Effect of Levetiracetam use duration on overall survival of isocitrate dehydrogenase wildtype glioblastoma in adults: an observational study

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Marc ZANELLO: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data

Number of characters in title: 140

Abstract Word count: 349

Word count of main text: 3449

References: 39

Figures: 1

Tables: 4

Statistical Analysis performed by: Johan PALLUD, MD, PhD

Search Terms: [14] All Clinical Neurology, [60] All Epilepsy/Seizures, [61] Antiepileptic drugs, [214] Primary brain tumor

Acknowledgements: N/A

Study Funding: The authors report no targeted funding

Disclosures: JP reports no disclosures relevant to the manuscript, GH reports no disclosures relevant to the manuscript, ED reports no disclosures relevant to the manuscript, SP reports no disclosures relevant to the manuscript, AM reports no disclosures relevant to the manuscript, MG reports no disclosures relevant to the manuscript, EG reports no disclosures relevant to the manuscript, FD reports no disclosures relevant to the manuscript, PV reports no disclosures relevant to the manuscript, CO reports no disclosures relevant to the manuscript, FC reports no disclosures relevant to the manuscript, AR reports no disclosures relevant to the manuscript, MZ reports no disclosures relevant to the manuscript.

Effect of Levetiracetam use duration on overall survival of *isocitrate dehydrogenase* wildtype glioblastoma in adults: an observational study

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Manuscript word count: 3445

Abstract word count: 349

Number of figures : 1

Number of color figures : 0

Number of tables : 4

Number of supplementary figures: 0

Number of supplementary tables: 0

Abbreviation list

HR: Hazard Ratio

IDH: Isocitrate Dehydrogenase

MGMT: O6-Methylguanine-DNA methyltransferase

WHO: World Health Organization

Funding Sources

None.

Conflicts of Interest

None.

Statistical Analysis conducted by Pr. Johan Pallud, MD, PhD.

Search Terms

Glioblastoma

IDH-wildtype

Levetiracetam

O6-methylguanine-DNA methyltransferase

Overall survival

Temozolomide

Author contribution

JP, ED, AM, FD, PV, AR, and MZ did the data collection.

JP, GH, AR, and MZ did the data analysis.

JP, GH, ED, SP, AM, MG, EG, FD, PV, CO, FC, AR, and MZ did the data interpretation.

JP, GH, ED, SP, AM, MG, EG, FD, PV, CO, FC, AR, and MZ wrote the report.

JP, GH, ED, SP, AM, MG, EG, FD, PV, CO, FC, AR, and MZ reviewed and approved the paper.

Author Disclosures

JP reports no disclosures relevant to the manuscript.

GH reports no disclosures relevant to the manuscript.

ED reports no disclosures relevant to the manuscript.

SP reports no disclosures relevant to the manuscript.

AM reports no disclosures relevant to the manuscript.

MG reports no disclosures relevant to the manuscript.

EG reports no disclosures relevant to the manuscript.

FD reports no disclosures relevant to the manuscript.

PV reports no disclosures relevant to the manuscript.

CO reports no disclosures relevant to the manuscript.

FC reports no disclosures relevant to the manuscript.

AR reports no disclosures relevant to the manuscript.

MZ reports no disclosures relevant to the manuscript.

Abstract

Objectives. The association between Levetiracetam and survival of *Isocitrate Dehydrogenase (IDH)* wildtype glioblastomas is controversial. We investigated whether the duration of Levetiracetam use during the standard chemoradiation protocol impacts overall survival of *IDH*-wildtype glioblastoma patients.

Methods. Observational single-institution cohort study (2010-2018). Inclusion criteria were: 1) patients ≥18 years old; 2) newly diagnosed supratentorial tumor; 3) histomolecular diagnosis of *IDH*-wildtype glioblastoma; 4) standard chemoradiation protocol. To assess the survival benefit of Levetiracetam use during the standard chemoradiation protocol (whole duration, part time, and never subgroups), a Cox proportional hazard model was constructed. We performed a case-matched analysis (1:1) between patients with Levetiracetam use during the whole duration of the standard chemoradiation protocol and patients with Levetiracetam use part time or never according to the following criteria: sex, age, epileptic seizures at diagnosis, RTOG-RPA class, tumor location, preoperative volume, extent of resection, and O6-Methylguanine-DNA methyltransferase promoter methylation status. Patients with unavailable O6-Methylguanine-DNA methyltransferase promoter methylation status (48.5%) were excluded.

Results. 460 patients were included. The median overall survival was longer in the 116 patients with Levetiracetam use during the whole duration of the standard chemoradiation protocol (21.0 months; 95%CI, 17.2-24.0) than in the 126 patients with part time Levetiracetam use (16.8 months; 95%CI, 12.4-19.0], and in the 218 patients who never received Levetiracetam (16.0 months; 95%CI, 15.5-19.4; p=0.027). Levetiracetam use during the whole duration of the standard chemoradiation protocol (adjusted Hazard Ratio (aHR) 0.69; 95%CI, 0.52-0.93; p=0.014), O6-Methylguanine-DNA methyltransferase promoter methylation (aHR 0.53; 95%CI, 0.39-0.71; p<0.001), and gross total tumor resection (aHR

0.57; 95%CI, 0.44-0.74; p<0.001) were independent predictors of a longer overall survival. After case matching (n=54 per group), a longer overall survival was found for Levetiracetam use during the whole duration of the standard chemoradiation protocol (HR=0.63; 95%CI, 0.42-0.94, p=0.023).

Discussion. Levetiracetam use during the whole standard chemoradiation protocol possibly improves overall survival of *IDH*-wildtype glioblastoma patients. It should be considered in the anti-tumor strategy of future multicentric trials.

Classification of Evidence. This study provides Class III evidence that in individuals with IDH-wildtype glioblastoma, levetiracetam use throughout the duration of standard chemotherapy is associated with longer median overall survival.

