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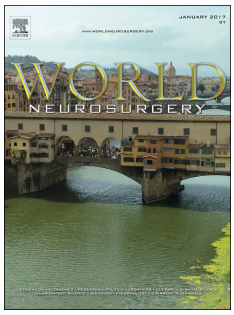

Genomic prognosticators and extent of resection in molecularly subtyped, WHO grade II and III gliomas - a single institution, nine-year review.

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Genomic prognosticators and extent of resection in molecularly subtyped, WHO grade II and III gliomas - a single institution, nine-year review.

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ABSTRACT

Background: WHO grade II and III *IDH* wild-type (*IDH-wt*) gliomas are often treated as WHO grade IV glioblastomas. However, cumulative evidence indicates that *IDH* mutation status alone is insufficient in predicting survival. The current study examines molecular and clinical markers to further prognostically stratify WHO grade II and III gliomas, in particular, *IDH-wt*.

Methods: A single institution's records were retrospectively reviewed for molecularly stratified WHO grade II and grade III gliomas over a nine-year period (2010-2019). Clinical data, *IDH1/IDH2* status, *EGFR* amplification and other molecular markers were recorded and correlated to the study outcomes. These were defined as progression-free survival (PFS), overall survival (OS) and time to malignant progression (TtMP).

Results: 167 and 42 WHO grade II and III gliomas, respectively, were identified, totalling 209 cases with 157 *IDH1/2* mutated and 52 *IDH* wild-type tumours. The presence of *IDH1/2* mutation was associated with longer OS ($p<0.0001$) and PFS ($p<0.0001$) but not with TtMP ($p=0.314$). Lack of *EGF*R amplification, younger age, greater extent of resection (EOR) (≥80%) were identified as independent, favourable prognostic factors. In the *IDH*-wt cohort, multivariate analysis indicated that older age ($p=0.003$) and lesser EOR (<80%) ($p=0.007$) are associated with worse OS. Additionally, *EGFR* amplification showed a trend toward shorter OS in the *IDH-wt* cohort ($p=0.073$).

Conclusions: *IDH1/2* mutation favours longer OS and PFS but does not protect from malignant progression. Lack of *EGF*R amplification, older age and greater EOR are favourable OS prognosticators. In the *IDH*-wt cohort, older age and lesser EOR were linked to worse OS.

INTRODUCTION

Gliomas have been traditionally treated according to the World Health Organisation (WHO) classification based, until recently, on phenotypes only, to assign a final diagnosis.[1] However, the updated WHO 2016 classification of tumours of the central nervous system (CNS) has introduced molecular profiles and incorporated genotypes. Consequently, a paradigm shift in the glioma definitions occurred, affecting prognosis, targetable mutations, enrolment to trials and, crucially, treatment options.[2,3] Notable result has been the treatment of WHO grades II or III low grade glioma (LGG) patients, not harbouring mutations in *isocitrate dehydrogenase* (*IDH*) *1* or *2*, as WHO grade IV glioblastomas, a drastic conceptual treatment shift based on a single molecular marker.[4,5]

Mutations in *IDH* 1 and 2 were initially described in 2008 in a subset of glioblastomas,[6] followed by their identification in approximately 70% of low grade gliomas (LGGs).[7] Multiple studies have reported distinctive clinical behaviour in the *IDH* mutant (*IDH-mut*) and *IDH* wild-type (*IDH-wt*) WHO grade II gliomas,[8-14] resulting in the introduction of *IDH* mutation as a major classifier in the 2016 update of the WHO Classification of CNS tumours, eight years after its initial identification.[2]

Numerous publications have recognised that a considerable proportion of *IDH-wt* LGGs, particularly astrocytic tumours, exhibit aggressive behaviour with rapid anaplastic transformation and limited overall survival (OS), not dissimilar to *IDH-wt* WHO grade IV glioblastomas.[15-17] *IDH-wt* LGGs, are characterized by molecular features of glioblastomas, including mutations in *TERT* promoter or *PIK3CA*, copy number alternations (gain of chromosome 7 and loss of chromosome 10) or abnormal epigenetic alterations.[18,19]

However, as new molecular and clinical data accumulate, it appears that not all *IDH-wt* WHO grade II and grade III gliomas carry a universally poor prognosis. Conversely, increasing evidence indicates that the *IDH-wt* cohort represents a heterogeneous group.[20-22] A meta-analysis investigating the outcome of *IDH-wt* WHO grade II gliomas reported the mean OS of 59 months (range: 9-120 months), which is in stark contrast to the median OS of glioblastoma patients, approximately 15 months.[23] Examining molecular markers, Aibaidula and colleagues reported on 166 *IDH-wt* WHO grade II and grade III gliomas categorised molecularly as either high grade (harbouring mutation in *EGFR, H3F3A*, or *TERTp*) or low grade (lacking *EGFR,*

H3F3A, or *TERTp* mutation) with striking differences in the median OS: 1.23 years versus 7.63 years, respectively.[20]

Therefore as new molecular signatures and genotypes emerge, it appears that *IDH* mutation status in isolation cannot indiscriminately and reliably dictate treatment, predict prognosis or overall survival. Our study examines original data spanning nine years collected from over 200 molecularly-analysed patients in a single institution. In doing so, we seek to assign significance to additional molecular signatures and clinical markers that could further stratify WHO grade II and III gliomas, in particular, *IDH-wt* and statistically correlate molecular genotypes to OS, PFS and TtMP. In addition, the current study examines the role of extent of resection (EOR).

MATERIAL AND METHODS

Tumour selection and clinical data

Pathology data and molecular profiling results were obtained from the Laboratory Information Management System from the Division of Neuropathology, National Hospital for Neurology and Neurosurgery, Queen Square, London. From a compressive data export, records of patients with a histologically confirmed diagnosis of WHO grades II and III gliomas were identified over a nine-year time period (January 2010 to January 2019).

Clinical data extracted and catalogued including standard demographics (***Table 1***), phenotypical/histological diagnosis and molecular/genotype subtyping, surgical intervention, including the extent of resection (EOR), adjuvant treatment (radiotherapy or chemotherapy or a combination) and time interval to radiological progression and/or histological evidence of malignant progression (TtMP). The OS was defined as the time between the histological diagnosis and death,[20] while PFS was defined as the time from the histological diagnosis until the first unequivocal radiological sign of progressive disease.[9] Finally, TtMP was defined as the time between the initial histological diagnosis and radiological signs of malignant transformation indicated by a contrast enhancement following administration of gadolinium, having excluded, when possible, a diagnosis of pseudo-progression, or a new histological diagnosis of a higher grade tumour from latest surgery.

EOR resection was assessed by two experienced neuroradiologists, blinded to histological and molecular subtyping, and type(s) of interventions(s) by reviewing immediate pre- and post-operative MRI images protocolled for standard tumour sequences. Any equivocal interpretation was resolved by consensus. EOR was stratified into four categories: gross total resection (GTR), defined as tumour resection ≥95%; subtotal resection (STR) defined as tumour resection ≥80 but <95%; partial resection (PR) defined as tumour resection ≥50 but < 80%, and biopsy (B) defined as planned or unplanned tumour debulking <50% or cases with pre-planned stereotactic biopsies. The analysis was conducted according to an 80% EOR threshold.[24] The definitions of EOR are variable in the literature. In a comprehensive study establishing percent resection and residual volume thresholds, the heterogeneity shown in GTR expressed as percent resections ranged from 90% to 100%, while that of the STR group ranges from 0% to 99%".[25] In keeping with other reported series we adopted a pragmatic threshold of GTR of ≥95% to account for post-operative changes.[24,26-27] Limited resections (<50%) have no effect in OS or PFS and were grouped, in our analysis, with stereotactic biopsies.[28]

Molecular analysis

Data on *IDH1/IDH2* status, *epidermal growth factor* (*EGFR)* amplification, *telomerase reverse transcriptase* promoter (*TERT*p) status, *phosphatase and tensin homolog* (*PTEN)* loss and O^6-*methylguanine-DNA methyltransferase* promoter (*MGMT*p) methylation status was retrospectively extracted from histopathological reports produced at the time of diagnosis. Cases diagnosed prior to 2016 WHO update of CNS tumours classification were re-evaluated according to current molecular subtyping to ascertain numbers of *IDH*-mutant astrocytoma, 1p/19q-co-deleted oligodendrogliomas, and *BRAF*-mutant low grade gliomas. All tumours had at this stage already been routinely tested for*: IDH1* and *IDH2* mutation, *EGFR* amplification, *TERT*p mutation, *BRAF* mutation, *PTEN* loss and *MGMTp* methylation status analysis. *EGFR* amplification results were obtained from all tested tumours and were categorised as present, regardless of copy number, or absent. Tumours were retrospectively tested for loss of *ATRX* expression. Also, a proportion of gliomas with non-informative molecular markers were subsequently tested with methylation arrays. The *IDH* status was performed by sequencing rather than by immunochemistry only. *EGFR* amplification and *PTEN* loss were assessed by

Comparative CT (threshold cycle) multiplex PCR. Additional details of the methodology and diagnostic approach employed has been previously published by our institution's neuropathology group.[29]

All *IDH-wt* tumours had a confirmed exclusion of an *IDH1* or *IDH2* mutation with 100% success rate in the cohort. *TERT* promoter mutations were routinely tested since 2016, although this failed in a proportion of the tumours, for reasons related to DNA quality and not the sample size. DNA yield was sufficient for all samples, including stereotactic biopsies, as only 200 ng of DNA is required mass for testing, a volume that can be extracted from 4×10 μm sections of 3 stereotactic biopsy cores. Molecular subtyping was available at the following rates: *IDH1* and *IDH2*, 209/209; *EGFR*, 196/209; *PTEN* loss, 198/209; *MGMTp* methylation, 196/209; *ATRX,* 133/209; *TERT*p, 78/209.

