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Association of plasma levetiracetam concentration, MGMT methylation and sex with survival of chemoradiotherapy-treated glioblastoma patients

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Abstract

Glioblastoma multiforme (GBM) is an aggressive brain tumor, often occurring with seizures managed with antiepileptic drugs, such as levetiracetam (LEV). This study is aimed at associating progression-free survival (PFS) and overall survival (OS) of GBM patients with LEV plasma concentration, MGMT promoter methylation, and sex

In this retrospective, non-interventional, and explorative clinical study, GBM patients underwent surgery and/or radiotherapy and received LEV during adjuvant temozolomide (TMZ) treatment. A high-performance liquid chromatography with UV-detection was used for therapeutic drug monitoring of LEV plasma concentrations. Follow-up average drug concentration was related to patients' clinical characteristics and outcomes.

Forty patients (42.5% female; mean age=54.73±11.70 years) were included, and GBM MGMT methylation status was assessed. All were treated with adjuvant TMZ, and LEV for seizure control. Patients harboring methylated MGMT promoter showed a longer median PFS (460 *vs.* 275 days, log-rank p<0.001). The beneficial effect of MGMT promoter methylation was more evident for females (p<0.001) and in patients with LEV concentration ≤20.6 µg/mL (562 days vs. 274.5 days, p=0.032). Female patients also showed longer OS (1220 *vs.* 574 days, p=0.03).

Also, higher LEV concentration (>20.6 µg/mL) synergized with MGMT promoter methylation by extending the OS (1014 vs. 406 days of patients with no methylation and low LEV average concentration, p=0.021). Beneficial effect of higher LEV plasma levels was more evident in males (p=0.024).

Plasma concentrations of LEV may support better outcomes for chemoradiotherapy when other positive prognostic factors are lacking and may promote overall survival by synergizing with MGMT promoter methylation and male sex.

Introduction

Although glioblastomas (GBMs) remain rare conditions (23.03 cases per 100.000 inhabitants/yearly [1]), sites, histopathological characteristics, and biological behaviors make them accountable for high morbidity and mortality [2] in adult patients [3], adolescents, and children [4].

Seizures are common manifestations in GBM patients and significantly impact their quality of life (QoL) [5,6]. Approximately 40-45% of GBM patients present epilepsy at onset,

often as a secondary generalized seizure that may precede radiologic evidence [7], while 15-20% develop seizures later [5,8] .

Less attention has been made to how epilepsy and its treatment can affect tumor burden, progression, outcome, and QoL [9]. However, evidence revealed that many brain tumor-related epilepsies (BTREs) mechanisms could also contribute to tumor growth and invasion [10]. Further, preclinical studies suggest an antineoplastic activity for antiepileptic drugs (AEDs) [10]. Recent *in vitro* experiments indicate levetiracetam (LEV) as a transcription inhibitor of the O^6-methylguanine-DNA methyltransferase repair protein gene (MGMT) [11,12]. LEV may be crucial to sensitize GBM cells to temozolomide-induced damage by counteracting MGMT repair activity [13]. As proof of this, hypermethylation of the CpG island halts transcription of the MGMT gene, compromising its efficiency to repair alkylation of O^6-methylguanine, resulting in either low levels or complete loss of MGMT expression and correlating with a survival benefit in glioma patients [14].

This retrospective, non-interventional and explorative clinical study is aimed at associating progression-free survival (PFS) and overall survival (OS) of forty GBM patients with levetiracetam plasma concentration, MGMT methylation, and sex to test the hypothesis that MGMT inhibition may be associated with certain LEV plasma concentrations, supporting a pharmacodynamic role in adjuvant temozolomide (TMZ) plus radiotherapy effect.

Patients and Methods

Patients and data collection

The cohort consisted of GBM patients who attended the University Hospital of Pisa between 2011 and 2018 to be treated according to the STUPP protocol [15] and receive LEV (see below).

According to clinical practice, postoperative magnetic resonance imaging (MRI) was performed. Therefore, patients received radiation therapy at a total dose of 60 Gy, with daily fractions of 2 Gy. TMZ was administered orally, at a dose of 75 mg/m^2 daily when concurrently

with radiotherapy, and 150-200 mg/m^2/day for five days, every 28 days thereafter. The chemoradiation time-to-treatment (TT) recorded the time elapsed from the GBM surgical resection to the first chemoradiotherapy. Follow-up MRI was performed just before the first chemoradiation - serving as a baseline - and every 3–6 months of treatment.

Clinical parameters, such as complete (CR), partial response (PR), stable disease (SD), and progressive disease (PD), were defined following RANO criteria [16]. Besides, during chemoradiotherapy and adjuvant chemotherapy, treatment-related adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events ver. 5.0 [17].

All patients also received LEV to prevent or manage BTREs. LEV was used as prophylaxis at 500 mg twice a day, regardless of seizure history. If a seizure event occurred, 500 mg/day of LEV was added (up to 3,000 mg/day). Also, due to relapsing LEV-resistant seizures, one patient took oxcarbazepine as an add-on treatment and another lamotrigine plus pregabalin.