Introduction

Glioblastomas, especially *Isocitrate Dehydrogenase (IDH) 1 and 2*-wildtype, are the most common malignant primary brain tumor in adults. Maximal safe resection, whenever feasible, followed by the standard combined chemoradiation protocol with Temozolomide is the recommended first-line treatment [1,2]. However, it provides limited survival benefits, making the discovery of new therapeutic targets crucial. Temozolomide is an oral alkylating agent that leads to DNA methylation resulting in anti-tumor downstream effects. *IDH*-wildtype glioblastomas associated with methylation of the *O6-Methylguanine-DNA methyltransferase* (*MGMT*) promoter are more sensitive to Temozolomide and are associated with a better prognosis [3].

The presence of epileptic seizures at diagnosis is a predictor of longer survival in patients with diffuse gliomas [4-6]. This clinical observation appears paradoxical since both epilepsy and diffuse gliomas share common pathogenic mechanisms and influence each other [4,5,7]. Furthermore, neuronal activity has been shown to promote glioma growth [8,9]. A first hypothesis would be that less aggressive diffuse gliomas - with expected prolonged survival - may be more conducive to the development of epileptogenic processes, which require surviving neurons and plasticity [7]. One alternative explanation is that drug therapies targeting the shared pathogenic mechanisms may affect both seizure and tumor control. It has been previously suggested that several antiepileptic drugs, mainly Sodium valproate [10-12], Levetiracetam [13-15], and Perampanel [16], can have anti-tumor effects and that Temozolomide chemotherapy can improve seizure control [17,18]. In patients with *IDH*-wildtype glioblastomas, epileptic seizures are common during the course of the tumor, with incidence ranging up to 30-60%, and require long-term use of antiepileptic drugs [4,5]. Therefore, antiepileptic drugs are often combined with Temozolomide during the standard combined chemoradiation protocol. Levetiracetam (which mostly targets the synaptic vesicle protein 2A) has been increasingly

158 used as the first antiepileptic drug in the setting of glioma-related epileptic seizures due to its
159 high therapeutic index, favorable pharmacokinetics, absence of interaction with
160 chemotherapeutic drugs, tolerability, and antiemetic effects [19-23].
161 Previous studies have shown contradicting results regarding the association between
162 Levetiracetam use during standard combined chemoradiation protocol with Temozolomide
163 and improved survival in patients with glioblastoma [13-15,24,25]. However, these previous studies
164 did not systematically control for the duration of Levetiracetam administration (limited to "at
165 baseline" or "continued") [21], the *IDH*-mutation status, and the *MGMT* promoter methylation
166 status. Therefore, we investigated whether Levetiracetam use during standard combined
167 chemoradiation protocol with Temozolomide and the duration of Levetiracetam
168 administration (continuous, part time, or never) impact overall survival in a homogeneous
169 single-institution cohort of *IDH*-wildtype glioblastomas in adults and controlling for MGMT
170 promoter methylation status.

Methods

Study design

An observational retrospective cohort study was conducted at a neurosurgery and oncology tertiary referral center between December 2010 and December 2018. The manuscript was written according to the Strengthening the Reporting of Observational Studies in Epidemiology checklist.

Participants

Inclusion criteria were: 1) patients ≥18 years old; 2) newly diagnosed tumor; 3) supratentorial hemispheric location; 4) histomolecular diagnosis of *IDH*-wildtype glioblastoma according to the 2016 WHO classification and cIMPACT-NOW update 3 with histopathological re-assessment for all cases diagnosed prior to 2018 [26,27]; 5) available pre- and postoperative MRI to quantify the extent of resection; 6) standard combined chemoradiation protocol with Temozolomide as first-line therapy [1]; 7) no inclusion in a clinical trial to exclude other oncological treatment than standard combined chemoradiation protocol; 8) data on antiepileptic drug delivery; and 9) available postoperative follow-up.

Variables and data sources

Patient- and tumor-related characteristics included: sex, age, clinical signs, Karnofsky Performance Status (KPS) score, revised Radiation Therapy Oncology Group - Recursive Partitioning Analysis (RTOG-RPA) classes [28], anti-epileptic drug therapy administration during standard combined chemoradiation protocol, Levetiracetam use during standard combined chemoradiation protocol, tumor location, tumor volume, *MGMT* promoter methylation status, extent of resection, and overall survival. No *post hoc* evaluation was performed for the purpose of the study.

The Levetiracetam use was defined as: 1) “during the whole duration” of the standard combined chemoradiation protocol, meaning a continuous use during the whole period of concomitant chemoradiotherapy and adjuvant chemotherapy cycles from surgery to the end of the sixth cycle of Temozolomide; 2) “part time” during the standard combined chemoradiation protocol, meaning a transient use, whatever its duration, shorter than the whole period of concomitant chemoradiotherapy and adjuvant chemotherapy with six cycles of Temozolomide; and 3) “never” during the standard combined chemoradiation protocol.

The tumor volume (cm^3) was calculated using manual segmentation of abnormal signal on post-contrast T1-weighted sequence by one blinded investigator (AR) for every tumor. The extent of resection was quantified using an early postoperative MRI (within 48 hours) and performing manual segmentation of residual enhancing tumor by the same blinded investigator. A gross total resection corresponded to 100% resection of the enhancing signal on post-contrast T1-weighted sequence. All other cases were considered partial resections.

Overall survival was measured from the date of surgery to the date of death from any cause. Surviving patients were censored at the date of last follow-up.

Statistical analyses

This study investigates whether Levetiracetam use during standard combined chemoradiation protocol with Temozolomide impact overall survival of adult patients harboring an *IDH*-wildtype glioblastoma (Class III evidence).

