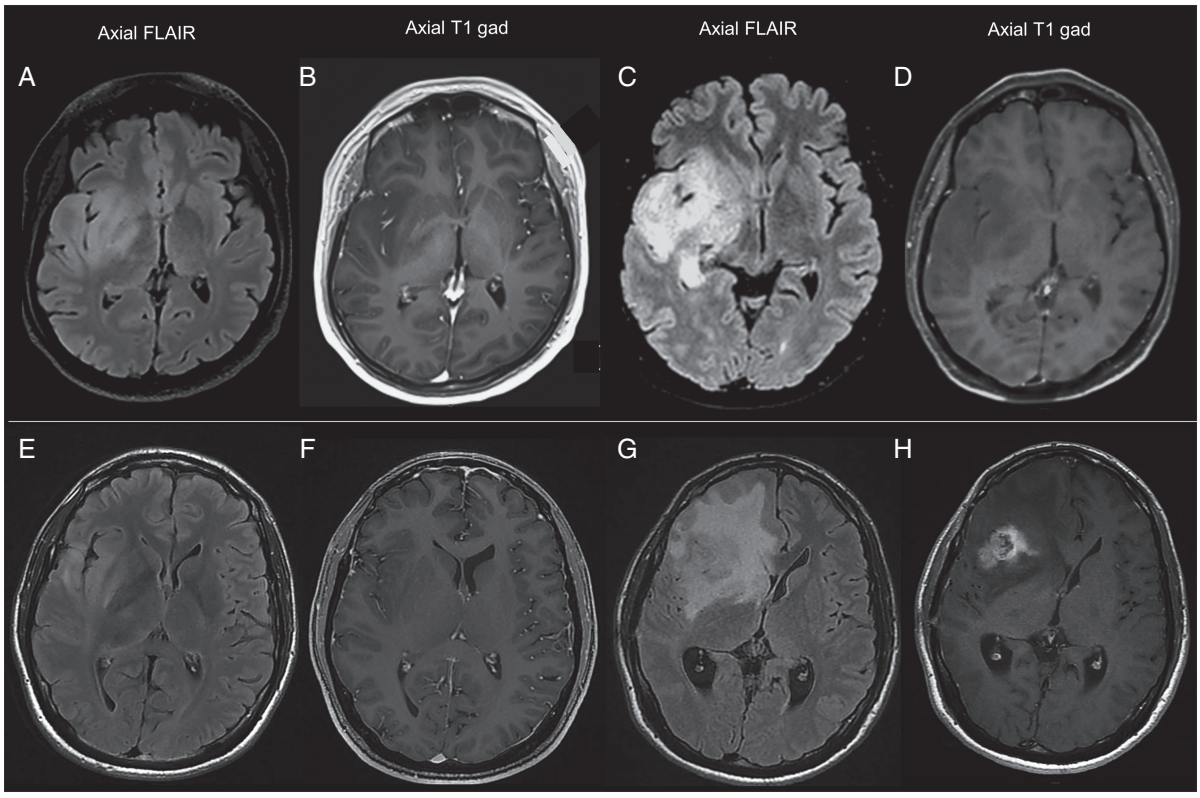

Fig. 2 Radiological patterns of progression in *IDH*wt grade II gliomas. *Panel A-D*: example of an infiltrative pattern of progression. Compared with images at diagnosis (panel A, B), images at progression (panel C, D) show a substantial extension of tumor infiltration along the right temporal and insular lobe and the ipsilateral thalamus (panel C), without the appearance of enhancing abnormalities (panel D). *Panel E-H*: example of a nodular pattern of progression. Compared with images at diagnosis (panel E, F), images at progression (panel G, H) show the appearance of a gross nodule of contrast enhancement in the right insula (panel H), surrounded by extensive perilesional edema (panel G, H).