The main inclusion criteria were as follows: availability of data regarding tumor histotype, isocitrate dehydrogenase-1 (IDH-1) gene mutation and MGMT promoter methylation status, surgery and chemoradiation timeline, a minimum of 1 completed chemo-radiotherapy cycle administered, the efficacy of first-line treatment and availability of blood sample.

Levetiracetam concentrations of samples were determined using high-performance liquid chromatography (HPLC) with UV detection. Two ml of blood were drawn and stored in EDTA tubes approximately 12 hours after the evening dose and after at least one week of treatment with LEV without dosage modification (steady-state conditions). Red blood cells and plasma were separated by centrifugation at 1800 g for 10 minutes and stored at –20 °C until analyzed. Plasma samples were prepared according to Chromsystems ® Kit (Chromsystems Instruments & Chemicals GmbH, Gräfelfing/Munich, Germany) procedure and instrumental parameters set as per the manufacturer's instructions [18]. The limit of quantification (LoQ) of the LEV HPLC assay was 0.5 mg/L, with linearity up to 1000 mg/L. The recovery from the plasma (our matrix) was 90%, and the

intra-assay coefficients of variation % (CV%) was <1.3%, whereas the inter-assay CV% was <3.7%. The analysis time was 7 minutes per sample.

The study was approved by the Comitato Etico di Area Vasta Nord Ovest (CEAVNO_Bocci_29-04-2020) and conducted according to the Helsinki Declaration's principles. All patients gave their signed informed consent before blood collection data analysis.

Statistical analysis

Categorical variables such as sex, Karnofsky performance status (KPS, i.e., score < or ≥ 70), IDH-1 mutation, and MGMT methylation status, as well as patient clinical outcome, were described by absolute and relative frequencies; quantitative factors as age, chemoradiation time-to-treatment (TT) after GBM resection and average LEV plasma concentration by mean±standard deviation (STD). For each patient, the average LEV plasma concentration referred to the mean plasma concentrations of the drug over the whole follow-up period.

Overall survival (OS) was defined as the time from the first surgery to death from any cause. Surrogate progression-free survival (PFS) was defined as the time from the first surgery to PD (as confirmed by a radiological study) or death from any cause. OS and PFS curves were illustrated using Kaplan–Meier analyses and log-rank tests. In addition, Cox proportional hazard models evaluated hazard ratio (HR) and 95% confidence interval (CI). Prior to univariate and multivariate analysis, the Z-score test assessed the population proportions for categorical variables to exclude those that showed a statistically significant difference.

Differences were considered significant at $p<0.05$. A post-hoc Bonferroni correction was assumed for multiple testing. Statistical analysis was performed using the open-source statistical language R (R Foundation for Statistical Computing, Wien, Austria) and the free and open statistical software program JAMOVI® (Version 1.1.9; retrieved from https://www.jamovi.org).

Results

Baseline characteristics for the study population are reported in **Table 1**.

Forty subjects with histologically proven GBM were included in this study (42.5% female). Their age ranged from 30 to 76 years (mean age=54.7±11.7 years). Everyone showed KPS≥70 at diagnosis. The IDH-1 mutation was present in 10% of tumors. Further, considering 9% as the cut-off value [19], nearly half of patients (47.5%) tested positive for methylation of the MGMT gene promoter (MGMT+).

All patients received postoperative treatment with radiotherapy plus TMZ after debulking surgery (mean TT=1.9±1. months), except for three patients who immediately underwent the concurrent chemoradiation regimen. For the entire cohort, the mean LEV concentration averages recorded throughout the routine therapeutic drug monitoring (TDM) was 20.6±11.3 µg/mL (**Figure 1**), while the median OS and PFS were 699 (95% CI 566–1090) and 401 (95% CI 307–483) days, respectively. Tumor progression was observed in thirty-six (90%) patients by the time of analysis, and twenty-seven (67.5%) of them died by the time of analysis. Overall, the median follow-up was 1102 days (95% CI 773-3123).

Patients harboring the methylated MGMT promoter at baseline of the STUPP protocol had significantly longer PFS (460 vs. 275 days, log-rank p<0.001; **Figure 2A**), and the univariate analyses confirmed the role of methylation as a predictor for a better median PFS (HR 0.29, 95% CI 0.13–0.65, p=0.002; **Table 2**).

The multiple comparison procedure (Bonferroni post-hoc test) also revealed that this beneficial effect was more enunciated within females (p<0.001). Although MGMT- women had the worst prognosis, with 7.13 times risk of rapid progression than MGMT+ men (95% CI 2.46-20.67, p<0.001) and 3.2 times risk than MGMT- men (95% CI 1.05-9.79, p=0.041), there were no more differences between MGMT+ women and MGMT+ men (HR 0.77, 95% CI 0.21-2.80, p=0.695) (**Figure 2B**). In addition, the median PFS was 453 days for women harboring the methylation and 216 days for women who did not (log-rank p=0.026; HR 0.11, 95% CI 0.02–0.48, p=0.003) , while

MGMT+ men did not show a significantly better PFS than MGMT- ones (460 vs. 379 days, log-rank p=0.071; HR 0.45, 95% CI 0.17–1.18, p=0.104) (**Figure 2B**).