Descriptive statistics were given as the mean ± standard deviation for continuous variables and as a percentage for categorical variables. To compare the Levetiracetam administration subgroups, univariate analyses were carried out using the chi-square or Fisher’s exact tests for comparing categorical variables, and the unpaired t-test or Mann–Whitney rank sum test for continuous variables, as appropriate. Unadjusted survival curves for overall survival were

plotted by the Kaplan-Meier method, using log-rank tests to assess significance for group comparison. A Cox proportional hazard model was constructed using a backward stepwise approach, adjusting for predictors previously associated at the $p<0.100$ level with mortality in unadjusted analysis.

To assess the survival benefit of Levetiracetam use during the whole duration of the combined standard chemoradiation protocol with Temozolomide in *IDH*-wildtype glioblastoma patients, we performed a case-matched analysis (1:1) with a control group of *IDH*-wildtype glioblastoma patients with Levetiracetam use part time or never during the combined standard chemoradiation protocol. Patients with unavailable *MGMT* promoter methylation status were excluded of the case matching. Each patient of the Levetiracetam use during the whole duration of the combined standard chemoradiation protocol subgroup was individually matched with a control patient of the Levetiracetam use part time or never during the combined standard chemoradiation protocol subgroup according to the following criteria: 1) sex; 2) age (within 5 years); 3) Epileptic seizures at diagnosis; 4) RTOG-RPA class (3-4 *versus* 5-6); 5) tumor location (same lobe); 6) preoperative volume (cutoff by median); 7) extent of resection (total *versus* partial); and 8) *MGMT* promoter methylation status. In addition, each patient of the Levetiracetam use during the whole duration of the combined standard chemoradiation protocol subgroup was individually matched with a control patient who never received Levetiracetam during the combined standard chemoradiation protocol subgroup according to the same criteria. A p-value <0.050 was considered significant. Analyses were performed using JMP 14.1.0 (SAS Institute Inc, Cary, North Carolina, USA).

Standard protocol approvals, registrations and patient consents

The study received required authorizations (IRB#1: 2021/01) from the human research institutional review board (IRB00011687). The requirement to obtain informed consent was waived according to French legislation (observational retrospective study).

Data availability

Data not provided in the article because of space limitations may be shared (anonymized) at the request of any qualified investigator for purposes of replicating procedures and results.

Results

Patient and tumor characteristics

A total of 460 consecutive adult patients (270 males, mean age 60.1±11.2 years) with a newly diagnosed *IDH*-wildtype supratentorial glioblastoma, who all underwent biopsy or resection followed by standard combined chemoradiation protocol with Temozolomide as first line therapy were included, corresponding to 86.8% of the 530 initially screened patients.

Characteristics of the population and subgroup analyses by Levetiracetam use are detailed in Table 1.

Epilepsy and anti-epileptic drug therapy

One hundred and sixty-two patients (35.2%) had epileptic seizures at the time of diagnosis. One hundred and seventy-one patients were taking at least one antiepileptic drug at the time of diagnosis (37.2%), consisting in one drug for 150 patients, two drugs for 20 patients, and three drugs for one patient. Of the 162 patients who had experienced epileptic seizures at diagnosis, 155 (95.7%) were on antiepileptic drug therapy, and of the 298 patients who had not experienced seizures, 16 (5.4%) were on antiepileptic drug therapy. One hundred and twenty-nine patients were taking Levetiracetam (representing 75.4% of the treated patients) at the beginning of the standard combined chemoradiation protocol. Levetiracetam was discontinued in 13 patients and introduced in 113 patients during the standard combined chemoradiation protocol. One hundred and sixteen patients (25.2%) received Levetiracetam during the whole duration of the standard combined chemoradiation protocol, 145 (31.5%) received Levetiracetam part time during the standard combined chemoradiation protocol, and 199 (43.3%) never received Levetiracetam during the standard combined chemoradiation protocol.

Survival Analysis

The median duration of follow-up was 16.5 months [95% CI, 3.1-44.1]. Three hundred and sixty-five patients (79.3%) died over the follow-up period. The median overall survival was 17.4 months [95% CI, 16.3-19.0] for the whole population.

Unadjusted Hazard Ratio's (HR) for overall survival in the whole series are detailed in Table 2. In a univariate analysis, Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (excluding the part time or never subgroups), together with younger age (<60 years old), absence of neurological deficit, Radiation Therapy Oncology Group Recursive Partitioning Analysis classes 3-4, Karnofsky Performance Status score ≥70, *MGMT* promoter methylation, and total tumor resection were associated with longer overall survival. The median overall survival was longer in the 116 patients with Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (21.0 months; 95% CI, 17.2-24.0) than in the 145 patients with Levetiracetam use part time during the standard combined chemoradiation protocol (16.8 months; 95% CI, 12.4-19.0], and the 199 patients who never received Levetiracetam (16.0 months; 95% CI, 15.5-19.4; p=0.027). Kaplan-Meier survival curves are shown in Figure 1. Adjusted HRs for overall survival in the whole series are detailed in Table 2. After multiple adjustments using Cox models, Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (aHR 0.69; 95%CI, 0.52-0.93; p=0.014), *MGMT* promoter methylation (aHR 0.53; 95%CI, 0.39-0.71; p<0.001), partial tumor resection (aHR 0.61; 95%CI, 0.40-0.90; p=0.0145), and gross total tumor resection (aHR 0.57; 95%CI, 0.44-0.74; p<0.001) were independent predictors of longer overall survival.

