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**Title**

The impact of MGMT methylation and IDH-1 mutation on long-term outcome for glioblastoma treated with chemoradiotherapy

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**Abstract**

**Background**

Increasingly, biomarkers have been identified which correlate with improved overall and progression-free survival (OS & PFS) in Glioblastoma, including MGMT methylation status and mutations in the IDH1 gene. In this study, we investigated the clinical and biological factors associated with long-term survival in glioblastoma patients treated with chemoradiotherapy.

**Method**

Demographic and clinical data was collected for all patients with Glioblastoma diagnosed between May 2004 and September 2007, treated with chemoradiotherapy and with associated tissue samples available for biomarker analysis. MGMT methylation was determined by pyrosequencing. IDH1 mutation was identified by R132H immunohistochemistry. Univariate Cox regression analysis of factors associated with survival, and Kaplan-Meier survival analysis was performed using the SPSS statistics package.

**Results**

100 patients were included in the study. Median follow-up was 12.2 months (range 1.6-102.4). Median OS was 12.1 months (95% CI: 10.8–13.3) and median PFS was 8.2 months (95% CI: 6.8–9.5). 2, 3 and 5-year survival was 18%, 9% and 6% respectively. Three patients are still alive at 7.4, 8.3 and 8.5 years after diagnosis.

Cox proportional-hazards regression identified independent prognostic factors for OS; female (p = 0.019), MGMT methylation (p < 0.0001) and IDH1 mutation (p = 0.023), and for PFS; MGMT methylation (p = 0.001) and IDH1 mutation (p = 0.018).

Kaplan-Meier survival analysis showed that MGMTmethylated/IDH1+ve was associated with significantly longer OS 66.8 months (95% CI: 0.0–167.8) and PFS 16.9 months (95% CI: 11.1–22.7), when compared with MGMTmethylated/IDH1-ve OS 15.5 months (95% CI: 11.6–19.4) and PFS 9.4 months (95% CI: 8–10.8) (Log-rank, P = 0.000), and MGMTunmethylated/IDH1-ve OS 11.1 months (95% CI: 8.5–13.7) and PFS 6.3 months (95% CI: 4.4–8.3) (log-rank, p = 0.000).

**Conclusions**

While the importance of MGMT methylation is well established, we demonstrate that the combination of MGMTmethylated/IDH1+ve is associated with considerably longer OS and PFS in this series of chemoradiotherapy treated Glioblastoma tumours. The long-term cognitive function and quality of life in these long-term survivors warrants investigation.

**Key Words:**

Glioblastoma, MGMT, Methylation, IDH1, Survival

**Introduction**

Glioblastoma is the most common, and most aggressive primary malignant brain tumour in adults [19,20]. It commonly occurs *de novo* as an advanced cancer, but also arises as a secondary glioblastoma in 5%, from progression from lower grade gliomas. Both primary and secondary GBMs are indistinguishable histologically but differ at the molecular level [25].

Current standard of care for glioblastoma following surgery is chemoradiotherapy using temozolamide and the median OS is 14.6 months with a 5-year long-term survival (LTS) rate of 9.8% with maximal treatment [30, 31]. Nevertheless glioblastoma remains a fatal disease and treatment strategies are essentially palliative, with the aim being to improve OS and PFS, whilst maintaining an acceptable quality of life.

Recent analysis of national data over a 5-year period for patients with Glioblastoma in England estimates incidence in England to be 3.43/100,000/year, while median overall-survival for patients up to 70 years was 14.8 months with a 5-year LTS rate of 6.6% with maximal treatment [4]. While debate exists as to the time-point when one can consider a patient a long-term survivor, it is clear that this group of glioblastoma patients are an important cohort to study [28].

It is well established and understood that MGMT promoter methylation has positive prognostic value, with tumours from LTS having much higher rates of MGMT promoter methylation than the general glioblastoma population (74% vs. 30-35%) [16, 30, 31], indeed we have previously reported that the extent of MGMT promoter methylation also correlates with survival [9]. Whilst the prognostic importance of methylation of MGMT has impacted on clinical practice, other genes of interest have not yet had such a convincing impact [21, 38].

The WHO classification of tumours of the central nervous system has recently been updated to include molecular parameters that along with histological features have defined new tumour entities, with specific reference to IDH status in Glioblastoma. IDH1 *wildtype* account for 90% of cases and are considered *de novo* Glioblastoma [19, 20]. Mutations in IDH1 are common in grade II and III astrocytomas and oligodendrogliomas and secondary glioblastoma [2, 15], and are associated with increased patient survival [11, 23, 26, 36]. Mutations in IDH1, most commonly an R132H substitution at the arginine residue [26, 40], have been reported in only 12% of patients with primary glioblastoma, and have been suggested to correlate poorly with LTS [1].