Statistical analysis

Frequency distribution and summary statistics were calculated for all clinical, histological and molecular variables. Fisher's exact test was used to define relationships between molecular markers and clinical parameters. PFS, OS, and TtMP were used to study the prognostic impact of molecular and clinical variables and were censored at the date of the last follow-up in May 2019. For survival analyses for patients with multiple biopsies, the date of the first biopsy was employed.

The associations between OS, PFS, TtMP and molecular and clinical markers were calculated using log-rank test and were presented as Kaplan–Meier plots. Cox regression models were fitted to assess the independent impact of the molecular and clinical markers. All statistical tests were two-sided. The threshold for statistical significance was set as $p = 0.05$. Analyses were conducted with SPSS Statistics Version 25.0 (IBM SPSS Statistics for Windows Version 25.0, Armonk, NY: IBM Corp.)

Ethical approval: As the study was a retrospective review of records, it was exempt from ethical approval according to the Joint Research Office of the Hospital and University. However, according to their recommendation, Departmental approval was obtained.

RESULTS

Clinical and molecular characteristics of the entire cohort

Data analysis resulted in a total of 209 cases of WHO grade II and III gliomas for the study period, which were all analysed for the *IDH1* mutations at codon 132 and *IDH2* mutations at codon 172. The sex ratio of the analysed cohort was 1.58 (128 males and 81 females), with a median age of 40.00 years (range 17-82). Cohort's clinical and molecular stratification, type and extent of intervention(s) are outlined in ***Table 1***. Phenotypically, the cohort consisted of 167 WHO grade II tumours (79.9%) and 42 WHO grade III tumours (20.1%). Following the application of WHO 2016 classification[2] cohort analysis resulted in 194 (92.8%) astrocytomas and 14 (6.7%) oligodendrogliomas.

Overall, out of 209 tumours, 151 (72.2%) *IDH1* and 6 (2.9%) *IDH2* mutations were found, and consequently 52 *IDH-wt* (24.9%) tumours were identified. Comparison of clinical and pathological characteristics of *IDH-wt* and *IDH-mut* tumours is presented in ***Table 1***. *The IDH-wt* cohort was comprised of older patients (median, 59.0 versus 36.0 years; mean, 55.9 versus 37.3 years, $p<0.0001$) and diagnosed with WHO grade III histology more frequently than *IDH-mut* (55.7% versus 7.6%, $p<0.0001$) as well as with astrocytoma histology (98.1% versus 91.1%, $p=0.02$). Additionally, *IDH-wt* tumours were more frequently biopsied (EOR < 50%) in comparison to *IDH-mut* cohort (15.4% versus 61.8%, $p<0.0001$) and more frequently received adjuvant therapy compared to *IDH-mut* patients (80.8% versus 56.1%, $p<0.01$). The surgical management in the entire cohort varied. According to the definitions described above, 103 (49.3%) patients underwent (B), 28 (13.4%) patients underwent PR, 27 (12.9%) patients underwent STR and 50 (23.9%) patients underwent GTR (***Table 1***). In addition to the surgical treatment, the majority of patients (62.2%) received adjuvant treatment; following analysis of clinicopathological and molecular data within the context of multidisciplinary meetings (MDT); 35 patients received radiotherapy only, 3 patients received chemotherapy only and 50 patients received both (***Table 1***). Median follow-up was 37.4 months (range 1.4-162.5 months) and 58 (27.8%) patients were deceased at the time of analysis.

Correlation between clinical and molecular markers and survival analysis for the entire cohort

The prognostic impact of *IDH1* and *IDH2* mutations in WHO grade II and grade III gliomas was analysed with the outcome measures of PFS, OS and TtMP. The absence of *IDH1/2* mutation was associated with a less favourable OS in the entire cohort (75th-percentile survival: 12.8 months (SEM: 2.6) months versus 92.2 months (SEM: 11.2), p<0.0001) (***Figure 1A***) (***Table 2***). Median OS for patients with *IDH-wt* tumours was 19.2 months, whereas median OS for patients with *IDH-mut* tumours was not reached (p<0.0001). Younger age (<50) (p<0.0001), greater EOR (≥80%) (p=0.00002) (***Figure 1E***), absence of adjuvant treatment (p=0.019), histological diagnosis of grade II tumour (p<0.0001), absence of *EGFR* amplification (p<0.0001) (***Figure C***) and *PTEN* retention (p=0.018) were all associated with a longer OS (***Table 2***). Due to test limitations and diagnostic necessities, TERT mutation data were only available for a proportion of the cohort (78/209; 37.3%), and therefore not included in the further analysis.

Patients with *IDH-wt* tumours had a shorter median PFS of 12.6 months (95% CI: 10.3-14.9) compared to patients with *IDH-mut* tumours with median PFS of 39.2 months (95% CI: 26.7-51.7) (p<0.0001) (***Figure 1B***) (***Table 3***). In the entire cohort, greater EOR (≥80%) (p=0.0005) (***Figure 1F***), younger age (<50) (p=0.014), histological diagnosis of grade II tumour (p<0.0001), oligodendroglioma histology (p=0.004), absence of adjuvant treatment (p<0.0001) and absence of *EGFR* amplification (p=0.001) (***Figure 1D***) were all associated with a longer PFS (***Table 3)***. Additionally, a trend of *PTEN* loss was associated with a less favourable PFS (median PFS: 23.9 months (95% CI: 14.1-33.7) versus 31.5 months (95% CI: 23.0-40.0), p=0.079).

Finally, with regards to TtMP, the absence of adjuvant treatment (p<0.0001) was associated with a longer TtMP. This may represent a selection bias as patients with more aggressive features tend to receive adjuvant treatment, although, theories of TMZ-induced hypermutation have been proposed[30]. Interestingly, TtMP was not affected by *IDH* mutation status (p=0.314), *EGFR* amplification (p=0.460) and EOR (p=0.080) (***Table 4***).

Cox regression analysis confirmed that *IDH* mutation was an independent, favourable prognosticator for OS (HR=0.25; 95% CI, 0.12-0.54, p=0.0004) (***Table 5***) and PFS (HR = 0.49; 95% CI, 0.28-0.84, p= 0.009) (***Table 6***) but not for TtMP (HR: 0.67; 95% CI. 0.27-1.70,

p=0.402) (***Table 7)***. Additionally, multivariate analysis confirmed that *EGFR* amplification was associated with less favourable OS (HR= 2.19; 95% CI, 1.08-4.44, p=0.030) (***Table 5***). Younger age (<50 years) and greater EOR (≥80%) were strongly linked to better OS (p=0.0002 and p=0.007, respectively) (***Table 5***). Absence of adjuvant treatment and greater EOR (≥80%) were independent, favourable prognostic factors for PFS (p=0.020; p=0.049, respectively) (***Table 6***).

Clinical and molecular characteristics of IDH-wt gliomas

Overall, 52 *IDH-wt* tumours were identified from the analysed cohort of 209 gliomas, including 22 (42.3%) WHO grade II gliomas and 30 (57.7%) WHO grade III gliomas. All examined tumours were astrocytomas. Mean and median ages of *IDH-wt* patients were 55.9 and 59.0 years, respectively, (range: 27-81); sex ratio was 3.3 (40 male and 12 females). Adjuvant treatment was administered to 42 (80.8%) patients, with 28 receiving both radiotherapy and chemotherapy, 11 receiving only radiotherapy and 3 only chemotherapy; 10 patients did not receive any adjuvant treatment. Median follow-up time was 16.7 months (range: 1.4-105.1 months) and 36 (69.2%) patients were deceased at the time of analysis. Clinical characteristics, type and extent of intervention(s) in the *IDH-wt* gliomas are outlined in ***Table 1***.

In terms of molecular markers, *TERTp* mutations, *PTEN* loss, *EGFR* amplification and *MGMT*p methylation status were examined in the *IDH-wt* cohort. Analysis of data resulted in 13 *TERTp* mutated tumours, 30 *EGFR*-amplified tumours, 17 tumours with *PTEN* loss and 18 *MGMT*p methylated tumours. The clinical characteristics of each group are outlined in ***Table 8***. The TERTp mutation was tested in 78 patients in total, mainly after 2014. As *TERTp* mutation status was available only for 14 *IDH-wt* tumours (*13 TERTp-mut*, and 1 *TERTp-wt*), this marker was excluded from further analysis. There were no significant associations between *PTEN* loss and *MGMT*p methylated tumours and histological grade or any other clinical characteristics in *IDH-wt* cohort.