and 176 mo for *IDH*mut 1p/19q codeleted gliomas, $P < 0.0001$) (Fig. 3, panel A). Higher preoperative KPS ($P = 0.04$) was associated with improved OS. Whole chromosome 10 loss (median OS: 88 vs 33 mo, $P = 0.03$), the +7/–10 signature (median OS: 88 vs 33 mo, $P = 0.02$), and chromosome 9p loss (median OS: 88 vs 19 mo, $P < 0.0001$) were associated with poorer OS (Supplementary Figure 2, panel B-D). A similar trend was observed for whole chromosome 7 gain (median OS: 88 vs 41 mo, $P = 0.2$), *TERT* promoter mutations (median OS: 88 vs 41 mo, $P = 0.2$), and *CDKN2A* deletions (median OS: 59 vs 42 mo, $P = 0.3$). Association matrix showed that whole chromosome 10 loss was associated with whole chromosome 7 gain ($P < 0.001$), *EGFR* amplifications ($P < 0.01$), chromosome 9p loss ($P < 0.01$), and *CDKN2A* deletions ($P < 0.01$) (Supplementary Figure 3, panel A-B).

IDHwt Grade III Gliomas

Table 2 compares the clinical and molecular features of *IDH*wt grade II ($n = 47$) and grade III ($n = 255$) gliomas. The median age at diagnosis for *IDH*wt grade III gliomas was 56.1 years-old (vs 55.0 years, $P = 0.26$). One-hundred-fifty-four patients (154/255, 60%) were male (vs 36/47 (77%), $P = 0.048$). Median preoperative KPS was 80 (vs 90, $P = 0.0025$). Surgery commonly consisted of biopsy (115/237, 49%) ($P = 0.31$). Initial treatment was represented by concomitant or sequential radiochemotherapy (129/220, 59%) or, less frequently, by chemotherapy (53/220, 24%) or radiotherapy (38/220, 17%) alone ($P < 0.001$).

Compared with *IDH*wt grade II, *IDH*wt grade III gliomas had a higher prevalence of *TERT* promoter mutations: 151/230 (66%) vs 23/45 (51%), $P = 0.0092$; *EGFR* amplifications; 73/235 (31%) vs 4/43 (9%), $P = 0.00088$; whole chromosome 7 gain: 91/173 (53%) vs 10/37 (27%), $P = 0.0062$; whole chromosome 10 loss: 111/222 (50%) vs 10/41 (24%), $P = 0.0010$; chromosome 9p loss: 57/222 (26%) vs 3/42 (7%), $P = 0.0082$; *CDKN2A* deletions: 72/235 (31%) vs 5/43 (12%), $P = 0.0033$; and *TP53* mutations (22/104 (21%) vs 0/22 (0%), $P < 0.0001$.

The median OS for *IDH*wt grade III gliomas was 19 months (vs 59 mo for *IDH*wt grade II gliomas, $P < 0.0001$) (Fig. 3, panel B). Younger age at diagnosis ($P = 0.0019$) and higher preoperative KPS ($P = 0.0030$) were associated with prolonged OS. *EGFR* amplifications (median OS: 22 vs 16