More recently, the combination of MGMT methylation and IDHI mutation has been shown to be associated with a significantly better outcome in a 2-gene predictor model; however, this cohort was not homogenously treated with chemoradiotherapy [22]. Therefore the aim of this study was to investigate the impact of MGMT methylation and IDH1 mutation on LTS in a cohort of uniformly treated glioblastoma patients with long-term follow-up.

**Methods & Materials**

**Case selection**

We have previously reported a series of 115 patients with newly diagnosed glioblastoma between May 2004 and September 2007 [9], and uniformly treated with chemoradiotherapy according to the Stupp protocol [31]. This cohort of patients was revisited to update clinical details (including follow-up) and perform IDH-1 immunohistochemistry if tissue samples permitted to provide a uniformly treated cohort with long-term follow-up and genetic analysis. For the current study patients were included if: (i) MGMT status was known; (ii) tissue was available for IDH1 analysis; and (iii) clinical data were available for extended survival analysis (Figure 1).

**Clinical data**

Clinical data was collected retrospectively for all patients within the study period from available patient notes and imaging. Demographic baseline data included age, sex, performance status, extent of surgery, time from diagnosis to radiotherapy, corticosteroid use, pathology, tumour location, MGMT methylation, and IDH1 mutation. Clinical data describing intensity of chemoradiotherapy treatment was also collected. Radiotherapy data included dose, fractions, duration, and reasons for interruption/delay or discontinuation of treatment. Concomitant and adjuvant temozolamide data included the reason for early discontinuation, number of cycles completed and dose.

**Pathology and tissues**

A consultant neuropathologist reanalysed tumour tissue samples to confirm that each was consistent with a diagnosis of glioblastoma WHO grade IV. Samples were selected for analysis after visual assessment >70% neoplastic cells and <50% necrosis. Methylene blue or haematoxylin and eosin stained smears were used as described earlier [34] scraping the tissue into DNA extraction buffer. Where available, snap frozen tissue was used. More than one tissue sample for each case, selected preferentially from different blocks and/or different fixation was analysed in most cases.

**DNA extraction and bisulphite treatment**

DNA extraction was performed with the DNeasy Blood and Tissue kit (Qiagen cat 69506, Crawley, UK). Quantfication was performed by spectrophotometry using a NanoDrop (NanoDrop ND-1000, Thermofisher Scientific, Loughborough, UK). Frozen, smear, and FFPE DNA yields were 6.9 ± 7.0 μg, 3.2 ± 4.1 μg, and 20.9 ± 22.2 μg, respectively. Using the EZ DNA methylation kit (Zymo, Orange, CA, USA, D5002), bisulphite modification was performed on 1 μg of DNA, with each experiment including universal methylated DNA (CpGenome Universal Methylated DNA S7821, Millipore, Watford, UK) as the positive control. Normal brain DNA was used as negative control.

**Analysis of MGMT promoter methylation**

The pyrosequencing assay was performed as described earlier [27]. To amplify bisulphite-treated DNA, the primers used were forward: 5’gGGATAGTTGGGATAGTT-3’ (the first g avoids hairpin loop formation) and reverse: 5’-biotin-ATTTGGTGAGTGTTTGGG-3’ which gives a 99-bp amplicon at genomic position 131155467 – 131 155 565. PCR analysis was performed in duplicate in a 25 μl reaction volume, which contained 300 pmol of forward and 300 pmol of revere primer, 2 μl 10 x buffer, 160 μm dNTPs, 0.5 U HotStar Taq polymerase (Qiagen) and 1-2 μl bisulphite-treated DNA. The following PCR conditions were used: 95**°**C-15 min; 40 cycles of 94**°**C-30 s, 50**°**C-45 s, 72**°**C-30 s; 72**°**C-10 min (Dyad, GRI, Braintree, UK). Before pyrosequencing, confirmation of the correct PCR product was performed, 3 μl of PCR products were analysed on 2% agarose gel, the remaining 22 μl was subjected to pyrosequencing on a PSQ96MA System (Biotage, Uppsala, Sweden) using the primer 5’-GGATATGTTGGGATAGT-3’ and PyroGold reagents (Biotage). To analyse the data, the Pyro Q-CpG software 1.0.9 (Biotage) was used.