Correlation between clinical and molecular markers and survival analysis in IDH-wt cohort

In our cohort of 52 *IDH-wt* patients, 36 patients died with a mean and median OS of 38.6 and 19.5 months, respectively (range: 1.4-100.2 months). Univariate analysis showed a positive

association between EOR (≥80%) and OS (75th-percentile survival: 37.4 (SEM: 25.2) months versus 9.5 (SEM: 3.6) months, p=0.009) (***Figure 2C***). Median OS of patients with EOR <80% was 16.5 months, while median OS of patients with EOR ≥80% was not reached (***Table 9***). Similarly, EOR was associated with longer PFS (p= 0.08) (***Figure 2D***) (***Table 10***). In the *IDH-wt* cohort, there was an association trend towards younger age (<50 years) with longer OS (p=0.082) (***Table 9)*** but not with PFS (p=0.688) (***Table 10***). Histological grade (WHO grade II versus grade III) was not associated with prolongation of either OS (p=0.841) or PFS (p=0.373). There was an association trend between *EGFR* amplification and shorter PFS (median: 11.2 (95% CI: 8.8-13.6) months versus 17.7 (95% CI: 7.1-28.3) months, p=0.082) as well as OS (median: 14.1 (95% CI: 9.6-18.6) versus 27.8 (95% CI: 14.6-41.0) months, p= 0.219) (***Figure 2A and 2B***). *PTEN* loss and *MGMTp* methylation status were not significantly associated with either PFS or OS (***Tables 9 and 10***).

Cox regression analysis confirmed that greater EOR (≥80%) was an independent, favourable prognostic factor for OS (HR = 0.124; 95% CI, 0.027-0.571, p=0.007) and similar trend was observed for PFS (HR=0.444; 95%, 0.179-1.002, p=0.080) (***Tables 11 and 12***). Additionally, younger age (p=0.003) and presence of adjuvant treatment (p=0.016) were also associated with longer OS but not with longer PFS (p=0.382; p=0.173, respectively). Similarly, to univariate analysis, histological grade (WHO grade II versus III) was not significantly associated with OS (p=0.302) or PFS (p=0.913). Multivariate analysis identified a trend linking *EGFR* amplification to shorter OS (HR=2.260; 95% CI, 0.928-5.504, p=0.073) and PFS (HR=1.850; 95% CI: 0.868-3.943, p=0.111) (***Tables 11 and 12***). Interestingly, both univariate (p=0.021) and multivariate (HR=19.09; 95% CI, 1.639-222.413, p=0.019) analyses indicated that female sex is associated with significantly shorter TtMP (***Tables 13 and 14***).

DISCUSSION

IDH status in the entire cohort and heterogeneity of IDH-wt cohort

We retrospectively investigated OS, PFS and TtMP in a single-institution cohort of 157 patients with *IDH1/2-mut* gliomas and 52 patients with *IDH-wt* gliomas. The absence of *IDH1/2* mutations was associated with worse OS (p<0.0001) (***Figure 1A***) (***Table 2***) and PFS (p<0.0001)

(***Figure 1B***) (***Table 3***), which was further confirmed in multivariate analyses (p=0.0004 and p=0.009, respectively) (***Tables 5 and 6***, respectively). Hence, our findings confirmed the significant prognostic value of the *IDH* mutation[8-13, 31-33].

The relationship between TtMP and *IDH* status remains controversial with emerging studies postulating starkly different outcomes. During characterisation of *IDH1* R132H mutations in 35 (WHO grade II and grade III) primary-recurrent astrocytomas Mu et al. found that *IDH1* R132H mutation confers a longer recurrence-free period when compared with *IDH1*-wt cohort (p<0.01).[34] This was further confirmed by the multivariate analysis (p<0.01).[34] Conversely, some authors suggested that *IDH* mutation is associated with a higher risk of malignant transformation despite conveying an overall better prognosis.[35,36] For example, WHO grade II oligodendroglial tumours with *IDH1* mutation were associated with a higher rate of malignant transformation, possibly involving p53, while compared to their *IDH-wt* counterparts.[35] On the other hand, our study found no significant association between TtMP and *IDH* mutation status using both uni- and multivariate analysis (p=0.31; and p=0.40, HR: 0.67, and 95% CI 0.27-1.70, respectively) (***Tables 4 and 7, respectively***). In line with our results, Yao et al. reported that *IDH1* mutation was associated with longer OS and PFS but was not a predictive marker for TtMP (p=0.61).[37] This result is further strengthened by a meta-analysis investigating outcomes of WHO grade II gliomas, which also reported a lack of association between *IDH* mutation status and TtMP (p=0.06).[23] It should not be omitted that other groups proposed that malignant transformation is independent of a single genetic event – *IDH* mutation – but rather depends on a wide spectrum of genomic changes centred on misregulations and changes to spliceosome machinery, transcription factors and chromatin remodelers.[38,39] However, malignant transformation remains a binary event in patients harbouring LGGs, which highlights a need for further studies aimed at identifying predictive biomarkers.

Additionally, our study demonstrated extensive heterogeneity in the *IDH-wt* cohort. In our study of 52 *IDH-wt* patients, 36 patients died with a mean and median OS of 38.6 and 19.5 months, respectively (range: 1.4-100.2 months). However, 4 patients with a follow-up between 50-80 months and 3 patients with a follow-up greater than 80 months were still alive at the time of analysis. Similarly, the mean and median PFS of patients were 23.6 months and 12.6 months, respectively (range: 1.4-100.2 months) but 3 patients still had not had any clinical or radiological progression at more than 35 months after their initial diagnosis. 42 out of 52 *IDH-wt* tumours in

our cohort harboured either *EGFR* amplification, *TERTp* mutation, or both – molecular features characteristic of glioblastomas. The remaining 10 *IDH-wt* cases were WHO grade II tumours, histologically identified as diffuse astrocytomas. Importantly, the OS of those 10 patients showed extensive variability with one patient surviving more than 100 months and two patients surviving more than 60 months but also 5 patients surviving less than 10 months. Our findings are in line with studies showing that although the majority of *IDH-wt* tumours are glioblastomas, this cohort also includes entities with more favourable prognosis including anaplastic astrocytomas with piloid features,[40] unsampled pilocytic astrocytomas or diffuse leptomeningeal glioneuronal tumours.[41] Consequently, these tumours may be currently overtreated as *IDH-wt* gliomas if we only use *IDH* mutation as a marker. Overall, our data support increasing evidence suggesting that *IDH* mutation status cannot exclusively and indiscriminately dictate outcomes and treatment protocols[20-23] and emphasise the need for additional markers.

EGFR amplification in IDH-wt WHO grade II and grade III gliomas

EGFR amplification is frequently detected in glioblastomas 40-50%[42] and has been associated with poor OS. Although this mutation is less common in LGGs, its prevalence ranging from 0-4% and 0-33% in WHO grade II and III astrocytomas, respectively[43,44], multiple studies reported shorter survival in the *EGFR*-amplified versus *EGFR* non-amplified gliomas.[20,45-47] Overall, 47 *EGFR*-amplified tumours were identified in our cohort (WHO grade II, 20; WHO grade III, 27). In line with previous reports, *EGFR* amplification in our study was associated with shorter OS ($p<0.0001$) (***Figure 1C***) (***Table 2***) and PFS ($p=0.001$) (***Figure 1D***) (***Table 3***) in the entire cohort. Furthermore, multivariate analysis showed that *EGFR* amplification is an independent, unfavourable predictor of OS in WHO grade II and grade III gliomas ($p=0.03$) (***Table 5***) but not PFS ($p=0.47$) (***Table 6***).

When considering our *IDH-wt* cohort separately, 30 *EGFR*-amplified tumours were detected (WHO grade II, 7; WHO grade III, 23). Importantly, the presence of *EGFR* amplification was more frequently found (almost twice as frequent) in *IDH*-wt cohort versus the *IDH-mut* cohort ($p<0.001$). In the *IDH-wt* cohort, the presence of *EGFR* amplification was associated with WHO histological grade; in particular, it was more frequently found in WHO grade III tumours (76.7% versus 33.3%; $p=0.003$) as other studies have also reported.[20] Patients with *EGFR*-amplified

IDH-wt tumours exhibited shorter median OS and PFS versus patients with non-amplified tumours (***Tables 9 and 10***) **(*Figure 2A and 2B*).** Additionally, multivariate analysis revealed a trend linking *EGFR* amplification to shorter OS and PFS (p=0.07, p=0.11, respectively) (***Tables 11 and 12***). Interestingly, multiple studies reported that *EGFR* amplification in gliomas is associated with poor prognosis in younger individuals (<46years, <50 years or <60 years, depending on the report) and a better prognosis in older patients[48-51]. As in our cohort older individuals (≥50 years) dominated within *IDH-wt* subgroup, this may partially explain why we found only a trend linking *EGFR* amplification to shorter OS and PFS.

As *EGFR* amplification is an independent, unfavourable predictor of OS in WHO grade II and grade III gliomas overall and other studies report it to be an independent negative prognostic factor for survival of patients with *IDH*-wt gliomas[20,45], our finding of *EGFR* amplification shortening OS supports its role as a classifier in *IDH-wt* gliomas. We acknowledge that statistical significance may not have been reached in our study due to the small sample size of our *IDH*-wt cohort.