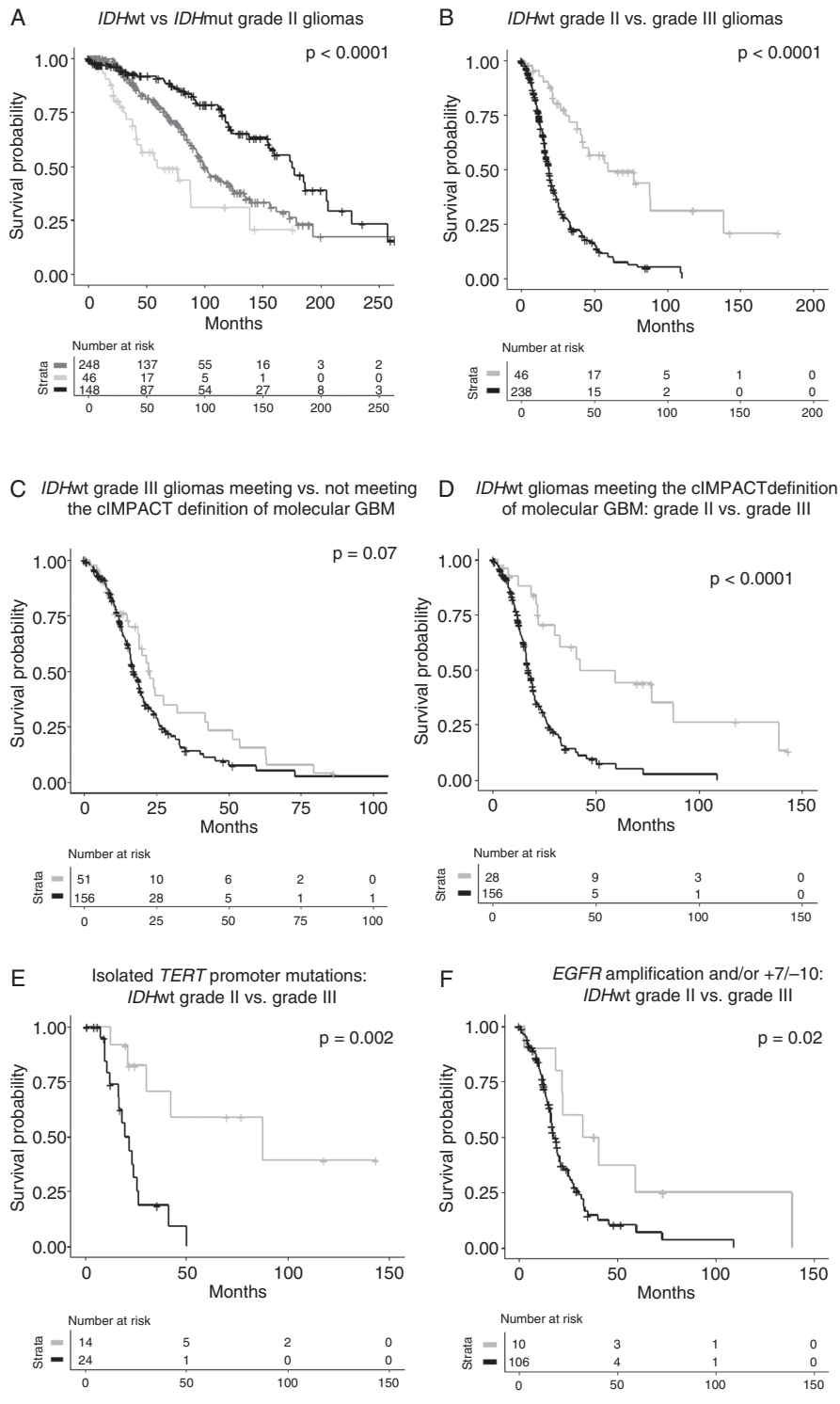

Fig. 3 Overall survival in *IDH*wt grade II gliomas compared with *IDH*mut grade II (panel A) and to *IDH*wt grade III (panel B) gliomas; overall survival in *IDH*wt grade II and III gliomas meeting the cIMPACT-NOW definition for "Diffuse astrocytic glioma, *IDH*-wildtype, with molecular features of glioblastoma (grade IV)" (panel C-F). *Panel A*: survival curves for *IDH*mut 1p/19q codeleted (black line) vs *IDH*mut 1p/19q non-codeleted (dark gray line) vs *IDH*wt (light gray line) grade II gliomas (median OS: 176 vs 101 vs 59 mo, $P < 0.0001$). *Panel B*: survival curves for *IDH*wt grade II (gray line) vs *IDH*wt grade III (black line) gliomas (median OS: 59 vs 19 months, $P < 0.0001$). *Panel C*: survival curves for patients with *IDH*wt grade III gliomas meeting (black line) and not meeting (gray line) the cIMPACT definition for molecular glioblastoma (median OS: 17 vs 23 months, $P = 0.07$). *Panel D*: survival curves for patients with *IDH*wt grade II (gray line) and *IDH*wt grade III (black line) gliomas meeting the cIMPACT definition of molecular glioblastoma (median OS: 42 vs 17 months, $P < 0.0001$). *Panel E*: survival curves for patients with *IDH*wt grade II (gray

mo, $P = 0.04$) and whole chromosome 10 loss (median OS: 23 vs 18 mo, $P = 0.03$) were associated with poorer OS, and a similar trend was observed for *TERT* promoter mutations (median OS: 22 vs 17 mo, $P = 0.05$) and *CDKN2A* deletions (median OS: 20 vs 16 mo, $P = 0.05$) (Supplementary Figure 2, panel E-H).