Unadjusted HRs for overall survival in the subgroup of patients with epileptic seizures at diagnosis are detailed in Table 3. In a univariate analysis, Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (excluding the part time or never

subgroups), together with Karnofsky Performance Status score ≥70, *MGMT* promoter methylation, and gross total tumor resection were associated with longer overall survival. The median overall survival was longer in the 108 patients with Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (21.0 months; 95% CI, 17.6-24.9) than in the 19 patients with Levetiracetam use part time during the standard combined chemoradiation protocol (19.2 months; 95% CI, 10.0-34.0], and the 35 patients who never received Levetiracetam (16.3 months; 95% CI, 13.5-18.3; p=0.019). Adjusted HRs for overall survival in the subgroup of patients with epileptic seizures at diagnosis are detailed in Table 3. After multiple adjustments using Cox models, Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (aHR 0.45; 95%CI, 0.27-0.73; p=0.001), Levetiracetam use part time during the standard combined chemoradiation protocol (aHR 0.56; 95%CI, 0.27-0.72; p=0.022), *MGMT* promoter methylation (aHR 0.53; 95%CI, 0.37-0.76; p<0.001) and gross total tumor resection (aHR 0.45; 95%CI, 0.31-0.66; p<0.001) were independent predictors of a longer overall survival.

In the subgroup of patients with Levetiracetam use during the whole duration of the standard combined chemoradiation protocol, the daily dose (≤500 *versus* 500-1000 *versus* >1000 mg/day) did not significantly influence the overall survival (p=0.260). In the subgroup of patients who did not receive the standard combined chemoradiation protocol (external dataset, data no shown), Levetiracetam use did not influence the overall survival (p=0.959).

After case matching (n=54 in both subgroups), a significantly longer overall survival was found for Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (HR=0.63 [0.42-0.94], p=0.023). The median overall benefit was 4.8 months, with a median of 21.0 months [95% CI, 17.2-23.0] in the Levetiracetam use during the whole duration of the standard combined chemoradiation protocol subgroup and 16.2 months [95% CI: 13.5-18.3] in the Levetiracetam use part time or never during the standard

combined chemoradiation protocol subgroup. Table 4 shows the characteristics of each matched pair. After case matching comparing patients with Levetiracetam use during the whole duration of the standard combined chemoradiation protocol with patients who never received Levetiracetam (n=40 in both subgroups), a significantly longer overall survival was found for Levetiracetam use during the whole duration of the standard combined chemoradiation protocol (HR=0.61 [0.38-0.97], p=0.037). The median overall benefit was 3.2 months, with a median of 19.5 months [95% CI, 17.2-23.0] in the subgroup of patients with Levetiracetam use during the whole duration of the standard combined chemoradiation protocol and 16.3 months [95% CI: 13.5-18.3] in the subgroup of patients who never received Levetiracetam during the standard combined chemoradiation protocol.

Discussion

Key results

In this homogeneous single-institution cohort of adults harboring a newly diagnosed supratentorial *IDH*-wildtype glioblastoma, we showed that Levetiracetam use during the whole duration of the standard combined chemoradiation protocol was an independent predictor of increased overall survival both in the whole series (n=460), in the subgroup of patients with epileptic seizures at diagnosis (n=162), and in case-matched analysis (n=54 pairs). Particularly, Levetiracetam's survival advantage was independent of the presence of epileptic seizures, the extent of resection and the *MGMT* promoter methylation status.

Interpretation

In the present study, the survival benefit of Levetiracetam is related to the duration of its administration during the standard combined chemoradiation protocol with Temozolomide, which suggests a direct effect. The survival benefit of Levetiracetam is independent of the presence of epileptic seizures at diagnosis, the extent of resection, and the *MGMT* promoter methylation status, suggesting that the underlying mechanisms are multifactorial and not solely *MGMT*-mediated. *In vitro* experiments suggested that Levetiracetam sensitizes glioblastoma cells to Temozolomide through the reduction of *MGMT* protein expression [29]. Levetiracetam increases histone deacetylase 1 transcription and recruits the HDAC1/mSin3A multiprotein corepressor complex to the p53-binding site in the *MGMT* promoter, thus downregulating its transcription [29-31]. In addition, the combination of Levetiracetam and Temozolomide has increased anti-tumor activity with a tumor suppression effect inducing glioma cell senescence and activation of the apoptotic pathway [31]. Neuronal activity, via functional neurogliomal chemical glutamatergic synapses between the presynaptic pyramidal neurons and postsynaptic glioma cells (AMPA receptors) identified in high-grade diffuse

gliomas, induces synchronized calcium releases that ultimately promotes glioma progression [32,33]. Since Levetiracetam displays anti-AMPA effects [34], its long-term use may have neuromodulating effects on these neurogliomal synapses, contributing to inhibition of glioma progression [13]. Lastly, chloride dynamics also play a role in regulating glioma cell growth, with intracellular chloride accumulation favoring tumor progression. The chloride equilibrium may be disrupted by chloride permeable $GABA_A$ receptor activation [4]. Levetiracetam has been shown to affect both intracerebral GABA concentration [35] and $GABA_A$ receptors, [36] likely affecting glioma cell dynamics. Similarly, a case report illustrating continuous regression of a glioblastoma in a patient who received Levetiracetam and Dexamethasone without Temozolomide or other cancer-targeted therapy, suggested that Levetiracetam could have a direct anti-tumor effect [37].

The present results confirm findings from previous studies, including series of 103, 332, and 418 patients with glioblastomas, respectively, who received standard combined chemoradiation protocol with Temozolomide, which all suggested that Levetiracetam use provided a survival benefit [13-15]. These observations conflict with those from two large studies where Levetiracetam use did not provide any significant survival benefit. The study by Happold et al., which aggregated 1869 patients with a newly diagnosed glioblastoma from four randomized clinical trials between 2000-2011 [24], included patients with varying oncological treatments (radiotherapy alone, standard combined chemoradiation protocol, Cilengitide, Bevacizumab), did not detail the *IDH1/2* mutation status, and did not provide the duration of Levetiracetam use. The study by Knudsen-Baas et al., which registered 1263 glioblastomas diagnosed in Norway between 2004-2010 [25], included patients with varying oncological treatments, did not detail the *IDH1/2* mutation and the *MGMT* promoter methylation statuses, and did not provide the duration of Levetiracetam use. In those two studies, the possible inclusion of *IDH*-mutant glioblastomas with better prognosis, of patients

with a short and restricted perioperative use of Levetiracetam according to guidelines [38,39] (identified as “part time use” in the present study) and of patients with oncological treatments different than the standard combined chemoradiation protocol with Temozolomide may explain why Levetiracetam was not shown to affect overall survival [24,25].