EOR as prognosticator in the entire cohort and within WHO grade II and grade III IDH-wt gliomas

The surgical management of gliomas, particularly LGGs, remains controversial with recent evidence supporting that EOR is an independent, prognostic factor for survival in all WHO grades gliomas.[52-56] Additionally, the role of a greater EOR in improving patient outcome was also recognised in WHO grade III gliomas.[54,56] In our study, comparing EOR<80% (B,PR) (n=131) to EOR≥80% (STR,GTR) resection (n=77) by univariate analysis indicated that greater EOR (≥80%) is associated with longer OS (p<0.0001) (***Table 2***) (***Figure 1E***), PFS (p=0.0005) (***Figure 1F***) (***Table 3***) and trend for TtMP (p=0.08) (***Table 4***). Multivariate analysis further confirmed that EOR ≥80% is an independent, favourable predictor for OS (p=0.007) (***Table 5***) and PFS (p=0.049) (***Table 6***) but not TtMP (p=0.78) (***Table 7***). Hence, our study overall confirmed the role of EOR as a significant prognosticator in glioma patients. Moreover, with respect to EOR as a predictor for TtMP, an extensive review by Sanai and Berger found that EOR was not consistently associated with TtMP, which is consistent with our results.[56]

Patients with EOR<50% were more likely to be found in the *IDH-wt* versus *IDH-mut* cohorts (84.6% and 37.8%, respectively, p<0.0001). In the *IDH-wt* cohort, EOR≥80% was found to be an independent predictor of OS in this cohort (p=0.007) (***Table 11***). A similar trend was observed in regard to PFS (p=0.08) (***Table 12***). In line with our results, Patel et al. reported that greater EOR was associated with a prolonged OS in *IDH-wt* patients.[57] However, their reported association to TtMP was not replicated in our study (***Tables 13 and 14***). Additionally, another study by Eseonu et al. evaluating a cohort of low-grade gliomas also championed an association between the extent of resection and survival of *IDH-wt* patients.[58] Similarly, Aibaidula et al. also reported that patients with totally resected *IDH-wt* tumours tended to have better clinical outcomes compared to patients with non-totally resected *IDH-wt* tumours (median OS: 2.86 vs 1.55 years, p =0.07).[20] However, total resection was not defined by % in their report, which may explain why their results did not reach statistical significance. Conversely, other studies did not support this association[59,60]. Kawaguchi et al, found that gross total resection is not significantly associated with improved overall survival among the *IDH-wt* cohort[59]. However, this study included only binary stratification of surgical resection (yes/no gross total resection) and measured residual glioma differently for enhancing and non-enhancing gliomas, which might have influenced their findings[59]. Importantly, these contrasting findings regarding *IDH-wt* LGGs may further reflect the clinical and molecular heterogeneity of this subgroup emphasised by our study. In light of these joint findings, we support striving for maximising EOR in patients with *IDH-wt* gliomas as it may positively impact their outcomes.

Limitations

Despite the nine-year period, the follow-up time of some patients was limited. Nevertheless, the median follow-up time was 37.4 months (range: 1.4-162.5 months). Another limitation was the sample size, particularly the *IDH-wt* cohort (n=52), but this appears comparable to other reported series. Additionally, due to limited availability and diagnostic necessities, it was not possible to obtain all molecular markers for each patient. Further studies are required for validation, preferably pooling comparable molecular and clinical data from large patient cohorts within prospective registries or trial settings.

CONCLUSIONS

We investigated a single-institution cohort of 209 WHO grade II and grade III patients. After stratification for clinical interventions and molecular prognosticators, we correlated specific markers with PFS, OS and TtMP. Our results indicated that *IDH-wt* gliomas remain a heterogeneous group. *IDH* mutation status alone cannot predict response to treatment or clinical course. Despite their biologically aggressive phenotype, EOR is a statistically significant prognosticator in *IDH-wt* gliomas. *EGFR* amplification is likely another classifier of *IDH-wt* gliomas as our study found a clear tendency despite the lack of statistical significance. Finally, *IDH* mutation does not appear to confer protection for a malignant transformation. As further molecular data are collected, additional sophisticated classifiers are likely to emerge.

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Figure Legends:

Figure 1. Kaplan–Meier survival analysis of *IDH* status, *EGFR* amplification, and EOR in relation to OS and PFS in the entire cohort. Survival analysis indicated that *IDH* mutation was associated with longer OS (A) and PFS (B) versus their wild-type counterparts ($p<0.0001$; $p<0.0001$, respectively). EGFR-amplified tumours tended to have shorter OS (C) and PFS (D) when compared to EGFR non-amplified tumours ($p<0.0001$; $p=0.001$, respectively). Finally, patients with EOR $\geq 80\%$ exhibited longer OS (E) and PFS (F) ($p=0.00002$; $p=0.0005$, respectively).

Figure 2. Kaplan–Meier survival analysis of *EGFR* amplification and EOR in relation to OS and PFS in the *IDH-wt* cohort. *IDH-wt* EGFR-amplified tumours tended to have shorter OS (A) and PFS (B) versus EGFR non-amplified tumours ($p=0.219$; $p=0.082$, respectively). Patients with *IDH-wt* tumours and EOR $\geq 80\%$ exhibited longer OS (C) and PFS (D) when compared to patients with EOR<80% ($p=0.009$; $p=0.080$, respectively).

References

1. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol.* 2007;114(2):97-109 https://www.doi.org/10.1007/s00401-007-0243-4.
2. Louis D, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* 2016;131(6):803-820 https://www.doi.org/10.1007/s00401-016-1545-1.
3. Young JS, Gogos AJ, Morshed RA, et al. Molecular characteristics of diffuse lower grade gliomas: what neurosurgeons need to know. *Acta Neurochir.* 2020;162(8):1929-1939 https://www.doi.org/10.1007/s00701-020-04426-2.
4. Brat D, Aldape K, Colman H, et al. cIMPACT-NOW update 3: recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV". *Acta Neuropathol*. 2018;136(5):805-810 https://www.doi.org/10.1007/s00401-018-1913-0.
5. Sepúlveda-Sánchez JM, Muñoz Langa J, Arráez MÁ, et al. SEOM clinical guideline of diagnosis and management of low-grade glioma. *Clin Transl Oncol.* 2018;20(1):3-15 https://www.doi.org/10.1007/s12094-017-1790-3.
6. Parsons D, Jones S, Zhang X, et al. An Integrated Genomic Analysis of Human Glioblastoma Multiforme. *Science*. 2008;321(5897):1807-1812 https://www.doi.org/10.1126/science.1164382.

7. Yan H, Parsons D, Jin G, et al. IDH1 and IDH2 Mutations in Gliomas. *New Engl J Med*. 2009;360(8):765-773 https://www.doi.org/10.1056/NEJMoa0808710.
8. Metellus P, Coulibaly B, Colin C, et al. Absence of IDH mutation identifies a novel radiologic and molecular subtype of WHO grade II gliomas with dismal prognosis. *Acta Neuropathol*. 2010;120(6):719-729 https://www.doi.org/ 10.1007/s00401-010-0777-8.
9. Houillier C, Wang X, Kaloshi G, et al. IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. *Neurology*. 2010;75(17):1560-1566 https://www.doi.org/10.1212/WNL.0b013e3181f96282.
10. Sanson M, Marie Y, Paris S, et al. Isocitrate Dehydrogenase 1 Codon 132 Mutation Is an Important Prognostic Biomarker in Gliomas. *J Clin Oncol*. 2009;27(25):4150-4154 https://www.doi.org/10.1200/JCO.2009.21.9832.
11. Elsir T, Qu M, Berntsson S, et al. PROX1 is a predictor of survival for gliomas WHO grade II. *Brit J Cancer*. 2011;104(11):1747-1754 https://doi.org/10.1038/bjc.2011.162.
12. Dubbink H, Taal W, van Marion R, et al. IDH1 mutations in low-grade astrocytomas predict survival but not response to temozolomide. *Neurology*. 2009;73(21):1792-1795 https://www.doi.org/10.1212/WNL.0b013e3181c34ace.
13. Gorovets D, Kannan K, Shen R, et al. IDH Mutation and Neuroglial Developmental Features Define Clinically Distinct Subclasses of Lower Grade Diffuse Astrocytic Glioma. *Clin Cancer Res*. 2012;18(9):2490-2501 https://www.doi.org/10.1158/1078-0432.CCR-11-2977.
14. Hartmann C, Hentschel B, Tatagiba M, et al. Molecular Markers in Low-Grade Gliomas: Predictive or Prognostic? *Clin Cancer Res*. 2011;17(13):4588-4599 https://www.doi.org/10.1158/1078-0432.CCR-10-3194.
15. Eckel-Passow J, Lachance D, Molinaro A, et al. Glioma Groups Based on 1p/19q,IDH, and TERT Promoter Mutations in Tumors. *New Engl J Med*. 2015;372(26):2499-2508 https://doi.org/10.1056/nejmoa1407279.
16. Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. *Acta Neuropathol*. 2010;120(6):707-718 https://doi.org/10.1007/s00401-010-0781-z.