Association matrix showed that whole chromosome 10 loss associated with whole chromosome 7 gain ($P < 0.001$), *EGFR* amplifications ($P < 0.01$), chromosome 9p loss ($P < 0.001$) and *CDKN2A* deletions ($P < 0.01$). *TERT* promoter mutations associated with whole chromosome 7 gain ($P < 0.001$), *EGFR* amplifications ($P = 0.04$), and older age at diagnosis ($P = 0.02$) (Supplementary Figure 3, panel C-D).

IDHwt Grades II and III Gliomas Meeting cIMPACT-NOW Criteria for "Diffuse Astrocytic Glioma, IDH-Wildtype, with Molecular Features of Glioblastoma (Grade IV)"

Twenty-nine patients in the group of *IDH*wt grade II gliomas (29/43, 67%) and 166 patients in the group of *IDH*wt grade III gliomas (166/224, 74%) met cIMPACT-NOW criteria for the definition of "diffuse astrocytic glioma, *IDH*-wildtype, with molecular features of glioblastoma (WHO grade IV)."[10]

Patients with *IDH*wt grade II gliomas and molecular features of glioblastoma were older (median age at diagnosis: 58.6 vs 34.5, $P = 0.00057$) and more frequently had fronto-temporo-insular tumors (19/29 (66%) vs 5/14 (36%), $P = 0.0055$) compared with patients with *IDH*wt grade II gliomas lacking defining molecular alterations (Table 1). Extent of resection ($P = 0.88$) and treatment schemes ($P = 0.74$) did not substantially differ between the 2 groups, as most patients had been treated before the publication of cIMPACT criteria. The median OS in patients with *IDH*wt grade II gliomas and molecular features of glioblastoma was 42 months (vs 57 months in patients with *IDH*wt grade II gliomas lacking defining features, $P = 0.2$). Neither age ($P = 0.31$) nor molecular features of GBM ($P = 0.17$) were associated with survival on the Cox model.

Patients with *IDH*wt grade III gliomas and molecular features of glioblastoma were older (median age at diagnosis: 58.8 vs 43.9 years, $P < 0.0001$) and more frequently received biopsy (80/149 (54%) vs 19/47 (40%), $P = 0.063$) compared with patients with *IDH*wt grade III gliomas lacking defining molecular alterations (Supplementary Table 1). The median OS patients with *IDH*wt grade III gliomas and molecular features of glioblastoma was 17 months (vs 23 mo for *IDH*wt grade III gliomas lacking defining features, $P = 0.07$) (Fig. 3, panel C). Age ($P = 0.0019$) was associated with survival on Cox model, while molecular features of GBM did not reach significance ($P = 0.058$).

Therefore, *IDH*wt grade II and *IDH*wt grade III gliomas meeting the definition for molecular glioblastoma clearly had different OS (42 vs 17 mo, $P < 0.0001$) (Fig. 3, panel D). Table 3 compares clinical and molecular features in the 2 groups. Most patients in the group of *IDH*wt grade II gliomas met the definition of molecular glioblastoma because of a single criterion, most frequently isolated *TERT* promoter mutations (16/26, 62%). Conversely, patients with *IDH*wt grade III gliomas generally met the definition of molecular glioblastoma because of multiple criteria as, besides *TERT* promoter mutations, most of them had additional defining alterations such as *EGFR* amplifications or the +7/–10 signature (93/131, 71%).

We then evaluated the ability of the different criteria to capture tumor malignant behavior. Isolated *TERT* promoter mutations, without *EGFR* amplifications or the +7/–10 signature, were associated with a median OS of 88 months in *IDH*wt grade II and 22 months in *IDH*wt grade III gliomas ($P = 0.002$) (Fig. 3, panel E). Conversely, the presence of *EGFR* amplifications and/or of the +7/–10 signature, regardless of the presence of *TERT* promoter mutations, was associated with a median OS of 37 months in *IDH*wt grade II and 18 months in *IDH*wt grade III gliomas ($P = 0.02$) (Fig. 3, panel F).

Discussion

From a large cohort of 1360 "lower grade" (ie, grades II and III) gliomas, we extracted 101 *IDH*wt grade II gliomas, whose radiological, molecular, and histological features were thoroughly revised to guarantee a rigorous patient selection. We ensured to exclude *IDH1*-mutant gliomas falsely negative on Sanger sequencing because of contamination with normal tissue, peripheral samples of high-grade *IDH*wt gliomas, and H3K27M-mutant midline gliomas. The analyses were then restricted to 47 patients who had a confirmed diagnosis of "diffuse astrocytoma, IDH-wildtype (grade II)" following centralized histological review, which was independently conducted by 2 expert neuropathologists through comprehensive morphological and immunohistochemical studies.