The present study reports, for the first time, a link between the overall survival and the use of Levetiracetam during the whole duration of the standard combined chemoradiation protocol with Temozolomide. This suggests Levetiracetam might display anti-tumor properties, in adjunct to standard combined chemoradiation protocol for newly diagnosed supratentorial *IDH*-wildtype glioblastomas in adults. On a practical basis, the present results suggest that: 1) the choice of the antiepileptic drug in patients with an *IDH*-wildtype glioblastoma should be carefully considered because it may affect overall survival; 2) Levetiracetam could be proposed as the first antiepileptic drug in patients presenting with epileptic seizures at diagnosis in the setting of a newly diagnosed supratentorial *IDH*-wildtype glioblastoma, due first to its efficacy, second to its good safety profile, third to its lack of interference with chemotherapy and fourth to its possible favorable effects on survival. In addition, the present results open the door for future questions that remain to be solved: 1) the introduction of Levetiracetam could be discussed at the time of diagnosis, eventually even in patients without epileptic seizures, while carefully weighing the risks of adverse effects related to Levetiracetam, including neuropsychiatric ones [40]; 2) Levetiracetam could be pursued during the whole duration of the standard combined chemoradiation protocol with Temozolomide, including the concomitant chemoradiotherapy and adjuvant chemotherapy periods, even in seizure-controlled patients.

Generalizability

Strengths of this study include the homogeneous data collection of a large real-life case series of newly diagnosed supratentorial *IDH*-wildtype glioblastomas in adults in the current era of standard combined chemoradiation protocol as first-line treatment and with Levetiracetam as the first antiepileptic drug for these patients. The homogeneous population is illustrated by the observed survival rates that are close to those reported in pivotal trials of glioblastomas in adults treated by standard combined chemoradiation protocol as first-line treatment [1,2]. This study controlled for histomolecular biases via re-assessment of all diffuse gliomas according to the 2016 updated WHO classification [26,27]. In addition, we provided a case-matched analysis to control for selection biases between Levetiracetam use subgroups, including MGMT promoter methylation status. We excluded *IDH*-mutant cases since mutations of the *IDH1/2* genes share a higher prevalence of epileptic seizures at diagnosis and during the course of the tumor with particular epileptogenic mechanisms and share a better prognosis [4]. Since the study is focused purely on newly diagnosed supratentorial *IDH*-wildtype glioblastomas in adults, the results cannot be extrapolated to recurrent glioblastomas, to *IDH*-mutant diffuse gliomas, and to the pediatric population.

Limitations

Main limitations include the retrospective design of the study, its single-center setting, the diagnosis of epileptic seizures on a clinical basis only, and data partially missing for *MGMT* promoter methylation status that reduced the number of patients entered in the case matching (n=54 per group). The potential biases induced by data missing regarding the MGMT promoter methylation status were limited by their systematic incorporation in statistical analyses as a specific category. Other limitations include the lack of information regarding the exact duration of Levetiracetam use “part time” during the standard combined chemoradiation protocol and in patients with early tumor recurrence, the lack of information regarding the

serum concentration of Levetiracetam of patients under study, the reasons for Levetiracetam introduction or arrest during the standard combined chemoradiation protocol with Temozolomide, and the antiepileptic drug management by the treating physicians. At a time where several antiepileptic drugs, such as Sodium valproate [10-12], Perampanel [16], and Levetiracetam,[13-15] have been suggested to affect *IDH*-wildtype glioblastoma prognosis, further prospective multicentric controlled trials aiming at investigating the survival benefit of each of these drugs compared to an antiepileptic drug devoid of such anti-tumor properties and to the absence of antiepileptic drug should be considered.

Conclusion

Beyond its effectiveness against epileptic seizures, Levetiracetam also possibly improves overall survival of newly diagnosed supratentorial *IDH*-wildtype glioblastoma adult patients treated by standard combined chemoradiation. Levetiracetam could also be envisioned as the first antiepileptic drug and as an anti-tumor adjunct during the whole duration of standard combined chemoradiation protocol with Temozolomide. Its potential favorable effect in patients that did not experience seizures remains to be established.

Fundings

This work was supported by grants from the European Research Council (consolidator grant #865592 to GH) and from the Cancéropole Ile-de-France (grant EMERG-2 2016 to JP).

Competing interests

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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.

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Table and Figure legends

Table 1.

Characteristics of the population (n=460).

Table 2.

Univariate and multivariate predictors of overall survival in the whole series (n=460).

Table 3.

Univariate and multivariate predictors of overall survival in the subgroup of patients with epileptic seizures at diagnosis (n=162).

Table 4.

Levetiracetam use subgroups during standard combined chemoradiation protocol with Temozolomide (whole duration *versus* part time or never) paired by matching criteria (n=54 pairs).

Figure 1. Kaplan-Meier estimates of overall survival.

A. Kaplan-Meier estimates of overall survival in the whole population (n=460).

B. Kaplan-Meier estimates of overall survival, according to Levetiracetam use during the combined standard chemoradiation protocol (whole duration of the combined standard chemoradiation protocol, n=116; part time, n=145; never, n=199).