17. Weller M, Weber R, Willscher E, et al. Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. *Acta Neuropathol*. 2015;129(5):679-693 https://doi.org/10.1007/s00401-015-1409-0.
18. Hasselblatt M, Jaber M, Reuss D, et al. Diffuse Astrocytoma, IDH-Wildtype: A Dissolving Diagnosis. *J Neuropathol Exp Neurol*. 2018;77(6):422-425 https://doi.org/10.1093/jnen/nly012.
19. Reuss D, Kratz A, Sahm F, et al. Adult IDH wild type astrocytomas biologically and clinically resolve into other tumor entities. *Acta Neuropathol*. 2015;130(3):407-417 https://doi.org/10.1007/s00401-015-1454-8.
20. Aibaidula A, Chan A, Shi Z, et al. Adult IDH wild-type lower-grade gliomas should be further stratified. *Neuro-Oncology*. 2017;19(10):1327-1337 https://doi.org/10.1093/neuonc/nox078.
21. Chan A, Yao Y, Zhang Z, et al. Combination genetic signature stratifies lower-grade gliomas better than histological grade. *Oncotarget*. 2015;6(25) https://doi.org/10.18632/oncotarget.4928.
22. Poulen G, Gozé C, Rigau V, Duffau H. Huge heterogeneity in survival in a subset of adult patients with resected, wild-type isocitrate dehydrogenase status, WHO grade II astrocytomas. *J Neurosurg*. 2019;130(4):1289-1298 https://doi.org/10.3171/2017.10.jns171825.
23. Di Carlo D, Duffau H, Cagnazzo F, et al. IDH wild-type WHO grade II diffuse low-grade gliomas. A heterogeneous family with different outcomes. Systematic review and meta-analysis. *Neurosurg Review*. 2018;43(2):383-395 https://doi.org/10.1007/s10143-018-0996-3.
24. Sanai N, Polley MY, McDermott MW, et al. An extent of resection threshold for newly diagnosed glioblastomas. *J Neurosurg.* 2011;115(1):3-8 https://doi.org/10.3171/2011.2.jns10998.
25. Chaichana K, Jusue-Torres I, Navarro-Ramirez R, et al. Establishing percent resection and residual volume thresholds affecting survival and recurrence for patients with newly diagnosed intracranial glioblastoma. *Neuro-Oncology* 2013;16(1):113-122 https://doi.org/doi:10.1093/neuonc/not137.

26. Bloch O, Han S, Cha S, et al. Impact of extent of resection for recurrent glioblastoma on overall survival. *J Neurosurg.* 2012:117(6):1032-1038 https://doi.org/doi:10.3171/2012.9.jns12504.
27. Gandhi S, Tayebi Meybodi A, Belykh E, et al. Survival Outcomes Among Patients With High-Grade Glioma Treated With 5-Aminolevulinic Acid-Guided Surgery: A Systematic Review and Meta-Analysis. *Front Oncol*. 2019;9:620. https://doi.org/10.3389/fonc.2019.00620.
28. Roelz R, Strohmaier D, Jabbarli R et al. Residual Tumor Volume as Best Outcome Predictor in Low Grade Glioma – A Nine-Years Near-Randomized Survey of Surgery vs. Biopsy. *Sci Rep*. 2016;6(1). https://doi.org/10.1038/srep32286.
29. Jaunmuktane Z, Capper D, Jones D, et al. Methylation array profiling of adult brain tumours: diagnostic outcomes in a large, single centre. *Acta Neuropathol Commun*. 2019;7(1) https://doi.org/10.1186/s40478-019-0668-8.
30. Daniel P, Sabri S, Chaddad A, et al. Temozolomide Induced Hypermutation in Glioma: Evolutionary Mechanisms and Therapeutic Opportunities. *Front Oncol.* 2019;9:41 https://doi.org/10.3389/fonc.2019.00041.
31. Thon N, Eigenbrod S, Kreth S, et al. IDH1mutations in grade II astrocytomas are associated with unfavorable progression-free survival and prolonged post-recurrence survival. *Cancer*. 2011;118(2):452-460 https://doi.org/10.1002/cncr.26298.
32. Zou P, Xu H, Chen P, et al. IDH1/IDH2 Mutations Define the Prognosis and Molecular Profiles of Patients with Gliomas: A Meta-Analysis. *PLoS ONE*. 2013;8(7):e68782 https://doi.org/10.1371/journal.pone.0068782.
33. Xia L, Wu B, Fu Z, et al. Prognostic role of IDH mutations in gliomas: a meta-analysis of 55 observational studies. *Oncotarget.* 2015;6(19) https://doi.org/10.18632/oncotarget.4008.
34. Mu L, Xu W, Li Q, et al. IDH1 R132H Mutation Is Accompanied with Malignant Progression of Paired Primary-Recurrent Astrocytic Tumours. *J Cancer*. 2017;8(14):2704-2712. https://doi.org/10.7150/jca.20665.
35. Kanamori M, Kumabe T, Shibahara I, et al. Clinical and histological characteristics of recurrent oligodendroglial tumors: comparison between primary and recurrent tumors in 18 cases. *Brain Tumor Pathol*. 2012;30(3):151-159 https://doi.org/10.1007/s10014-012-0119-8.

36. Leu S, von Felten S, Frank S, et al. IDH mutation is associated with higher risk of malignant transformation in low-grade glioma. *J Neuro-Oncol.* 2016;127(2):363-372 https://doi.org/10.1007/s11060-015-2048-y.
37. Yao Y, Chan A, Qin Z, et al. Mutation Analysis of IDH1 in Paired Gliomas Revealed IDH1 Mutation Was Not Associated with Malignant Progression but Predicted Longer Survival. *PLoS ONE*. 2013;8(6):e67421 https://doi.org/10.1371/journal.pone.0067421.
38. Johnson B, Mazor T, Hong C, et al. Mutational Analysis Reveals the Origin and Therapy-Driven Evolution of Recurrent Glioma. *Science*. 2013;343(6167):189-193 https://doi.org/10.1126/science.1239947.
39. Park C, Park I, Lee S, et al. Genomic dynamics associated with malignant transformation in IDH1 mutated gliomas. *Oncotarget*. 2015;6(41) https://dx.doi.org/10.18632%2Foncotarget.6189.
40. Reinhardt A, Stichel D, Schrimpf D, et al. Anaplastic astrocytoma with piloid features, a novel molecular class of IDH wildtype glioma with recurrent MAPK pathway, CDKN2A/B and ATRX alterations. *Acta Neuropathol*. 2018;136(2):273-291 https://doi.org/10.1007/s00401-018-1837-8.
41. Deng M, Sill M, Chiang J, et al. Molecularly defined diffuse leptomeningeal glioneuronal tumor (DLGNT) comprises two subgroups with distinct clinical and genetic features. *Acta Neuropathol.* 2018;136(2):239-253 https://doi.org/10.1007/s00401-018-1865-4.
42. Libermann TA, Nusbaum HR, Razon N et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature*. 1985;313(5998):144–7 http://doi.org/10.1038/313144a0.
43. Kordek R, Biernat W, Alwasiak J, Maculewicz R, Yanagihara R, Liberski PP. p53 protein and epidermal growth factor receptor expression in human astrocytomas. *J Neurooncol*. 1995;26(1):11-16. http://doi.org/10.1007/BF01054764.
44. Mottolese M, Natali PG, Coli A, et al. Comparative analysis of proliferating cell nuclear antigen and epidermal growth factor receptor expression in glial tumours: correlation with histological grading. *Anticancer Res*. 1998;18(3B):1951-1956.
45. Vuong H, Tran T, Ngo H, et al. Prognostic significance of genetic biomarkers in isocitrate dehydrogenase- wild- type lower- grade glioma: the need to further stratify this tumor entity – a meta- analysis. *Eur J Neurol*. 2018;26(3):379-387 https://doi.org/10.1111/ene.13826.

46. Liu L, Bäcklund LM, Nilsson BR, et al. Clinical significance of EGFR amplification and the aberrant EGFRvIII transcript in conventionally treated astrocytic gliomas. *J Mol Med (Berl)*. 2005;83(11):917-926. https://doi.org/10.1007/s00109-005-0700-2.
47. Järvelä S, Helin H, Haapasalo J, Järvelä T, Junttila TT, Elenius K, Tanner M, Haapasalo H, Isola J. Amplification of the epidermal growth factor receptor in astrocytic tumours by chromogenic in situ hybridization: association with clinicopathological features and patient survival. *Neuropathol Appl Neurobiol*. 2006 Aug;32(4):441-50. https://doi.org/10.1111/j.1365-2990.2006.00758.x.
48. Smith JS, Tachibana I, Passe SM, et al. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. *J Natl Cancer Inst*. 2001;93(16):1246-1256. https://doi.org/10.1093/jnci/93.16.1246
49. Korshunov A, Sycheva R, Golanov A. The prognostic relevance of molecular alterations in glioblastomas for patients age < 50 years. *Cancer*. 2005;104(4):825-832. http://doi.org/10.1002/cncr.21221.
50. Shinojima N, Tada K, Shiraishi S, et al. Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. *Cancer Res*. 2003;63(20):6962-6970.
51. Batchelor TT, Betensky RA, Esposito JM, et al. Age-dependent prognostic effects of genetic alterations in glioblastoma. *Clin Cancer Res*. 2004;10(1 Pt 1):228-233. http://doi.org/10.1158/1078-0432.ccr-0841-3.
52. Jakola A, Skjulsvik A, Myrmel K, et al. Surgical resection versus watchful waiting in low-grade gliomas. *Ann Oncol*. 2017;28(8):1942-1948 https://doi.org/10.1093/annonc/mdx230.
53. Ius T, Isola M, Budai R, et al. Low-grade glioma surgery in eloquent areas: volumetric analysis of extent of resection and its impact on overall survival. A single-institution experience in 190 patients. *J Neurosurg*. 2012;117(6):1039-1052 https://doi.org/10.3171/2012.8.jns12393.
54. Fujii Y, Muragaki Y, Maruyama T, et al. Threshold of the extent of resection for WHO Grade III gliomas: retrospective volumetric analysis of 122 cases using intraoperative MRI. *J Neurosurg*. 2018;129(1):1-9 https://doi.org/10.3171/2017.3.jns162383.
55. Xia L, Fang C, Chen G, et al. Relationship between the extent of resection and the survival of patients with low-grade gliomas: a systematic review and meta-analysis. *BMC Cancer*. 2018;18(1) https://doi.org/10.1186/s12885-017-3909-x.