Representing less than 15% of low-grade diffuse gliomas,[4,8,20] *IDH*wt grade II gliomas are uncommon, especially in women, and are associated with older age at diagnosis, fronto-temporo-insular location and a highly invasive behavior,[3,6,7] with frequent infiltration of adjacent cortex and deep gray matter.[21] This highly infiltrative pattern accounts for the prevalence of biopsy over resection and the scarce tissue availability for translational studies. With no *TP53* mutations and, as expected,[12] no 1p/19q codeletions, our *IDH*wt grade II gliomas correspond to the previously defined "triple negative" grade II gliomas[3]: usually large and highly infiltrative fronto-temporal-insular tumors, which could be merely defined as *IDH*wt grade II diffuse gliomas.

line) and grade III (black line) gliomas meeting the cIMPACT definition for molecular glioblastoma because of isolated *TERT* promoter mutations (median OS: 88 vs 22 mo, $P = 0.002$). *Panel F*: survival curves for patients with *IDH*wt grade II (gray line) and grade III (black line) meeting the cIMPACT definition for molecular glioblastoma because of *EGFR* amplifications and/or the +7/–10 signature (median OS: 37 vs 18 mo, $P = 0.02$).

Table 2 Clinical and molecular features in *IDH*wt grade II ($n = 47$) and *IDH*wt grade III ($n = 255$) gliomas

	*IDH*wt Grade II And III Gliomas, Whole Cohort ($n = 302$)	*IDH*wt Grade II Gliomas ($n = 47$)	*IDH*wt Grade III Gliomas ($n = 255$)	*P*-value*
Age, y, median (range)	56.1 (8.5–84.1)	55.0 (19.6–82.1)	56.1 (8.5–84.1)	0.26
Male	190/302 (63%)	36/47 (77%)	154/255 (60%)	**0.048**
Preoperative KPS, median (range)	90 (20–100)	90 (70–100)	80 (20–100)	**0.0025**
Extent of resection				
Biopsy	142/270 (53%)	27/44 (61%)	115/237 (49%)	0.31
Partial resection	60/270 (22%)	9/44 (20%)	51/237 (22%)	
Gross total resection	68/270 (25%)	8/44 (18%)	60/237 (25%)	
Initial treatment				
Chemotherapy alone	67/258 (26%)	14/38 (37%)	53/220 (24%)	**<0.001**
Concomitant or sequential RT-CHT	147/258 (57%)	18/38 (47%)	129/220 (59%)	
Radiotherapy alone	39/258 (15%)	1/38 (3%)	38/220 (17%)	
Surveillance	5/258 (2%)	5/38 (13%)	0/220 (0%)	
Molecular profile				
TERT promoter mutation	176/274 (64%)	23/45 (51%)	151/230 (66%)	**0.0092**
EGFR amplification	77/275 (28%)	4/43 (9%)	73/235 (31%)	**0.00088**
7+	102/206 (50%)	10/37 (27%)	91/173 (53%)	**0.0062**
10-	119/259 (46%)	10/41 (24%)	111/222 (50%)	**0.0010**
7+/–10	85/236 (36%)	7/41 (17%)	77/199 (39%)	**0.018**
9p loss	59/260 (23%)	3/42 (7%)	57/222 (26%)	**0.0082**
CDKN2A deletion	76/274 (27%)	5/43 (12%)	72/235 (31%)	**0.0033**
TP53 mutation	22/123 (18%)	0/22 (0%)	22/104 (21%)	**<0.0001**
Median OS, mo	21.9	59.1	19.1	**< 0.0001**

7+ = whole chromosome 7 gain; 9p = chromosome 9p; 10- = whole chromosome 10 loss; 7+/–10 = whole chromosome 7 gain and whole chromosome 10 loss; RT-CHT = radiochemotherapy. **P*-values refer to the comparison between IDHwt grades II and III gliomas; in bold statistically significant results.