12 CpG sites are yielded from pyrosequencing within the promoter region of the MGMT gene. For data analysis, the average percentage methylation across the 12 CpG sites was calculated from duplicate PCR reactions, to give an average methylation per sample. To compare with clinical data, the glioblastoma was considered methylated if at least one sample had average methylation ≥9% (≥ mean ± 2 s.d for non-neoplastic brain) in more than one independent bisulphite modification. If average methylation was <9% in all samples, the case was considered unmethylated. The average methylation per case was calculated by averaging the mean methylation per sample for methylated samples for that case. Methylation-specific PCR assays were carried out as described by [13].

**Analysis of IDH1 immunohistochemistry**

IDH1 immunohistochemistry was performed with a Bond III autostainer (Leica Microsystems, Wetzlar, Germany) and biotin-free detection system (DS9800; Leica Microsystems, Wetzlar, Germany). The anti-Human IDH1 R132H mouse monoclonal antibody (DIA-H09; Dianova, Hamburg, Germany), diluted to 1:20 was used for staining. A polarising microscope (DN2500M; Leica Microsystems, Wetzlar, Germany) at 20x magnification was used to assess IDH1 status.

**Statistics**

Statistical analysis was performed using SPSS statistics 22.0 (Chicago, IL, USA). Unsupervised hierarchical cluster analysis was performed in Gene Spring, using Euclidean distance and centroid linkage. Survival data were calculated from the date of diagnosis. Kaplan-Meier survival curves were obtained and differences in PFS or OS were tested for statistical significance using the log-rank or breslow test where appropriate. Univariate Cox regression analysis was used to determine whether age, sex, performance status, extent of surgery, MGMT methylation, and IDH1 mutation had prognostic significance with respect to outcome. Cox proportional-hazards regression analysis was used to identify independent prognostic factors for OS and PFS.

**Results**

**Clinical, demographic and treatment characteristics**

115 patients with newly diagnosed glioblastoma were identified within the study period. 15 patients were excluded, 9 patients as they did not have IDH1 immunohistochemistry analysis of tumour tissue, 3 patients as they did not have MGMT promoter methylation determined by pyrosequencing, and 3 patients as they were not treated with the current standard of care chemoradiotherapy. A final cohort of 100 patients met all the criteria for inclusion in this study. All 100 patients commenced chemoradiotherapy following biopsy or cytoreductive surgery. The demographic and clinical characteristics of the patients at baseline, along with the genetic analysis are summarised (Table 1). The promoter region of MGMT was methylated greater than 9% in 53% of patients. IDH1 was mutated in 5% of patients.

All patients commenced radiotherapy and concomitant chemotherapy in accordance with the protocol (data are summarised in Table 1). Median radiotherapy dose was 60 Gy (25-60). Median number of fractions was 30 (14-60). Median duration was 42 days (13-59). 6% had an interruption or delay to radiotherapy at some point, 94% completed radiotherapy. 100% started temozolomide, 11% discontinued temozolomide early. Adjuvant temozolamide was commenced in 74% of patients. The median number of cycles completed was 5 (1-8). 35 patients completed all 6 cycles (48%). 69 patients had their dose increased at cycle 2 (95%). 39 patients discontinued chemotherapy early (53%). Figure 1 summarises the included cohort of patients (n=100), segregated by genotype and extent of chemoradiotherapy.

**Analysis of variables affecting progression-free and overall survival**

Median follow-up was 12.2 months (range 1.6-102.4). Median OS was 12.1 months (95% CI: 10.8–13.3) and median PFS was 8.2 months (95% CI: 6.8–9.5). 2, 3 and 5-year survival rates were 18%, 9% and 6% respectively. Three patients are still alive at 7.4, 8.3 and 8.5 years after diagnosis.

Univariate analysis of demographic and clinical variables on OS and PFS was undertaken and the data are summarised in table 2. Variables returning a significantly longer median OS included age less than 50 years at the time of diagnosis, female sex, PS 0 when compared with PS 1,2, or 3, and extent of surgery (biopsy vs. debulking). Variables returning a significantly longer median PFS also included age less than 50 years at the time of diagnosis, female sex, PS 0 when compared with PS 1,2, or 3, and extent of surgery (biopsy vs. debulking). Cox proportional-hazards regression was used to investigate which of the variables identified in a univariate fashion were independent prognostic factors for OS and PFS. For OS; female (p = 0.019), MGMT methylation (p < 0.0001) and IDH1 mutation (p = 0.023) were identified, and for PFS; MGMT methylation (P = 0.001) and IDH1 mutation (P = 0.018).