56. Sanai N, Berger M. Glioma extent of resection and its impact on patient outcome. *Neurosurgery*. 2008;62(4):753-766 https://doi.org/10.1227/01.neu.0000318159.21731.cf.
57. Patel T, Bander E, Venn R, et al. The Role of Extent of Resection in IDH1 Wild-Type or Mutant Low-Grade Gliomas. *Neurosurgery*. 2017;82(6):808-814 https://doi.org/10.1093/neuros/nyx265.
58. Eseonu CI, Eguia F, ReFaey K, et al. Comparative volumetric analysis of the extent of resection of molecularly and histologically distinct low grade gliomas and its role on survival. *J Neurooncol*. 2017;134(1):65-74. https://doi.org/10.1007/s11060-017-2486-9.
59. Kawaguchi T, Sonoda Y, Shibahara I, et al. Impact of gross total resection in patients with WHO grade III glioma harboring the IDH 1/2 mutation without the 1p/19q co-deletion. *J Neurooncol*. 2016;129(3):505-514. https://doi.org/10.1007/s11060-016-2201-2.
60. Patel SH, Bansal AG, Young EB, et al. Extent of Surgical Resection in Lower-Grade Gliomas: Differential Impact Based on Molecular Subtype. *AJNR Am J Neuroradiol*. 2019;40(7):1149-1155. https://doi.org/10.3174/ajnr.A6102.

Table 1. Patients and treatment characteristics

Variables	All patients Number of pts (%) (n = 209)	*IDH 1* or *2* mutation Number of pts (%) (n = 157)	No *IDH 1* or *2* mutation Number of pts (%) (n = 52)
Age (years)			
<50	156 (74.6)	139(88.5)	17 (32.7)
≥50	53 (25.4)	18(11.5)	35 (67.3)
Median	40.0	36.0	59.0
Mean (±SD)	41.9±13.8	37.3±10.3	55.9±13.5
Range	(17-82)	(17-82)	(27-81)
Sex			
Female	81 (38.8)	69 (43.9)	12 (23.1)
Male	128 (61.2)	88 (56.1)	40 (76.9)
Histological grade according to WHO			
Grade II	167 (79.9)	145 (92.4)	22 (42.3)
Grade III	42 (20.1)	12 (7.6)	30 (57.7)
Histological appearance			
Astrocytoma	194 (92.8)	143 (91.1)	51 (98.1)
Oligodendroglioma	14 (6.7)	14 (8.9)	0
Not available	1(0.5)	0	1 (1.9)
Extent of Surgery			
Biopsy	103 (49.3)	59 (37.6)	44 (84.6)
Resection	105(50.2)	97 (61.8)	8 (15.4)
PR	28/105	28/97	n
STR	27/105	25/97	2/8
GTR	50/105	44/97	6/8
Not available	1(0.5)	1(0.6)	n
Adjuvant Treatment			
Yes	130 (62.2)	88 (56.1)	42(80.8)
RT	46/130	35/88	11/42
CT	6/130	3/88	3/42
RT+CT	78/130	50/88	28/42
No	79(37.8)	69 (43.9)	10 (19.2)

CT: Chemotherapy; RT: Radiotherapy, P: Partial, ST: Subtotal, GT: Gross Total. Bold values indicate significant *P* value ($p<0.05$)

Table 2: OS - univariate analysis

Variables	Number patients	Median, months (95% CI)	75th percentile survival, months (SEM)	log rank	HR (95% CI)
Age (years)				**p<0.0001**	
<50	156	-	92.2 (16.7)		
≥50	53	24.2 (1.8-46.6)	12.9 (2.9)		5.78 (3.42-9.80)
Sex				p=0.783	
Female	81	92.2 (79.4-105.1)	59.8 (18.1)		0.93 (0.53-1.60)
Male	128	-	37.4 (14.8)		
Histological grade according to WHO				**p<0.0001**	
Grade II	167	111.1 (-)	66.0 (11.1)		
Grade III	42	27.0 (10.8-43.)	12.9 (2.6)		3.03 (1.77-5.20)
Histological appearance				p=0.249	
Astrocytoma	194	-	42.3 (11.4)		1.96 (0.61-6.27)
Oligodendroglioma	14	111.1 (-)	98.8 (33.7)		
Extent of Resection				**p<0.0001**	
<80%	131	98.8 (66.8-130.8)	20.3 (3.8)		
≥80%	77	-	111.1 (25.0)		0.21 (0.09-0.46)
Adjuvant Treatment				**p=0.019**	
Yes	130	100.2 (78.1-122.3)	29.9 (10.4)		2.16 (1.12-4.18)
No	79	-	-		
***IDH* Status**				**p<0.0001**	
Mut	157	-	92.2 (11.2)		0.11 (0.06-0.17)
Wt	52	19.5 (11.4-27.7)	12.6 (2.6)		
***EGFR* amplification**				**p<0.0001**	
Yes	47	27.0 (7.7-46.3)	12.9 (2.3)		4.03 (2.38-6.82)
No	149	-	79.9 (13.9)		
***PTEN* Loss**				**p=0.018**	
Yes	41	65.0 (-)	59.8 (11.8)		1.93 (1.11-3.37)
No	157	111.1(-)	18.0 (6.2)		
***MGMT*p methylation**				p=0.516	
Yes	101	111.1 (87.5-134.8)	59.8 (10.9)		0.84 (0.49-1.43)
No	95	101.1 (-)	37.4 (15.3)		

Bold values indicate significant *P* value (p<0.05)

Table 3. PFS - univariate analysis

Variables	Number patients	Median, months (95% CI)	75 % percentile survival, months (SEM)	log rank	HR (95% CI)
Age (years)				**p=0.014**	
<50	156	32.7 (24.3 – 41.1)	17.0 (2.7)		
≥50	53	16.4 (11.0-21.8)	9.5 (2.1)		1.58 (1.09-2.28)
Sex				p=0.167	
Female	81	37.6 (24.3-50.9)	17.0 (2.9)		0.78 (0.55-1.11)
Male	128	26.6 (18.6-34.6)	11.9 (1.6)		
Histological grade according to WHO				**p<0.0001**	
Grade II	167	34.3 (25.0-43.6)	16.4 (2.2)		
Grade III	42	11.9 (5.7-18.1)	7.8 (2.0)		2.11 (1.42-3.12)
Histological appearance				**p=0.004**	
Astrocytoma	194	26.6 (21.1-32.1)	11.6 (1.1)		3.13 (1.40-7.24)
Oligodendroglioma	14	89.2 (60.4-118.0)	64.4 (16.2)		
Extent of Resection				**p=0.0005**	
<80%	131	20.0 (13.5-26.5)	9.4 (1.0)		
≥80%	77	47.8 (28.2-67.4)	25.4 (2.8)		0.52 (0.35-0.75)
Adjuvant Treatment				**p<0.0001**	
Yes	130	22.8 (18.2-27.4)	10.8 (0.8)		2.25 (1.51-3.36)
No	79	61.6 (35.5 -87.7)	23.9 (5.9)		
***IDH* Status**				**p<0.0001**	
Mut	157	39.2 (26.7-51.7)	19.2 (2.8)		0.38 (0.26-0.55)
Wt	52	12.6 (10.3-14.9)	7.8 (2.4)		
***EGFR* amplification**				**p=0.001**	
Yes	47	16.4 (11.4-21.4)	7.7 (2.5)		1.88 (1.30-2.72)
No	149	37.6 (26.7 - 48.5)	16.1 (2.8)		
***PTEN* Loss**				p=0.079	
Yes	41	23.9 (14.1-33.7)	7.1 (2.7)		1.43 (0.96 -2.13)
No	157	31.5 (1)	13.3 (2.1)		
***MGMT*p methylation**				p=0.533	
Yes	101	30.7 (21.8-39.6)	58.4 (10.9)		0.90 (0.63-1.27)
No	95	27.6 (18.5-36.7)	29.9 (14.8)		

Bold values indicate significant *P* value (p<0.05)

Table 4. TtMP - univariate analysis

Variables	Number patients	Median, months (95% CI)	75 % percentile survival, months (SEM)	log rank	HR (95% CI)
Age (years)				p=0.367	
<50	131	100.4 (71.1-129.7)	32.1 (11.0)		
≥50	34	-	59.0(17.0)		0.72 (0.35-1.48)
Sex				p=0.991	
Female	68	87.1 (-)	51.9 (17.8)		1.00 (0.56-1.77)
Male	97	118.2 (85.1-151.4)	32.7 (11.4)		
Histological appearance				p=0.218	
Astrocytoma	150	118.2 (75.4-161.0)	32.1 (10.3)		2.05 (0.64-6.61)
Oligodendroglioma	14	-	70.4 (10.9)		
Extent of Resection				p=0.080	
<80%	100	118.2 (77.6-158.9)	28.1 (10.0)		
≥80%	65	-	66.6(11.1)		0.58 (0.31-1.08)
Adjuvant Treatment				**p<0.0001**	
Yes	92	73.7 (52.5-95.0)	24.9 (5.7)		4.34 (1.95-9.67)
No	73	-	-		
***IDH* Status**				p=0.314	
Mut	143	118.2 (74.5-162.0)	51.9 (11.7)		0.69 (0.34-1.43)
Wt	22	-	10.0 (5.7)		
***EGFR* amplification**				p=0.460	
Yes	20	-	59.0 (26.1)		0.72 (0.30-1.72)
No	132	118.2 (61.5-174.9)	32.7 (13.5)		
***PTEN* Loss**				p=0.195	
Yes	23	74.0 (49.7-98.3)	27.3 (12.9)		1.56 (0.79-3.05)
No	131	121.1 (64.7-177.5)	43.0 (12.7)		
***MGMTp* methylation**				p=0.113	
Yes	83	121.1 (73.1-69.1)	55.7 (7.2)		0.64 (0.37-1.12)
No	69	100.4 (66.2-134.7)	27.3 (7.3)		