Compared with *IDH*wt grade III gliomas, *IDH*wt grade II gliomas had a lower burden of molecular alterations, including *EGFR* amplifications (9% vs 31%), whole chromosome 7 gain (27% vs 53%), whole chromosome 10 loss (24% vs 50%), *TERT* promoter mutations (51% vs 66%), *TP53* mutations (0% vs 21%), chromosome 9p loss (7% vs 26%), and *CDKN2A* deletions (12% vs 31%). Their median OS was 59 months that, while much shorter than *IDH*mut grade II gliomas, was 3 times the median OS of *IDH*wt grade III tumors (19 months, $P < 0.0001$). These findings reveal deep differences between *IDH*wt grade II and *IDH*wt grade III gliomas in terms of both genetic profile and outcome.

The distinction between histological grades II and III gliomas primarily relies on proliferative activity expressed as mitotic index, which is known to have a heavier prognostic impact in *IDH*wt than in *IDH*mut gliomas.[4,22] The 2016 WHO classification does not provide clear thresholds for the evaluation of mitotic count. The use of different thresholds for grading assessment margin could explain the inconsistent results obtained in different studies on prognosis and molecular profile of *IDH*wt grade II and III gliomas and, in our view, represents an urgent issue to address in the forthcoming WHO classification.[23] The grading criteria used here have been widely used in the past and have been adopted by the cIMPACT to separate grade II and III *IDH*mut diffuse astrocytomas[14]: in the same way, they allow here a prognostic stratification of *IDH*wt gliomas, suggesting that the same mitotic threshold is associated with different outcome in both *IDH*mut and *IDH*wt gliomas.

Among the tumors meeting the cIMPACT-NOW definition of "Diffuse astrocytic glioma, *IDH*-wildtype, with molecular features of glioblastoma, WHO grade IV,"[10] only those with grade III histology had a survival similar to *IDH*wt glioblastomas, while tumors with grade II histology had a survival almost 3 times longer.[7,9] In fact, more than half of the patients with *IDH*wt grade II gliomas in our series met cIMPACT-NOW criteria because of isolated *TERT* promoter mutations and this was not predictive of poor outcome. *TERT* promoter mutations are detected in *IDH*mut 1p/19q codeleted diffuse gliomas a well as in several other primary brain tumors and are associated with different prognostic significance depending on tumor histology and *IDH* status.[24,25] These considerations suggest some caution when assimilating *IDH*wt grade II gliomas to molecular glioblastomas, especially if the sole criterion met is an isolated *TERT* promoter mutation. In addition, our

Table 3 Comparison between *IDH*wt grade II ($n = 29$) and *IDH*wt grade III ($n = 166$) gliomas meeting cIMPACT-NOW criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma (grade IV)" (molecular GBM)

	*IDH*wt Grade II and III Gliomas Meeting the definition of molecular GBM ($n = 195$)	*IDH*wt Grade II Gliomas Meeting the Definition of Molecular GBM ($n = 29$)	*IDH*wt Grade III Gliomas Meeting the Definition of Molecular GBM ($n = 166$)	*P*-value*
Age, y, median (range)	58.7 (20.8–83.0)	58.6 (20.8–82.1)	58.8 (22.2–83.0)	0.86
Male	124/195 (64%)	24/29 (83%)	100/166 (60%)	**0.022**
Preoperative KPS, median (range)	90 (60–100)	90 (70–100)	80 (60–100)	**0.026**
Extent of resection				
Biopsy	99/177 (56%)	19/28 (68%)	80/149 (54%)	0.33
Partial resection	31/177 (18%)	5/28 (18%)	26/149 (17%)	
Gross total resection	47/177 (27%)	4/28 (14%)	43/149 (29%)	
Treatment				
Chemotherapy alone	41/169 (24%)	10/25 (40%)	30/144 (21%)	**<0.0001**
Concomitant or sequential RT-CHT	98/169 (58%)	12/25 (48%)	87/144 (60%)	
Radiotherapy alone	27/169 (16%)	0/25 (0%)	27/144 (19%)	
Surveillance	3/169 (2%)	3/25 (12%)	0/144 (0%)	
Molecular profile				
TERT promoter mutation	171/187 (91%)	23/28 (82%)	148/159 (93%)	0.12
EGFR amplification	77/180 (43%)	4/26 (15%)	73/155 (47%)	**0.0037**
7+	96/141 (68%)	10/26 (38%)	86/116 (74%)	**0.00089**
10-	114/170 (67%)	10/26 (36%)	105/145 (72%)	**0.00045**
7+/–10	83/151 (55%)	7/26 (27%)	76/126 (60%)	**0.00194**
9p loss	47/170 (28%)	3/26 (12%)	44/145 (30%)	0.057
CDKN2A deletion	57/180 (32%)	3/26 (12%)	54/155 (35%)	**0.023**
Number of c-IMPACT criteria met				
One	52/154 (34%)	20/26 (77%)	33/129 (26%)	**<0.0001**
Two or 3	102/154 (66%)	6/26 (23%)	96/129 (74%)	
Reason for meeting c-IMPACT criteria:				
TERT promoter mutation *without EGFR* amplification or +7/–10	43/156 (28%)	16/26 (62%)	28/131 (21%)	**<0.0001**
EGFR amplification and/or +7/–10 *without TERT* promoter mutation	15/156 (10%)	5/26 (19%)	10/131 (8%)	
TERT promoter mutation *plus EGFR* amplification and/or +7/–10	98/156 (63%)	5/26 (19%)	93/131 (71%)	
Median OS, mo	19.2	42.2	17.2	**<0.0001**