600 **Table 1. Characteristics of the population (n=460).**
601

Parameter		Whole series n=460		Levetiracetam use Whole duration n=116		Levetiracetam use Part time n=145		Levetiracetam use Never n=199		p-value
		n	%	n	%	n	%	n	%	
Sex	Female	190	41.3	35	30.2	67	46.2	88	44.2	**0.016**
	Male	270	58.7	81	69.8	78	53.8	111	55.8	
Age, year (mean ± SD)		460	60.1 ± 11.2	116	58.7 ± 11.2	145	60.9 ± 10.6	199	60.4 ± 11.5	0.183
Time to surgery, month (mean ± SD)		460	1.5 ± 1.8	116	1.5 ± 2.0	145	1.6 ± 1.6	199	1.5 ± 1.9	0.469
Signs of increased intracranial pressure at diagnosis	No	186	40.4	20	17.2	72	49.7	94	47.2	**<0.001**
	Yes	274	59.4	96	82.8	73	50.3	105	52.8	
Neurological deficit at diagnosis	No	142	30.9	68	58.6	35	24.1	39	19.6	**<0.001**
	Yes	318	69.1	48	41.4	110	75.9	160	80.4	
KPS score at diagnosis	≥ 70	394	85.7	114	98.3	112	77.2	168	84.4	**<0.001**
	< 70	66	14.3	2	1.7	33	22.8	31	15.6	
RTOG-RPA class at diagnosis	3-4	266	57.8	71	61.2	76	52.4	119	59.8	0.274
	5-6	194	42.2	45	38.8	69	47.6	80	40.2	
History of epileptic seizures at diagnosis	No	298	64.8	8	6.9	126	86.9	164	82.4	**<0.001**
	Yes	162	35.2	108	93.1	19	13.1	35	17.6	
First antiepileptic drug administered	None	289	62.8	0	0	123	84.8	166	83.4	
	Levetiracetam	129	28.0	116	100	13	9.0	0	0	
	Valproic acid	16	3.5	0	0	3	2.1	13	6.5	
	Other	26	5.6	0	0	6	4.1	20	10.1	
Levetiracetam dose (mg/day) at diagnosis	500	6	4.7	3	2.6	3	23.1	0	0	
	1000	107	82.9	98	84.4	9	69.2	0	0	
	1500	8	6.2	7	6.0	1	7.7	0	0	
	2000	4	3.1	4	3.5	0	0	0	0	
	3000	4	3.1	4	3.5	0	0	0	0	
Anatomic location	Frontal	131	28.5	30	25.9	50	34.5	51	25.6	0.159
	Temporal	171	38.3	54	46.5	47	32.4	75	37.7	
	Parietal	104	22.6	21	18.1	30	20.7	53	26.6	
	Other	49	10.6	11	9.5	18	12.4	20	10.1	
Tumor volume, cm^3 (mean ± SD) *		460	39.2 ± 41.6	116	18.8 ± 23.9	145	47.4 ± 45.1	199	45.2 ± 43.2	**<0.001**
MGMT promoter methylation status	No	133	28.9	28	24.1	39	26.9	66	33.2	0.397
	Yes	104	22.6	25	21.6	34	23.4	45	22.6	
	Missing	223	48.5	63	54.3	72	49.7	88	44.2	
First-line treatment										
Extent of resection £	Biopsy	138	30.0	34	29.3	48	33.1	56	28.1	0.148
	Subtotal removal	105	22.8	23	19.8	41	28.3	41	20.6	
	Total removal	217	47.2	59	50.9	56	38.6	102	51.3	
Number of adjuvant Temozolomide cycles (mean ± SD)		460	5.0 ± 3.7	116	5.0 ± 3.2	116	5.2 ± 4.4	116	4.8 ± 3.5	0.877
Number of adjuvant Temozolomide cycles	< 6	238	51.7	65	56.0	64	44.1	109	54.8	0.204
	6	117	25.5	22	19.0	39	26.9	56	28.1	
	> 6	105	22.8	29	25.0	42	29.0	34	17.1	
Treatment at progression										
Progression	No	89	19.3	15	12.9	32	22.1	42	21.1	0.126
	Yes	371	80.7	101	87.1	113	77.9	157	78.9	
Surgical resection	No	410	89.1	15	12.9	15	10.3	20	10.1	0.709
	Yes	50	10.9	101	87.1	130	89.7	179	89.9	
Radiotherapy	No	419	91.1	100	86.2	136	93.8	183	91.9	0.100
	Yes	41	8.9	16	13.8	9	6.2	16	8.1	
Chemotherapy	No	232	50.4	46	39.7	79	54.5	107	53.8	**0.027**
	Yes	228	49.6	70	60.3	66	45.5	92	46.2	

602
603 CI: Confidence Interval; HR: Hazard Ratio; KPS: Karnofsky Performance Status; MGMT: O6-methylguanine–DNA methyltransferase; OS: Overall Survival; RTOG: Radiation Therapy Oncology
604 Group; RPA: Recursive Partitioning Analysis
605 * Quantitative assessment based on preoperative tumor volume using contrast-enhanced three-dimensional T1-weigthed sequence.
606 £ Quantitative assessment based on preoperative tumor volume and postoperative (MRI performed within 48h) tumor volume using contrast-enhanced three-dimensional T1-weigthed sequence.

Table 2. Univariate and multivariate predictors of overall survival in the whole series (n=460).