Methylated MGMT also seemed to offer an advantage in median PFS within the subgroup of patients with an average concentration of LEV throughout the TDM ≤ 20.6 μg/mL (562 days vs. 274.5 days, log-rank p=0.032; HR 0.23, 95% CI 0.08–0.63, p=0.004), but not for those holding higher values (**Figure 2C**). The cut-off value was calculated as the mean of the average LEV concentrations recorded during the TDM.

In a condition of low plasma LEV (≤ 20.6 μg/mL), the MGMT methylation benefit was evident in male patients, who lived longer than both women (562 vs. 167 days, log-rank p=0.003) and men (562 vs. 307 days, log-rank p=0.02) without methylation (**Figure 3A** and **B**, respectively).

Concerning the OS, no significant association appeared with the MGMT promoter methylation (761 vs. 606, log-rank p=0.172; HR 0.55, 95% CI 0.24–1.29, p=0.173), whereas women survived significantly longer than men (median OS 1220 vs. 574 days, log-rank p=0.03; HR 0.39, 95% CI 0.16–0.94, p=0.037) (**Figure 4A** and **Table 2**).

Stratified analyses confirmed MGMT methylation to be unessential for OS compared to sex (**Figure 4B**), whereas high LEV concentrations appeared to affect the gender-related effect (**Figure 4C**). In detail, women with low plasma LEV lived longer than comparable men (1220 vs. 566 days, log-rank p = 0.036; HR 0.28, 95% CI 0.08-0.93, p = 0.037), and OS was magnified when females held an average LEV concentration above 20.6 (1836 vs. 566 days, log-rank p = 0.046; HR 0.21, 95% CI 0.05-0.81, p = 0.024). Contrariwise, no significant differences emerged either comparing males treated with high LEV concentrations or within gender groups.

Survival analyses also revealed that methylated patients with higher average LEV plasma concentration (>20.6 μg/mL) had a longer OS than their counterpart with no methylation and low LEV average concentration (1014 vs. 406 days, log-rank p=0.021; HR 0.31, 95% CI 0.09-0.91, p = 0.044) (**Figure 5A**). The effect was more evident in male patients. MGMT+ men with high plasma LEV lived twice as long as those with low LEV concentrations, whether they were methylated

(1090 vs. 574 days, log-rank p=0.027; HR 0.10, 95% CI 0.01-0.93, p = 0.043) or not (1090 vs. 406 days, log-rank p=0.011; HR 0.08, 95% CI 0.01-0.73, p = 0.025) (**Figure 5B**). In condition of low plasma LEV, no differences emerged between MGMT+ and MGMT- male patients.

Finally, multivariate analyses (estimated from 600 bootstrapped replications of the data, concordance index=0.97) not only strengthened the role of the methylated MGMT promoter as an independent prognostic variable for PFS (HR 0.41, 95% CI 0.17–0.98, p=0.044) but also uncovered a significant influence of average LEV plasma concentration on OS (HR 0.94, 95% CI 0.90–0.98, p=0.026) (**Table 2**). At the same time, age was also negatively correlated with OS (HR 1.07, 95% CI 1.01–1.13, p=0.020) (**Table 2**). Variables that did not pass the Z-score test were not considered.

Discussion

To our knowledge, this is the first study evaluating the role of LEV plasma levels in predicting time-to-event outcomes, along with other known prognostic factors such as MGMT methylated status and sex. MGMT methylation provides an advantage in treatment response by sensitizing tumor cells to the action of TMZ [14]. TMZ is an alkylating agent prodrug that supplies a methyl group to the purine bases of DNA (O^6-guanine; N^7-guanine, and N^3-adenine). The methyl group of O^6-methylguanine can be removed by MGMT (direct repair) in tumors expressing this protein. Thus, silencing MGMT before chemotherapy with alkylating agent prevents O^6-methylguanine repair and sensitizes the tumor to TMZ [14].

Sex also appears to play a prognostic role. For decades, it has been known that men are more likely than women to develop GBM [20], and to date, women also appear to respond better than men to standard therapy [21]. Reasons are still unclear, although a growing body of evidence documents a relevant role of sex in cancer biology and clinical response. As an example, researchers at the Washington University School of Medicine have found that different molecular profiles in cancers of men and women are related to differences in survival [22]. Their analyses showed the association between the cell-cycle gene signature and longer survival in men and the

association between the integrin gene signature and longer survival in women. Similar observations on sex-related differences have also been published for the MGMT promoter methylation and the IDH-1/2 mutation [22–24].

Our explorative and retrospective results are consistent with the studies above. The survival benefit of methylated MGMT was clear in PFS for the overall population and more evident in females. Moreover, women survived longer than men.

Simultaneously, recent observations have shown that prophylactic antiepileptic drugs during adjuvant chemotherapy - such as LEV - can affect the outcome of GBM patients, significantly improving median PFS compared to patients who did not receive the AED [25–27]. Nevertheless, these studies did not provide information about the duration of AEDs use, nor about their management. Indeed, opinions on the antitumor effect of LEV are controversial, and other papers [28,29] have demonstrated that no better outcomes derive from using LEV.