Genetic variables were also analysed in a univariate fashion to investigate their influence on OS and PFS (Table 3). MGMT promoter methylation (n=53) was associated with significantly longer median OS and PFS. Median OS with MGMT promoter methylation was 16.7 months (95% CI: 12.6–20.7) compared to 11.1 months (95% CI: 8.5-13.7) without (log-rank, p < 0.0001). Median PFS with MGMT promoter methylation was 9.7 months (95% CI: 7.9–11.4) compared to 6.3 months (95% CI: 4.4-8.3) without (log-rank, p < 0.0001). 2, 3 and 5-year survival was 34%, 17% and 11%; compared with 0% at 2-year survival and beyond without MGMT promoter methylation (Table 2).

IDH1 mutation was only seen in 5 patients and was associated with dramatic and significantly longer median OS and PFS. Median OS with IDH1 mutation was 66.8 months (95% CI: 0.000–167.8) compared to 11.7 months (95% CI: 10.7-12.7) without (log-rank, p = 0.004). Median PFS with IDH1 mutation was 16.9 months (95% CI: 11.1 – 22.7) compared to 7.6 months (95% CI: 6.6 – 8.6) without (log-rank, p = 0.003). 2, 3 and 5-year survival was 60%, 60% and 60% with IDH1 mutation; compared with 16%, 6% and 3% without (Table 2).

When considering both MGMT promoter methylation and IDH1 mutation together (n=5), this genotype was associated with significantly longer median OS 66.8 months (95% CI: 0.0 – 167.8) and PFS 16.9 months (95% CI: 11.1 – 22.7), compared with either MGMT promoter methylation and IDH1 wild-type (n=48) median OS 15.5 months (95% CI: 11.6 – 19.4) and PFS 9.4 months (95% CI: 8 – 10.8) (log-rank, p = 0.015), and unmethylated MGMT promoter and IDH1 wild-type (n=47) OS 11.1 months (95% CI: 8.5 – 13.7) and PFS 6.3 months (95% CI: 4.4 – 8.3) (log-rank, p < 0.0001) (Table 2).

**Clinical and biological factors associated with long-term survival**

LTS in glioblastoma is generally defined as survival over 3 years. Within our cohort the 3 and 5-year survival rates were 9% and 6% respectively. The clinical and demographic characteristics of these patients, along with their MGMT methylation status and IDH1 mutation status (n=9) are summarised (Table 3). Median age was 45 years, with only 3 patients over 50 years (50, 60, 61). Female sex (n=6) was more common. Performance status at time of diagnosis was 0 (n=4), 1 (n=4), and 2 (n=1). There were no common patient factors and tumours were located in all lobes of the brain. Histology was glioblastoma (n=7) and glioblastoma with oligodendroglia component (n=2). Of note, there were only two glioblastoma with oligodendroglia component cases in the entire cohort. All patients underwent cytoreductive surgery, radiotherapy and concomitant temozolomide, and adjuvant temozolomide apart from one patient (Patient No. 3) who completed only 2 cycles of adjuvant temozolomide due to toxicity effects. All patients had methylation of the MGMT promoter region greater than 9%. When considering the extent of methylation (Low, medium, or high), most patients were high (n=5), or medium (n=2), with the remainder low (n=2). Three of the patients with a mutation in the IDH1 gene (n=5) were observed in this sub-cohort of LTS. All survived over 5 years (n=3). The median progression-free survival was 33.2 months. Treatment at progression is described and was CCNU (n=5), carmustine implants (n=1), temozolamide (n=1), and no further treatment at follow-up (n=2). Three patients are still alive at 7.4, 8.3 and 8.5 years after diagnosis.

**Discussion**

Glioblastoma remains a devastating diagnosis and cytoreductive surgery and chemoradiotherapy provides only palliative treatment for the majority of patients. In a minority of cases, long-term survival is achieved and this is of considerable importance for understanding the underlying clinical and biological factors associated with a sustained response to treatment. In this study, we present a homogenous cohort of patients with glioblastoma treated uniformly with chemoradiotherapy, and known MGMT methylation and IDH1 mutation status. This has allowed us to assess the impact of these biomarkers on PFS and OS in routine clinical practice for patients receiving the current standard of care treatment. We have demonstrated that both MGMT promoter methylation and IDH1 mutation are independent prognostic factors for OS and PFS, and that the combination of both genetic modifications is associated with significantly longer OS and PFS than either alone.