Parameters		Median OS (months)	Overall survival (months)					
			Unadjusted Hazard Ratio			Adjusted Hazard Ratio		
			uHR	CI95%	p-value	aHR	CI95%	p-value
Clinical parameters								
Sex	Female	18.7	1 (ref)					
	Male	17.0	1.12	0.91 - 1.39	0.270			
Age (year)	< 60	18.0	1 (ref)					
	≥ 60	16.5	1.24	1.01 - 1.52	**0.044**	1.06	0.83 - 1.36	0.638
Signs of increased intracranial pressure at diagnosis	No	17.9	1 (ref)					
	Yes	16.5	1.11	0.90 - 1.38	0.314			
Neurological deficit at diagnosis	No	21.0	1 (ref)					
	Yes	16.5	1.31	1.04 - 1.64	**0.018**	1.32	0.99 - 1.75	0.053
RTOG-RPA class at diagnosis	3-4	19.5	1 (ref)					
	5-6	14.2	1.53	1.24 - 1.87	**<0.001**	1.23	0.89 - 1.69	0.215
KPS score at diagnosis	≥ 70	18.0	1 (ref)					
	< 70	13.2	1.69	1.26 - 2.28	**<0.001**	1.23	0.84 - 1.79	0.282
History of epileptic seizures at diagnosis	No	16.5	1 (ref)					
	Yes	19.0	0.83	0.67 - 1.03	0.084	0.81	0.57 - 1.17	0.259
Levetiracetam use during standard chemoradiation regimen	Never	16.5	1 (ref)					
	Part time (stopped or introduced)	16.8	0.93	0.73 - 1.18	0.562	1.04	0.80 - 1.35	0.777
	Whole duration	21.0	0.73	0.55 - 0.96	**0.027**	0.69	0.52 - 0.93	**0.014**
Anatomic location	Frontal	17.0	1 (ref)					
	Temporal	19.5	0.99	0.77 - 1.29	0.974			
	Parietal	17.4	1.05	0.78 - 1.42	0.729			
	Other	15.0	1.15	0.80 - 1.67	0.438			
Tumor volume (cm^3) *	< 30	18.3	1 (ref)					
	≥ 30	16.8	1.11	0.90 - 1.36	0.329			
MGMT promoter methylation status	No	16.2	1 (ref)					
	Yes	24.0	0.49	0.37 - 0.63	**<0.001**	0.53	0.39 - 0.71	**<0.001**
	Missing	16.0	0.82	0.65 - 1.04	0.107	0.75	0.57 - 0.99	**0.046**
Extent of resection at first time surgery £	Biopsy	12.5	1 (ref)					
	Partial removal	16.0	0.79	0.60 - 1.05	0.107	0.61	0.40 - 0.90	**0.015**
	Total removal	21.6	0.51	0.40 - 0.65	**<0.001**	0.57	0.44 - 0.74	**<0.001**
Surgical resection at progression	No	16.9	1 (ref)			1 (ref)		
	Yes	24.0	0.69	0.49 - 0.99	**0.043**	0.81	0.56 - 1.18	0.277
Radiotherapy at progression	No	17.0	1 (ref)			1 (ref)		
	Yes	31.0	0.52	0.35 - 0.79	**0.002**	0.80	0.51 - 1.24	0.314
Chemotherapy at progression	No	15.0	1 (ref)			1 (ref)		
	Yes	20.0	0.77	0.62 - 0.96	**0.023**	0.84	0.66 - 1.07	0.154

CI: Confidence Interval; HR: Hazard Ratio; KPS: Karnofsky Performance Status; MGMT: O6-methylguanine–DNA methyltransferase; OS: Overall Survival; RTOG: Radiation Therapy Oncology Group; RPA: Recursive Partitioning Analysis

612 **Table 3. Univariate and multivariate predictors of overall survival in the subgroup of**
613 **patients with epileptic seizures at diagnosis (n=162).**
614

Parameters		Median OS (months)	Overall survival (months)					
			Unadjusted Hazard Ratio			Adjusted Hazard Ratio		
			uHR	CI95%	p-value	aHR	CI95%	p-value
Clinical parameters								
Sex	Female	20.0	1 (ref)					
	Male	18.0	1.19	0.92 - 1.54	0.187			
Age (year)	< 60	19.0	1 (ref)					
	≥ 60	17.2	1.42	0.99 - 2.04	0.057	1.16	0.86 - 1.56	0.324
Signs of increased intracranial pressure at diagnosis	No	20.0	1 (ref)					
	Yes	16.9	1.18	0.76 - 1.83	0.472			
Neurological deficit at diagnosis	No	21.0	1 (ref)					
	Yes	17.0	1.20	0.84 - 1.72	0.306			
RTOG-RPA class at diagnosis	3-4	21.0	1 (ref)					
	5-6	16.0	1.29	0.89 - 1.86	0.177			
KPS score at diagnosis	≥ 70	19.0	1 (ref)					
	< 70	9.5	2.39	1.21 - 4.74	**0.012**	1.31	0.88 - 1.95	0.182
Levetiracetam use during standard chemoradiation regimen	Never	16.3	1 (ref)					
	Part time (stopped or introduced)	19.2	0.67	0.35 - 1.27	0.220	0.56	0.27 - 0.72	**0.022**
	Whole duration	21.0	0.59	0.38 - 0.92	**0.019**	0.45	0.27 - 0.73	**0.001**
Anatomic location	Frontal	21.0	1 (ref)					
	Temporal	19.0	1.21	0.78 - 1.88	0.393			
	Parietal	18.2	1.20	0.71 - 2.02	0.493			
	Other	17.6	0.93	0.42 - 2.03	0.853			
Tumor volume (cm^3) *	< 30	21.0	1 (ref)					
	≥ 30	16.9	1.37	0.92 - 2.05	0.121			
MGMT promoter methylation status	No	17.0	1 (ref)					
	Yes	28.5	0.33	0.19 - 0.57	**<0.001**	0.53	0.37 - 0.76	**<0.001**
	Missing	29.0	0.39	0.09 - 1.62	0.196	0.66	0.43 - 1.04	0.070
Extent of resection £	Biopsy	15.0	1 (ref)					
	Partial removal	16.0	0.79	0.46 - 1.34	0.384	0.70	0.32 - 1.52	0.370
	Total removal	21.7	0.58	0.36 - 0.93	**0.024**	0.45	0.31 - 0.66	**<0.001**
Surgical resection at progression	No	16.0	1 (ref)			1 (ref)		
	Yes	20.8	0.44	0.25 - 0.77	**0.004**	0.89	0.55 – 1.41	0.604
Radiotherapy at progression	No	16.0	1 (ref)			1 (ref)		
	Yes	35.2	0.58	0.32 - 1.04	0.066	0.45	0.31 - 1.04	0.068
Chemotherapy at progression	No	12.4	1 (ref)					
	Yes	19.5	1.02	0.69 - 1.45	0.894			