Back in 2010, Bobustuc and colleagues reported that LEV enhances histone deacetylases (HDACs) transcription and inducts the HDAC1/mSin3A corepressor, which in turn inhibits the MGMT promoter region and results in greater efficacy of TMZ anticancer effects [12]. More recently, another *in vitro* study confirmed this biological underpinning and suggested that LEV increases the effects of TMZ on GBM stem cells by directly activating antiproliferative and proapoptotic pathways [30].

Although preclinical data suggests a MGMT-mediated effect, it is still unclear which plasma concentrations of LEV can improve the survival of patients with GBM. We hypothesized that both LEV pharmacodynamics and pharmacokinetics might contribute to differences among patients harboring different molecular and clinical signatures.

Our results suggest that maintaining higher LEV plasma concentrations (>20.6 µg/mL) makes up for the lack of benefit of both sex and MGMT methylation, canceling the significant survival disparities between male and female patients, as well as between MGMT+ and MGMT-

ones. By contrast, disparities remain evident in the subgroup of patients with low average LEV plasma levels during the therapeutic drug monitoring.

Interestingly, although methylated MGMT predicted response to treatment, offering a PFS benefit (especially in females), it had no OS prognostic value for patients undergoing chemoradiation. Moreover, no survival significant difference emerged in the use of levetiracetam for unselected patients either. Data are not surprising since other studies and meta-analyses have already confirmed the absence of conclusive solid statistical evidence for both MGMT promoter methylation [31–35] and the use of LEV [25–29]. Nevertheless, the multivariate model showed that using LEV had a significant effect on survival when synergistically analyzed with MGMT promoter methylation status and sex. Survival analyses revealed that methylated patients treated with higher LEV concentrations lived longer than their counterparts with no methylation and low LEV average concentration, who lived off more than non-methylated patients whose LEV concentrations did not reach the threshold. Differences were confirmed for males.

LEV is a drug of choice for patients with BTREs [36] as it has few pharmacological interactions and low toxicity profiles. Therefore, routine monitoring of LEV is not recommended for all patients [37]. However, our results suggested the importance of TDM in designated patients.

Although the overall long-term clinical benefit of LEV is disappointing due to its lack of efficacy in unselected patients, a tailored approach can improve outcomes. Therapeutic reference for LEV ranges from 10 to 40 μg/mL, as reported by the consensus guidelines for TDM in neuropsychopharmacology [38]. However, this paper reports that an adopted LEV therapeutic range of at least 20-40 μg/mL could aid in decision making and better management of GBM [39] when other positive prognostic factors are lacking. Not only that, but it may also be beneficial to maintain a high individual therapeutic range in MGMT+ males in order to improve their survival.

Obvious limitations stem from the retrospective and explorative nature of our study, including the small sample size due to the low incidence of the disease. All patients had a KPS>70 and just 4 out of 40 harbored the mutated IDH-1; moreover, the age and timing of

chemoradiotherapy could change. Thus, a proper statistical approach was used to avoid type 1 errors. For instance, we conducted a multivariate analysis also including well-recognized prognostic factors such as age and TT after GBM resection.

Also, since the volume of residual disease is a predictor of outcome in adult patients with GBM [40] and since the depth of surgery is inevitably limited by brain anatomy [41], we selected patients who had undergone debulking surgery, and who had the minimal residual disease (subclinical, albeit visible) on MRI performed just before the first chemoradiation (which served as a baseline to mitigate selection bias). The bias is unavoidable [15]. A radiomic study could be suggestive to investigate the peritumoral niche, quantify the residual tumor volume obtained from serial MRI samples, and correlate them with molecular data to finally identify patients at risk of progression during treatment (as we have proposed for NSCLC [42]).

The seizure frequency after the surgery and during chemoradiotherapy was not thoroughly investigated. Nevertheless, considering that almost all patients received LEV monotherapy, the seizure incidence, and severity of patients included in the present study were likely low.

LEV Posology was defined clinically, aiming for maximum seizure control. However, we have not estimated the LEV concentration/dose ratio as the drug has simple - first-order - kinetics (after a single dose and during long-term administration) and does not suffer from other confounding factors when in monotherapy. LEV is wholly and rapidly absorbed orally, reaching maximum plasma levels within 1 hour after intake [43]. The protein binding degree is <10% [43], and excretion is largely through kidneys [43]. Previous studies confirmed that concomitant AEDs might reduce the LEV concentration/dose ratio [44,45], while LEV and TMZ did not affect each other's kinetics, although they share a primary renal excretion [46]. Again, excluding two, all patients received LEV monotherapy and did not suffer from other confounders, such as impaired kidney function. Further, our analyses were performed on repeated measurements for LEV steady-state concentrations, which are normalized data free from other influences.

Large prospective controlled studies are needed to assess the generalizability of the findings.

In conclusion, in this retrospective and explorative analysis, high plasma levels of LEV, MGMT promoter methylation, and female sex were associated with OS and PFS extension in patients with GBM treated with TMZ and radiotherapy. Our results suggest that the pharmacokinetic monitoring of LEV in patients with GBM should be carefully considered and should deal with patient sex and tumor molecular signature, because it may affect survival. In the era of personalized medicine, the identification of the most appropriate treatment and the proper diagnostic workflow, including both the molecular tests and the therapeutic drug monitoring, remains a key and necessary step also in rare diseases such as GBM.