The importance of IDH status has been recognised in the restructured WHO classification of tumours of the central nervous system in that it now defines two tumour entities. Glioblastoma IDH-wildtype, accounting for 90% of Glioblastoma cases, which correspond clinically to *de novo* or primary cases occurring in patients over 55 years of age, and Glioblastoma IDH-mutant, accounting for 10% of Glioblastoma cases, which correspond closely to secondary glioblastoma with a history of prior lower grade diffuse Glioma, preferentially in younger patients.

**Long-term survival: definition and prediction**

It is important to consider when a patient with glioblastoma becomes a long-term survivor, however this term is often poorly defined. Whilst most studies use a cut-off of 3 years or 5-years, it has been suggested that 2.5-years should be duration after which one is considered to be a long-term survivor [28]. This was calculated based on the observation that the first quarter of the 2nd year of survival observes the peak incidence of mortality, with the risk of death decreasing to half this rate at 2.5 years. Whilst this may be a valid observation with some merit, in this study, we chose to examine both the 3 and 5-year time points to enable comparison with previously published studies [5, 16].

Our current understanding of LTS in glioblastoma suggest that most of the associated factors are fixed demographic, clinical, and genomic variables; for instance younger age at diagnosis, frontal lobe tumour location, smaller tumour volume, and cytoreductive surgery followed by radiotherapy with concomitant and adjuvant temozolomide [5]. In our series we also demonstrated some of these predictors of LTS. Younger age at the time of diagnosis was associated with significantly longer PFS and OS, as was female sex. When we compared performance status 0 with performance status 1,2 or 3 we also observed significantly longer PFS and OS. This was also observed in those patients undergoing debulking surgery, rather than just biopsy. However, analysis of our sub-cohort of LTS patients did not identify any common demographic and clinical features. However, it is likely that the sub-group is too small to draw any conclusions within the scope of this study.

**MGMT methylation and IDH1 mutation**

We have demonstrated the impact of MGMT methylation on OS and PFS, observing significantly longer median OS and PFS. These data compare favourably with other studies [12, 13, 14, 21, 30, 31, 37, 38] and we have previously shown that a greater extent of methylation is associated with significantly longer OS and PFS [9]. When we consider patients within our cohort with mutation of the IDH1 gene (n=5) we observed significantly longer median OS and PFS. Three of these patients survived longer than 5-years and two of these were alive at follow-up. All five patients had methylated MGMT promoter regions. Our finding of a 5% mutation of IDH1 is comparable with that reported by (Nobusawa *et al*, 2009) who observed a 3.7% mutation rate [23], however a mutation rate of 12% in primary glioblastoma is frequently reported [1]. Nobusawa *et al*, 2009 reported median OS of 27.1 months. Unfortunately, in this study we were unable to comment on those patients with an IDH1 mutation but without MGMT promoter methylation, as there were no IDH1 mutated, MGMT unmethylated cases. This combination whilst observed, is not common.

It has previously been suggested that non-LTS patients who have mutations in IDH1 tend to lack methylation [10]. However, as we have shown, two patients who had mutations in IDH1 but were not LTS patients still had methylation of the MGMT promoter region. In a larger cohort of patients, it would be possible to further investigate both the frequency of both MGMT methylation combined with IDH-1 mutation, and the significance of extent of MGMT promoter methylation in combination with mutations in the IDH1 gene to better understand the impact on survival. However, regardless of these observations, it is still clear that mutations in IDH1 drive longer OS, rather than MGMT methylation, either independently, or in combination with IDH1.

Genomic analysis, laboratory studies, and case series have all demonstrated the strong association between MGMT methylation and IDH1 mutation in that the two often occur together. Furthermore, the phenotype consisting of both modifications is associated with better survival. A recent publication by (Molenaar *et al*, 2014) demonstrated that this 2-gene predictor was an independent prognostic variable compared to either alone [22]. This is consistent with our study; however, their study (Molenaar *et al*, 2014) included recurrent tumours, and so did not consist of a cohort of patients treated with chemoradiotherapy as standard of care.