Bold values indicate significant *P* value (p<0.05)

Table 5. OS – Multivariate Analysis

Variables	HR (95% CI)	*P* value
Age (continuous)	1.04 (1.02-1.07)	**p=0.0002**
Histological grade according to WHO		p=0.273
Grade II	0.667 (0.32-1.38)	
Grade III	1	
Extent of Resection		**p=0.007**
<80%	1	
≥80%	0.323 (0.14-0.74)	
Adjuvant Treatment		p=0.600
Yes	0.813 (0.38-1.76)	
No	1	
***IDH* Status**		**p=0.0004**
Mut	0.252 (0.12-0.54)	
Wt	1	
***EGFR* amplification**		**p=0.030**
Yes	2.19 (1.08-4.44)	
No	1	
***PTEN* Loss**		p=0.875
Yes	1.052 (0.56-1.99)	
No	1	

Bold values indicate significant *P* value (p<0.05)

Table 6. PFS – multivariate analysis

Variables	HR (95% CI)	*P* value
Age (continuous)	1.00 (0.98-1.02)	p=0.969
Histological grade according to WHO		p=0.784
Grade II	1	
Grade III	1.076 (0.64-1.82)	
Extent of Resection		**p=0.049**
<80%	1	
≥80%	0.665 (0.44-0.998)	
Adjuvant Treatment		**p=0.020**
Yes	1.72 (1.09-2.72)	
No	1	
***IDH* Status**		**p=0.009**
Mut	0.487 (0.28-0.84)	
Wt	1	
***EGFR* amplification**		p=0.468
Yes	1.190 (0.74-1.906)	
No	1	
***PTEN* Loss**		p=0.573
Yes	1.145(0.72-1.83)	
No	1	

Bold values indicate significant *P* value (p<0.05)

Table 7. TtMP – multivariate analysis

Variables	HR (95% CI)	*P* value
Age (continuous)	0.993 (0.968-1.019)	p=0.580
Extent of Resection		p=0.781
<80%	1	
≥80%	0.908 (0.459-1.794)	
Adjuvant Treatment		**p=0.001**
Yes	4.338(1.780-10.575)	
No	1	
***IDH* Status**		p=0.402
Mut	0.674 (0.268-1.695)	
Wt	1	
***EGFR* amplification**		p=0.266
Yes	0.601 (0.246-1.4)	
No	1	

Bold values indicate significant *P* value (p<0.05)

Table 8. Patients and treatment characteristics of *IDH*-wt according to molecular markers stratification

Variables	All *IDH* wt patients (n = 52) (%)	*EGFR* amp (n=30) (%)	*TERTp* mut (n=13) (%)	*PTEN* loss (n=17) (%)	*MGMT*p methylation (n=18) (%)
Age (years)					
<50	17 (32.7)	10 (33.3)	5 (38.5)	6 (35.3)	4 (22.2)
≥50	35 (67.3)	20 (66.7)	8 (61.5)	11 (64.7)	14 (77.8)
Median	59.0	60.0	55.0	64.0	61.5
Mean (±SD)	55.9±13.5	55.9±14.0	53.8±12.0	58.3±15.3	60.2±13.4
Range	(27-81)	(28-81)	(27-69)	(33-81)	(33-81)
Sex					
Female	12 (23.1)	8(26.7)	3 (23.1)	2 (11.8)	4 (22.2)
Male	40 (76.9)	22 (73.3)	10 (76.9)	15 (88.2)	14 (77.8)
Histological grade according to WHO					
Grade II	22 (42.3)	7 (23.3)	3 (23.1)	5 (29.4)	8 (44.4)
Grade III	30 (57.7)	23 (76.7)	10 (76.9)	12 (70.6)	10 (55.6)
Histological appearance					
Astrocytoma	51 (98.1)	30 (100)	13 (100)	17 (100)	18 (100)
Oligodendroglioma	n	n	n	n	n
Not available	1 (1.9)	n	n	n	n
Extent of Surgery					
Biopsy	44 (84.6)	27 (90)	9 (69.2)	16 (94.1)	15 (83.3)
Resection	8 (15.4)	3 (10)	4 (30.8)	1 (5.9)	3 (16.7)
PR	n	n	n	n	n
STR	2/8	1/3	1/4	1/1	1/3
GTR	6/8	2/3	3/4	n	2/3
Not available	n	n	n	n	n
Adjuvant Treatment					
Yes	42(80.8)	26 (86.7)	12 (92.3)	14 (82.4)	14 (77.8)
RT	11/42	9/26	n	6/14	5/14
CT	3/42	0/26	3/12	2/14	1/14
RT+CT	28/42	17/26	9/12	6/14	8/14
No	10 (19.2)	4 (13.3)	1 (7.7)	3 (17.6)	4 (22.2)

CT: Chemotherapy; RT: Radiotherapy, P: Partial, ST: Subtotal, GT: Gross Total.

Table 9. OS in the *IDH-wt* cohort - univariate analysis

Variables	Number patients	Median, months (95% CI)	75 % percentile survival, months (SEM)	log rank	HR (95% CI)
Age (years)				p=0.082	
<50	17	29.9 (25.4-34.4)	19.5 (6.3)		
≥50	35	16.4 (12.8-20.1)	9.5 (4.3)		1.94 (0.91-4.13)
Sex				p=0.348	
Female	12	19.5 (10.0-29.0)	9.5 (5.8)		1.43 (0.68-3.01)
Male	40	17.0 (4.0-29.9)	12.6 (2.6)		
Histological grade according to WHO				p=0.841	
Grade II	22	19.5 (11.2-27.8)	13.4 (1.1)		
Grade III	30	18.0 (2.8-33.3)	9.5 (5.4)		0.94 (0.48-1.80)
Extent of Resection				**p=0.009**	
<80%	44	16.5 (11.8-21.1)	9.5 (3.6)		
≥80%	8	-	37.4 (25.2)		0.18 (0.04-0.77)
Adjuvant Treatment				p=0.354	
Yes	42	22.1 (13.0-31.1)	12.9 (0.8)		0.66 (0.27-1.60)
No	10	7.8 (0-21.7)	2.3 (1.2)		
***EGFR* amplification**				p=0.219	
Yes	30	14.1 (9.6-18.6)	12.9 (2.3)		1.53 (0.77-3.02)
No	21	27.8 (14.6-41.0)	79.9 (13.9)		
***PTEN* Loss**				p=0.399	
Yes	17	14.1 (7.3-20.9)	5.1(2.3)		1.34 (0.68-2.66)
No	35	22.1 (12.3-31.9)	12.9 (0.6)		
***MGMT*p methylation**				p=0.473	
Yes	18	16.4 (0-37.8)	9.5 (6.0)		1.28 (0.65-2.53)
No	32	19.5 (11.7-27.4)	12.6 (4.4)		

Bold values indicate significant *P* value (p<0.05)

Table 10. PFS in the *IDH-wt* cohort - univariate analysis

Variables	Number patients	Median, months (95% CI)	75 % percentile survival, months (SEM)	log rank	HR (95% CI)
Age (years)				p=0.688	
<50	17	17.2 (1.2-33.1)	9.2 (3.4)		
≥50	35	12.6 (9.8-15.4)	7.8 (2.3)		1.14 (0.60-2.16)
Sex				p=0.207	
Female	12	10.1 (8.7-11.5)	9.2 (4.9)		1.54 (0.78-3.04)
Male	40	13.3 (10.9-15.7)	6.6 (3.1)		
Histological grade according to WHO				p=0.373	
Grade II	22	13.9 (8.3-19.5)	9.6 (2.3)		
Grade III	30	11.6 (10.7-12.5)	6.0 (2.5)		1.32 (0.72-2.41)
Extent of Resection				p=0.080	
<80%	44	11.9 (10.1-13.7)	6.0 (2.4)		
≥80%	8	27.6 (0.02-55.2)	11.6 (4.7)		0.48 (0.21-1.11)
Adjuvant Treatment				p=0.862	
Yes	42	12.6(8.2-17.0)	9.6 (2.0)		0.93 (0.41-2.11)
No	10	7.8 (0-21.6)	2.3 (1.2)		
***EGFR* amplification**				p=0.082	
Yes	30	11.2 (8.8-13.6)	4.7 (1.2)		1.72 (0.93-3.20)
No	21	17.7 (7.1-28.3)	11.6 (1.8)		
***PTEN* Loss**				p=0.526	
Yes	17	11.9 (0-23.9)	3.5 (1.1)		1.23 (0.65-2.33)
No	35	13.3 (11.2-15.4)	10.0 (0.8)		
***MGMT*p methylation**				p=0.640	
Yes	18	11.3 (3.2-19.4)	7.8 (3.6)		1.16 (0.62-2.18)
No	32	12.6 (10.6-14.6)	9.6 (3.6)		