Authors' contributions

FC and GB designed the research study. NG, FSG, FP performed the clinical research and collected the samples. FC, GL, PO, MB conducted the laboratory experiments. FC, ADP, RD analyzed the data. FC, and GB wrote the manuscript. All authors read and approve the final manuscript.

Declaration of competing interests

The authors declare that they have no competing interests

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Figure Legends

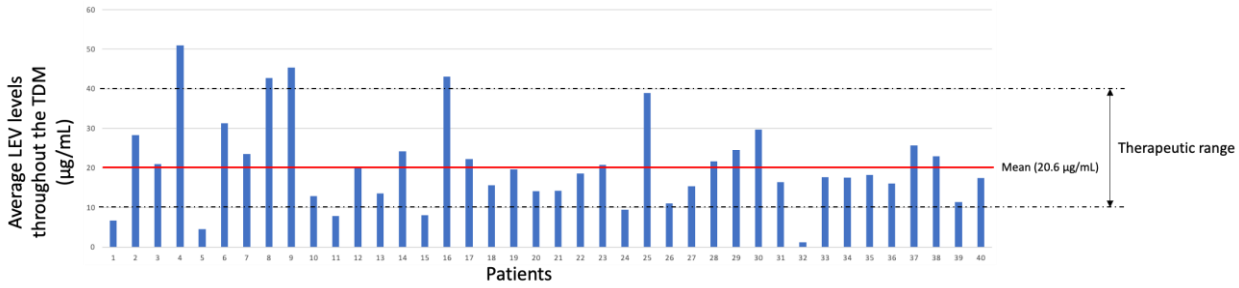

Figure 1. LEV blood levels for 40 patients undergoing TDM. Blue bars represent the average LEV trough plasma concentrations (y-axis) counted for each patient's TDM (x-axis). The red line describes the mean of the LEV average concentrations throughout the TDM. *LEV*, levetiracetam; *TDM*, therapeutic drug monitoring.