In contrast to our study where IDH1 mutation was a strong prognostic factor a recent publication suggested that IDH1 mutation is a weak prognostic factor for survival [1]. Interestingly, within the long-term survival cohort two patients had a histological diagnosis of glioblastoma with oligodendroglia component WHO grade IV. While this histological diagnosis is often considered alongside typical GBM tumours, it is likely that it is predictive of a better outcome [35]. While both cases had methylation of the MGMT promoter region, only one had a mutation of the IDH1 gene.

**MGMT methylation and IDH1 mutation correlates with a subgroup of glioblastoma**

Mutations in the IDH genes are tightly linked to the proneural phenotype and secondary glioblastomas, and virtually all tumours with IDH1 mutation are proneural and associated with increased methylation, so-called G-CIMP for glioma CpG island methylation phenotype [24]. Two recent independent studies demonstrated that IDH1 mutation is the cause of the G-CIMP hypermethylation phenotype in diffuse gliomas. Introduction of mutant IDH1 into immortalized primary human astrocytes was sufficient to cause the hypermethylation phenotype [6, 17, 32]. This further supports the notion that glioblastoma is highly heterogeneous and should be considered as multiple pathologies based on genetic and epigenetic mutations and modifications respectively. It would appear that the cohort of patients we have described in this study are likely to represent a proneural phenotype [24, 33]. Regardless of the fact that patients with both IDH1 mutation and MGMT promoter methylation appear to have better overall survival, it is necessary to develop drug therapies specific to this G-CIMP hyper methylation phenotype induced by mutated IDH. While no in vivo agents have yet to be explored, a number of potential strategies have been explored in vitro including suppressing the expression of mutated IDH, or inhibiting the production of 2-HG with metabolites such as oxaloacetate [7]. Despite these advances in the understanding of the epigenetic and genetic heterogeneity, only MGMT methylation so far been has been shown to impact survival, and has limited clinical implications for treatment stratification, other than in the elderly [21]. It is increasingly recognised that the subgroups of glioblastoma from epigenetic and genetic clustering of genome analysis are providing better-defined cohorts that may impact how we treat and study these patients.

**Study limitations**

Although this study represents a uniformly treated cohort of patients there are several limitations. As previously described, the mutation rate of IDH1 is relatively low in primary GBM, compared with secondary GBM. In this series, the mutation rate was only 5% when assessed by immunohistochemistry to identify the most frequent mutation in the IDH1 gene (R132), however, we acknowledge that this is not the only method for identifying such mutations. Therefore, it is likely that some mutations in IDH1 have not been identified within this cohort. With increasing interest in LTS in GBM it is important to consider the QoL associated with increased survival. Unfortunately, we do not have data on the QoL in our sub-cohort of LTS patients, and future studies should include these assessments.

**Conclusions**

While the importance of MGMT methylation is well established, being both prognostic and useful for treatment stratification, we have demonstrated that the combination of MGMTmethylated/IDH1+ve is associated with considerably longer OS and PFS than either alone, in our series of chemoradiotherapy treated Glioblastoma patients. IDH1 status has recently been incorporated into the restructured WHO classification of tumours of the central nervous system, reflecting the importance of this molecular marker on defining Glioblastoma entities. Whilst long-term survival is a highly desirable outcome for both the patient and doctor, we do not yet understand the long-term cognitive function and quality of life for patients surviving with glioblastoma and this warrants further investigation to ensure that these outcomes are acceptable to these patients.

**Declarations**

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Conflict of Interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

For this type of study formal consent is not required.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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**Appendix**

Figure 1 – Summary of case selection. 100 cases were included within the study, from an original cohort of 115 patients. 3 genetic subgroups were identified. Completion of treatment frequency is summarised for each subgroup. DNS = did not start, DNF = did not finish, ADJ = adjuvant, RT = radiotherapy, TZM = Temozolomide, MGMT = O6-Methylguanine-DNA methyltransferase, IDH1 = Isocitrate dehydrogenase 1 (+ = mutant, - = wild-type).

Table 1 -Demographic & clinical characteristics of the patients at baseline, genetic analysis, and intensity of treatment. PS = performance status, Dx = diagnosis, Rt = radiotherapy, GB = Glioblastoma.

Table 2 - Analysis of variables affecting OS, PFS, and 2, 3 and 5-year survival. Superscript denotes statistical test, 1 = log-rank, 2 = breslow. N.S = not significant.

Table 3 - Long-term survival cohort. All patients have survived at least 3-years (n=9), the bottom 6 patients have survived at least 5-years (n=6). PS = performance status, RT = radiotherapy, GB = Glioblastoma, GBO = Glioblastoma + oligodendroglia component.