RT-CHT = radiochemotherapy; +7 = whole chromosome 7 gain; –10 = whole chromosome 10 loss. **P* values refer to the comparison between the 2 subgroups identified by initial WHO grade; in bold statistically significant results.

findings suggest that other molecular markers, besides the ones identified by the cIMPACT-NOW consortium, could be helpful for the prognostic stratification of *IDH*wt lower grade diffuse gliomas, such as chromosome 9p loss that was as a strong predictor of poor outcome in our cohort.

The uncertainties on the malignant behavior of *IDH*wt grade II gliomas, together with their rarity, have prevented to reach a consensus on the standard of treatment for these tumors. As clinical trials conducted in low-grade gliomas failed to indicate a clear treatment strategy for this small population,[26–28] the guidelines of the European Association of Neuro-Oncology leave an ample discretional margin on the choice of individual treatment schemes, suggesting that this choice should

rely on age, KPS, and *MGMT* promoter methylation status,[29] which are recognized prognostic indicators in *IDH*wt diffuse gliomas. The absence of clinical standard advocates a careful multidisciplinary approach based on close clinical surveillance and case-by-case decisions. DNA methylation profiles identified 3 subgroups of *IDH*wt grade II gliomas, one of them with molecular similarities to pilocytic astrocytomas and more favorable outcome.[30] However, as most of our patients had a simple biopsy due to highly infiltrative pattern, the scarce available material did not allow such analysis. In our study, we found actionable molecular targets in 7 patients (7/29, 24%), including 1 *BRAF* V600 mutation, 1 activating *FGFR1* mutation, and 5 *FGFR*3 fusions. *FGFR3-TACC3* fusions have been reported in only 3% of *IDH*wt grades II–IV gliomas but they are of high interest for clinicians as they can be targeted by specific inhibitors.[19] Moreover *FGFR3-TACC3* fusions characterize a subgroup of *IDH*wt gliomas with a specific molecular and metabolic profile.[31,32] While grade II *IDH*wt represent less than 10% of *IDH*wt gliomas, we found here a clear overrepresentation of *FGFR3* fusions compared with what is expected for the whole population of *IDH*wt gliomas, with 4 *FGFR3-TACC* fusions and one *FGFR3-MYH14* fusion out of 29 patients tested (17%; $P < 0.01$). The systematic screening for fusion genes is therefore of special interest to better classify this population and to provide novel therapeutic targets.

In conclusion, our results highlight the importance of histological grade for the prognostic stratification of *IDH*wt lower-grade gliomas and warns on the importance of carefully integrating molecular features and histology for diagnostic, prognostic, and theranostic purposes. There is a clear need for further molecular characterization, search for actionable targets, and new clinical trials in this population.

Supplementary Material

Supplementary data are available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

Keywords

diffuse low grade gliomas | FGFR3 | gene fusion | IDH-wildtype | molecular markers

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