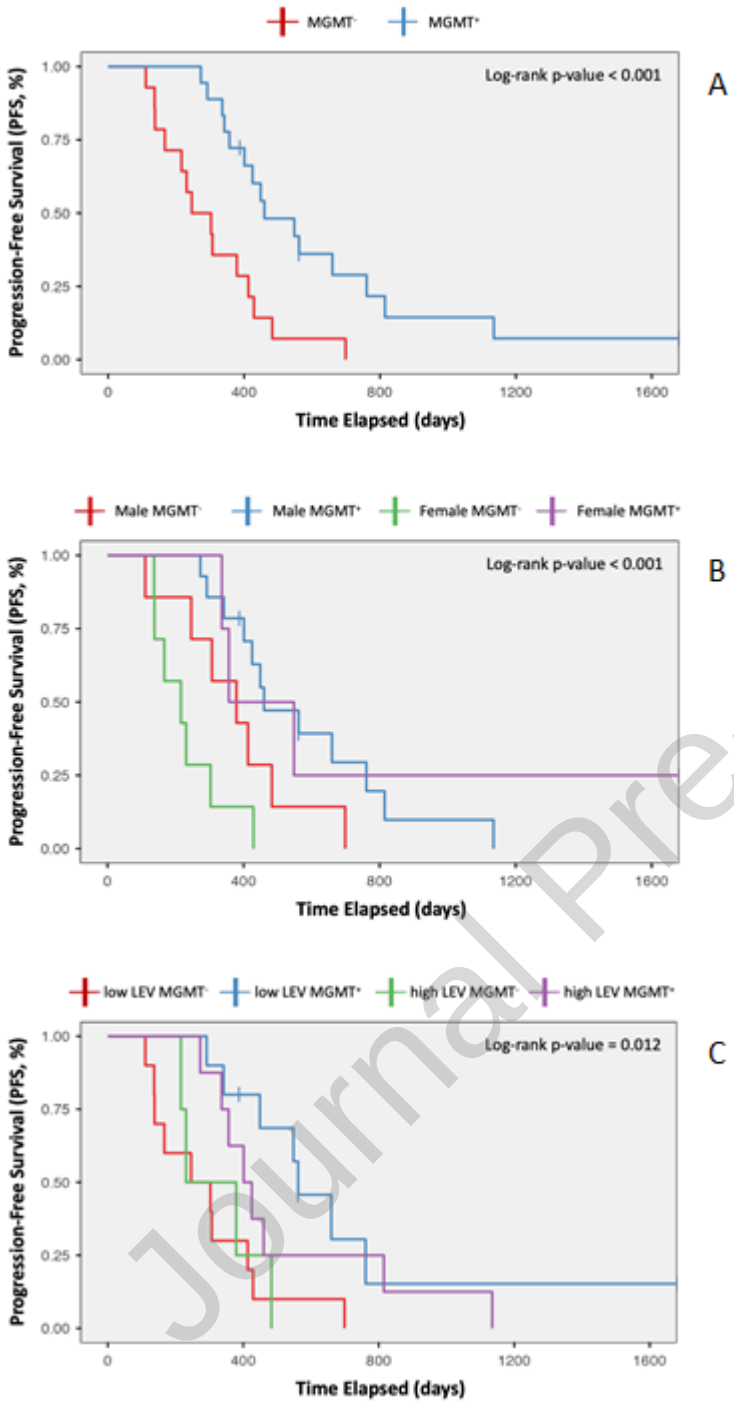

Figure 2. PFS of GBM patients undergoing STUPP protocol, stratified by MGMT methylation status **(A)**, as well as by MGMT methylation status with respect to sex (**B**) and LEV average concentration throughout the TDM (**C**). Crosses correspond to the censored patients. *PFS*, Progression-Free Survival; *MGMT*, O6-methylguanine-DNA methyltransferase; *MGMT*$^{+}$, methylated MGMT promoter; *MGMT*$^{-}$, non-methylated MGMT promoter; *GBM*, glioblastoma;

LEV, levetiracetam; *high LEV*, >20.6 µg/mL; *low LEV*, ≤20.6 µg/mL; *TDM*, therapeutic drug monitoring.

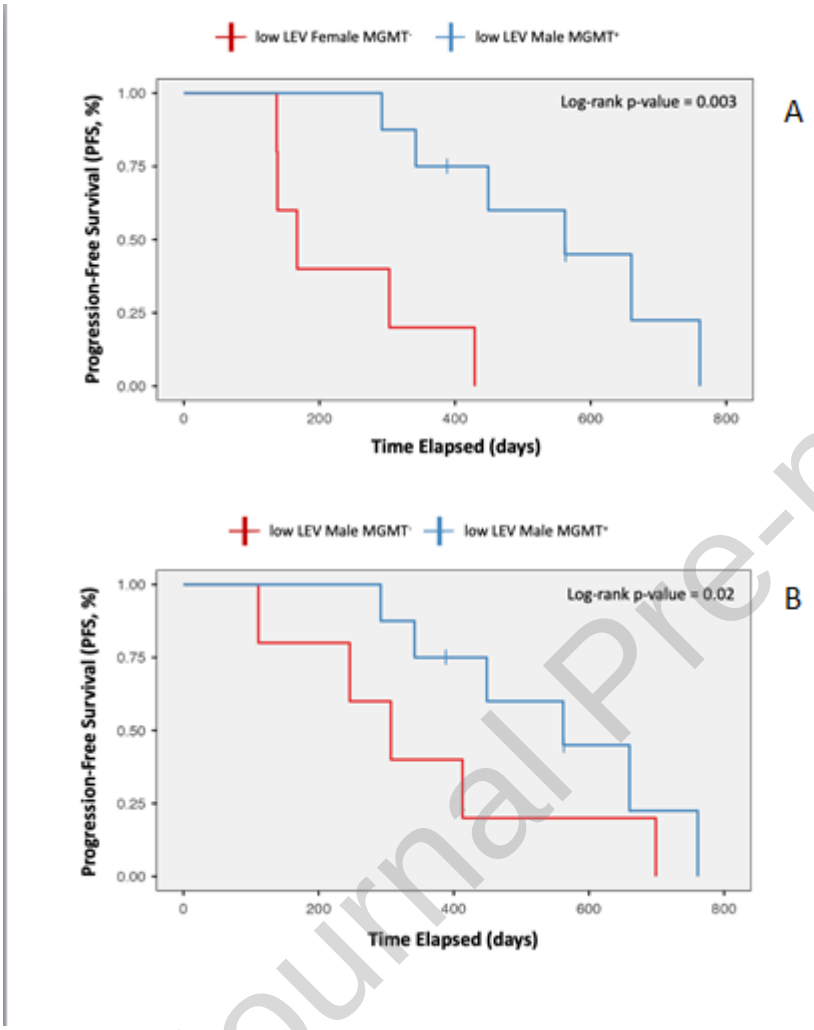

Figure 3. PFS differences between **(A)** and within **(B)** genders in a subgroup of glioblastoma patients undergoing STUPP protocol and with low LEV average concentration throughout the TDM, according to MGMT methylation status. *PFS*, Progression-Free Survival; *MGMT*, O6-methylguanine-DNA methyltransferase; *MGMT*$^{+}$, methylated MGMT promoter; *MGMT*$^{-}$, non-methylated MGMT promoter; *LEV*, levetiracetam; *low LEV*, ≤20.6 µg/mL; *TDM*, therapeutic drug monitoring.