Bold values indicate significant *P* value (p<0.05)

Table 11: OS in the *IDH*-wt cohort – multivariate analysis

Variables	HR (95% CI)	*P* value
Age (continuous)	1.047 (1.015-1.079)	**p=0.003**
Sex		p=0.412
Female	1.396 (0.629-3.099)	
Male	1	
Histological grade according to WHO		p=0.302
Grade II	1	
Grade III	0.648 (0.285-1.477)	
Extent of Resection		**p=0.007**
<80%	1	
≥80%	0.124 (0.027-0.571)	
Adjuvant Treatment		**p=0.016**
Yes	0.273 (0.095-0.783)	
No	1	
***EGFR* amplification**		p=0.073
Yes	2.26 (0.928-5.504)	
No	1	

Bold values indicate significant *P* value (p<0.05)

Table 12: PFS in the *IDH-wt cohort* – multivariate analysis

Variables	HR (95% CI)	*P* value
Age (continuous)	1.011 (0.987-1.035)	p=0.382
Sex		p=0.236
Female	1.557 (0.748-3.240)	

Table 13. TtMP in the *IDH*-wt cohort - univariate analysis

Male	1	
Histological grade according to WHO		p=0.913
Grade II	1	
Grade III	0.960 (0.463-1.990)	
Extent of Resection		p=0.080
<80%	1	
>80%	0.444 (0.179-1.002)	
Adjuvant Treatment		p=0.173
Yes	0.528 (0.210-1.323)	
No	1	
***EGFR* amplification**		p=0.111
Yes	1.850 (0.868-3.943)	
No	1	

Bold values indicate significant *P* value (p<0.05)

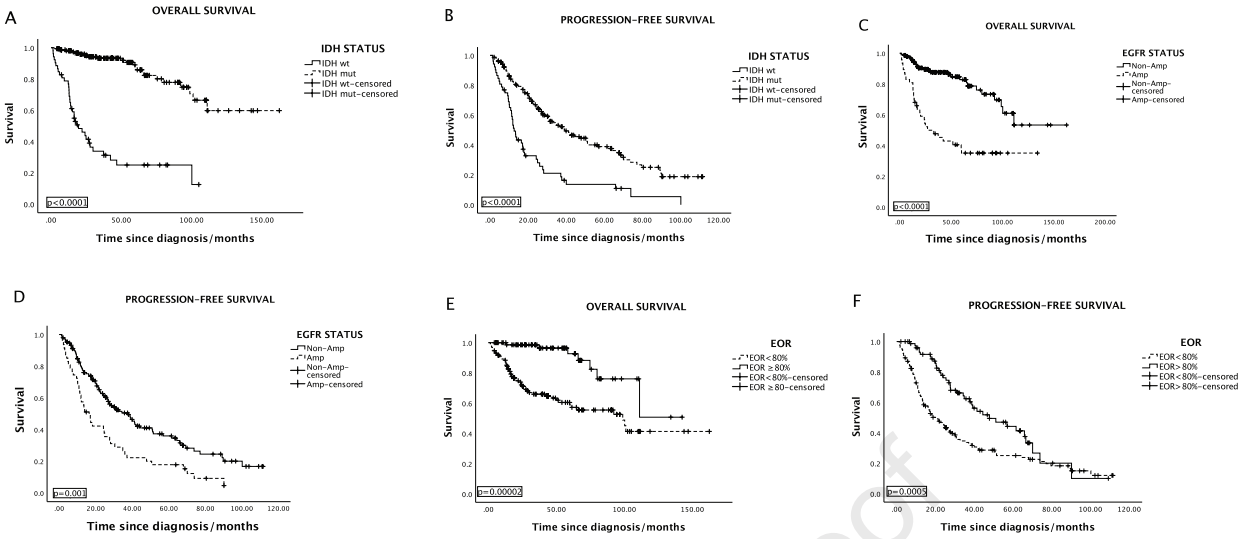
Variables	Number patients	Median, months (95% CI)	75 % percentile survival, months (SEM)	log rank	HR (95% CI)
Age (years)				p=0.303	
<50	5	28.1 (0-68.6)	9.2 (9.3)		
≥50	17	-	17.7 (7.3)		0.49 (0.12-1.96)
Sex				**p=0.021**	
Female	4	2.7 (0-11.0)	28.1 (20.5)		4.61 (1.12-19.04)
Male	18	-	0.8 (-)		
Extent of Resection				p=0.679	
<80%	19	-	9.6 (4.4)		
≥80%	3	-	17.7 (-)		0.65 (0.08-5.18)
Adjuvant Treatment				p=0.238	
Yes	16	43.0 (-)	9.2 (6.0)		3.26 (0.41-26.06)
No	6	-	-		
***EGFR* amplification**				p=0.482	
Yes	7	-	28.1 (-)		0.57 (0.11-2.82)
No	14	-	10.0 (0.6)		
***PTEN* Loss**				p=0.298	
Yes	5	28.1 (0-82.5)	2.7 (2.1)		2.06 (0.51 -8.27)
No	17	-	17.7 (7.4)		
***MGMT*p methylation**				p=0.595	
Yes	8	43.0 (-)	9.2 (16.7)		1.45 (0.36-5.83)
No	12	-	10.0 (5.8)		

Bold values indicate significant *P* value (p<0.05)

Table 14. TtMP in the *IDH-wt* cohort – multivariate analysis

Variables	HR (95% CI)	*P* value
Age (continuous)	1.01 (0.951-1.140)	p=0.383
Sex		**p=0.019**
Female	19.091 (1.639-222.413)	
Male	1	
Extent of Resection		p=0.758
<80%	1	
≥80%	1.461 (0.131-16.338)	
Adjuvant Treatment		p=0.326
Yes	3.311 (0.304-36.056)	
No	1	
***EGFR* amplification**		p=0.128
Yes	0.214 (0.029-1.562)	
No	1	

Bold values indicate significant *P* value (p<0.05)



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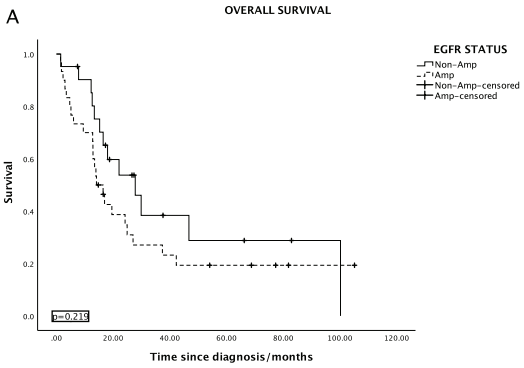

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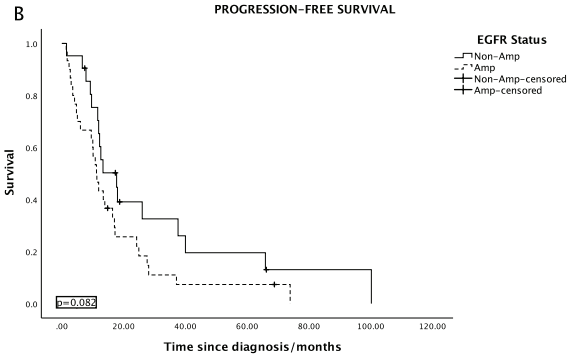

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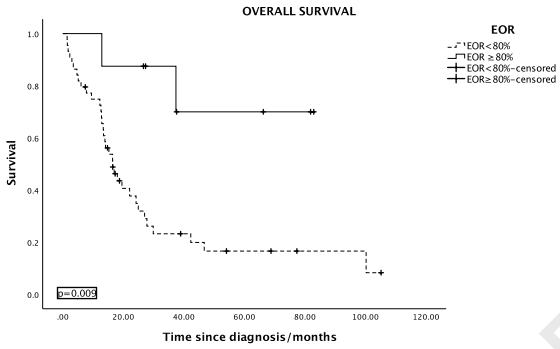

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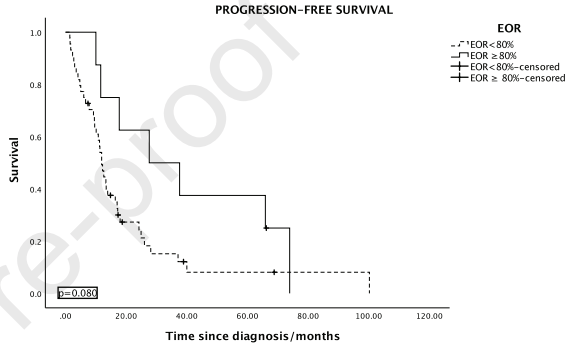

Abbreviation list

B biopsy

CNS central nervous system

CT chemotherapy

EGFR epidermal growth factor

EOR extent of resection

GTR gross total resection

IDH isocitrate dehydrogenase

LGG low grade glioma

MGMT O^6-methylguanine-DNA methyltransferase

Mut mutant

OS overall survival

PFS progression-free survival

PR partial resection

PTEN phosphatase and tensin homolog

RT radiotherapy

STR subtotal resection

TERT telomerase reverse transcriptase

TtMP time to malignant progression

WHO World Health Organisation

Wt wild-type

Disclosure-Conflict of Interest

Declarations of interest: none