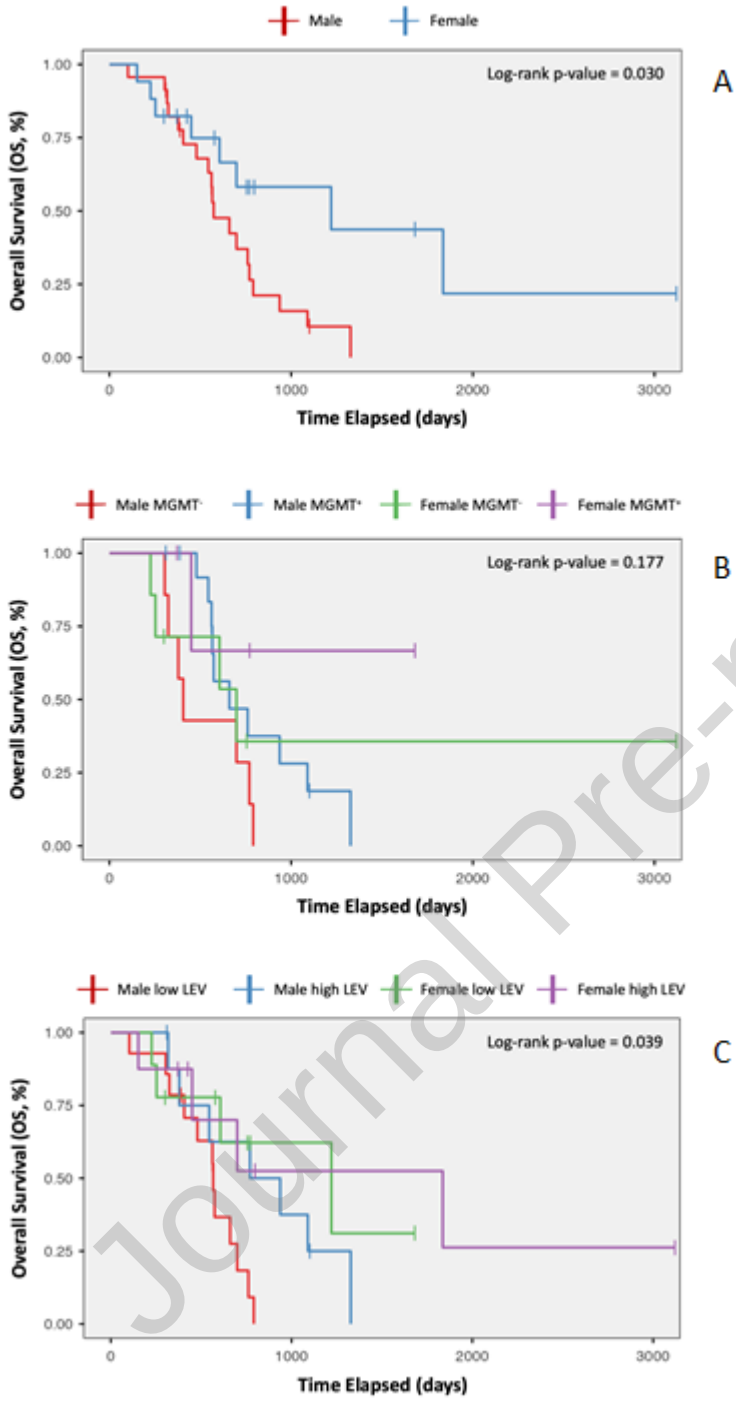

Figure 4. OS of GBM patients undergoing STUPP protocol, stratified as per sex **(A)**, as well as per sex with respect to MGMT methylation status (**B**) and LEV average concentration throughout the TDM (**C**). Crosses correspond to the censored patients. *OS*, Overall Survival; *MGMT*, O6-methylguanine-DNA methyltransferase; *MGMT*$^{+}$, methylated MGMT promoter; *MGMT*$^{-}$, non-methylated MGMT promoter; *GBM*, glioblastoma; *LEV*, levetiracetam; *high LEV*, >20.6 μg/mL; *low LEV*, ≤20.6 μg/mL; *TDM*, therapeutic drug monitoring.

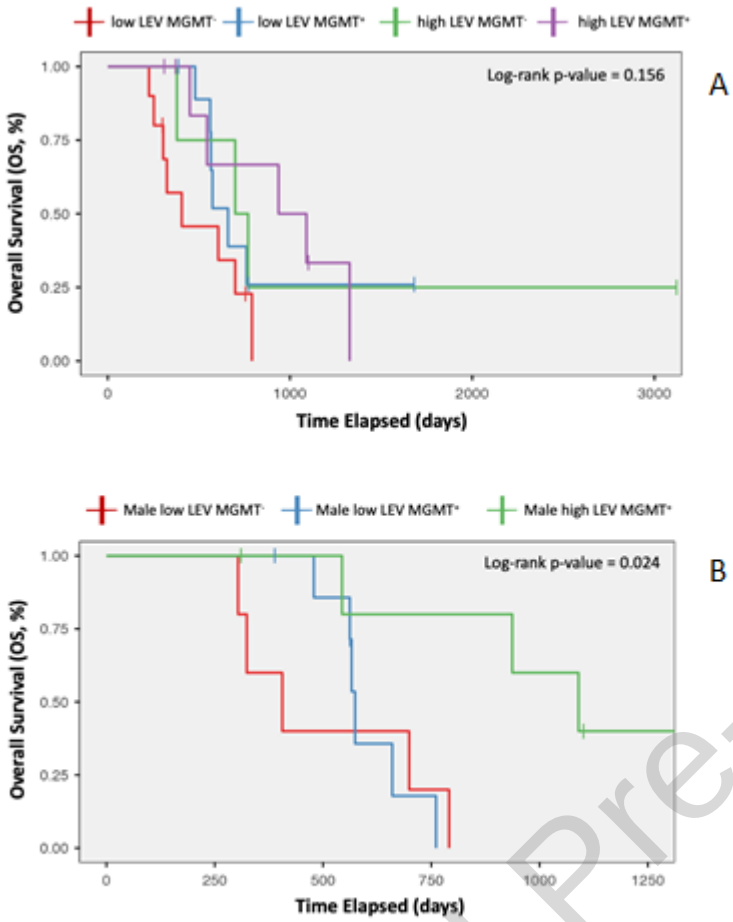

Figure 5. OS of GBM patients undergoing STUPP protocol, stratified as per MGMT methylation status with respect to LEV average concentration throughout the TDM **(A)**, with a focus on males **(B)**. Crosses correspond to the censored patients. *OS*, Overall Survival; *MGMT*, O6-methylguanine-DNA methyltransferase; *MGMT*$^{+}$, methylated MGMT promoter; *MGMT*$^{-}$, non-methylated MGMT promoter; *LEV*, levetiracetam; *high LEV*, >20.6 µg/mL; *low LEV*, ≤20.6 µg/mL; *TDM*, therapeutic drug monitoring.

Table 1. Clinical Characteristics of Patients

	Overall (N=40)
Sex, %	
Female	17 (42.5%)

Male	23 (57.5%)
Age, years	
Mean (STD)	54.7 (11.7)
Range	30.0 - 76.0
Karnofsky Performance Status, %	
>70	40 (100%)
IDH-1 mutation, %	
Missing data	8
Mutated	4 (10%)
Wild Type	28 (70%)
MGMT promoter methylation, %	
Missing data	8
No	14 (35%)
Yes	18 (45%)
Chemoradiation TT after GBM resection, months	
Missing data	3
Mean (STD)	1.9 (1.0)
Range	0.0 - 5.0
Seizure Event	
Missing data	28
Focal Seizure	5 (12.5%)
Secondarily Generalized Seizure	7 (17.5%)
Anatomical location	
Missing data	31
Right Temporal	2 (5%)
Right Fronto-Temporal	1 (2.5%)
Right Fronto-Temporo-Parietal	1 (2.5%)
Right Parietal	1 (2.5%)
Left Frontal	1 (2.5%)
LeftTemporo-Parietal	1 (2.5%)
Left Parietal	1 (2.5%)
Others	1 (2.5%)
LEV average concentration throughout the TDM, µg/mL	
Mean (STD)	20.6 (11.3)
Range	1.2 – 51.0
PD, %	
No	4 (10%)
Yes	36 (90%)

PFS, days	
Median (95% CI)	401.0 (307.0 - 483.0)
Deceased, %	
No	13 (32.5%)
Yes	27 (67.5%)
OS, days	
Median (95% CI)	699.0 (566.0 – 1090.0)

N, number of patients; STD, standard deviation; IDH-1, isocitrate dehydrogenase-1; MGMT, O^6-methylguanine-DNA methyltransferase; TT, time-to-treatment; GBM, glioblastoma; LEV, levetiracetam; TDM, therapeutic drug monitoring; PD, progressive disease; PFS, progression-free survival; OS, overall survival; CI, confidence interval.

Table 2. Univariate and Multivariate analyses of prognostic factors for both PFS and OS.

	PFS				OS			
	Univariate		**Multivariate**		**Univariate**		**Multivariate**	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Age	1.01 (0.98-1.05)	0.353	1.00 (0.96-1.05)	0.878	1.05 (1.01-1.09)	*0.021**	1.07 (1.01-1.13)	*0.020**
Female sex	0.97 (0.49-1.94)	0.935	1.38 (0.57-3.33)	0.476	0.39 (0.16-0.94)	*0.037**	0.48 (0.15-1.53)	0.216
MGMT promoter methylation	0.29 (0.13-0.65)	*0.002**	0.41 (0.17-0.98)	*0.044**	0.55 (0.24-1.29)	0.173	0.45(0.15-1.36)	0.157
Chemoradiation TT after GBM resection	0.61 (0.40-0.93)	*0.022**	0.63 (0.39-1.03)	0.064	0.71 (0.47-1.09)	0.119	0.78 (0.47-1.29)	0.329
LEV average concentration throughout the	0.99 (0.96-	0.623	0.99 (0.95-	0.484	0.97 (0.94-	0.097	0.94 (0.90-0.98)	*0.026**

TDM	1.04)	1.04)	1.01)

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; MGMT, O^6-methylguanine-DNA methyltransferase; TT, time-to-treatment; GBM, glioblastoma; LEV, levetiracetam; TDM, therapeutic drug monitoring.

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CRediT authorship contribution statement

Federico Cucchiara and Guido Bocci designed the research study.

Noemi Giannini, Filippo Sean Giorgi, Francesco Pasqualetti performed the clinical research and collected the samples.

Federico Cucchiara, Giacomo Luci, Paola Orlandi, Marta banchi conducted the laboratory experiments.

Federico Cucchiara, Antonello Di Paolo, Romano Danesi analyzed the data.

Federico Cucchiara and Guido Bocci wrote the manuscript.

All authors read and approve the final manuscript.

Graphical abstract