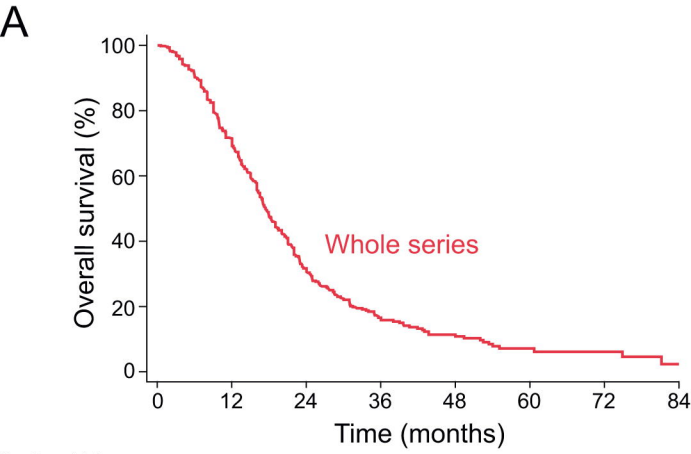
615 CI: Confidence Interval; HR: Hazard Ratio; KPS: Karnofsky Performance Status; MGMT: O6-methylguanine–DNA methyltransferase; OS: Overall Survival; RTOG: Radiation Therapy Oncology
616 Group; RPA: Recursive Partitioning Analysis

Table 4. Levetiracetam use subgroups during standard combined chemoradiation protocol with Temozolomide (whole duration versus part time or never) paired by matching criteria (n=54 pairs).

	Levetiracetam part time or never								Levetiracetam whole duration							
	Sex	Age	Epileptic seizures	RTOG-RPA class	Lobe	Volume	Extent of resection	MGMT promoter methylation status	Sex	Age	Epileptic seizures	RTOG-RPA class	Lobe	Volume	Extent of resection	MGMT promoter methylation status
Complete match in 8/8 criteria																
1	F	60	Yes	3 4	Frontal	< median	Partial	Unmethylated	F	65	Yes	3 4	Frontal	< median	Partial	Unmethylated
2	F	71	Yes	3 4	Parietal	> median	Total	Methylated	F	70	Yes	3 4	Parietal	> median	Total	Methylated
3	M	48	Yes	3 4	Frontal	< median	Total	Unmethylated	M	44	Yes	3 4	Frontal	< median	Total	Unmethylated
4	F	55	Yes	3 4	Temporal	< median	Total	Unmethylated	F	50	Yes	3 4	Temporal	< median	Total	Unmethylated
5	F	56	Yes	3 4	Temporal	< median	Total	Unmethylated	F	58	Yes	3 4	Temporal	< median	Total	Unmethylated
6	F	62	Yes	3 4	Temporal	< median	Partial	Unmethylated	F	66	Yes	3 4	Temporal	< median	Partial	Unmethylated
7	F	57	Yes	3 4	Temporal	< median	Total	Methylated	F	59	Yes	3 4	Temporal	< median	Total	Methylated
8	M	61	Yes	3 4	Frontal	< median	Total	Methylated	M	57	Yes	3 4	Frontal	< median	Total	Methylated
9	M	56	No	3 4	Temporal	> median	Total	Unmethylated	M	54	No	3 4	Temporal	> median	Total	Unmethylated
10	M	49	Yes	3 4	Temporal	< median	Partial	Unmethylated	M	51	Yes	3 4	Temporal	< median	Partial	Unmethylated
11	M	67	Yes	3 4	Temporal	< median	Total	Unmethylated	M	68	Yes	3 4	Temporal	< median	Total	Unmethylated
12	M	29	Yes	3 4	Temporal	< median	Total	Unmethylated	M	34	Yes	3 4	Temporal	< median	Total	Unmethylated
13	M	60	Yes	3 4	Temporal	< median	Total	Unmethylated	M	62	Yes	3 4	Temporal	< median	Total	Unmethylated
14	M	57	Yes	3 4	Parietal	> median	Total	Unmethylated	M	55	Yes	3 4	Parietal	> median	Total	Unmethylated
15	M	55	No	5 6	Temporal	< median	Partial	Methylated	M	57	Yes	5 6	Temporal	< median	Partial	Methylated
16	M	56	No	5 6	Temporal	> median	Partial	Methylated	M	61	No	5 6	Temporal	> median	Partial	Methylated
17	M	50	Yes	3 4	Temporal	< median	Total	Unmethylated	M	55	Yes	3 4	Temporal	< median	Total	Unmethylated
18	M	45	Yes	3 4	Temporal	< median	Total	Methylated	M	49	Yes	3 4	Temporal	< median	Total	Methylated
Incomplete match in 1/8 criterion *																
19	F	70	**No**	5 6	Frontal	< median	Partial	Methylated	F	67	**Yes**	5 6	Frontal	< median	Partial	Methylated
20	M	68	**No**	3 4	Temporal	> median	Total	Unmethylated	M	66	**Yes**	3 4	Temporal	> median	Total	Unmethylated
21	M	75	**No**	3 4	Temporal	> median	Partial	Methylated	M	71	**Yes**	3 4	Temporal	> median	Partial	Methylated
22	M	**72**	Yes	5 6	Temporal	< median	Total	Methylated	M	**60**	Yes	5 6	Temporal	< median	Total	Methylated
23	F	55	**No**	3 4	Temporal	< median	Total	Methylated	F	55	**Yes**	3 4	Temporal	< median	Total	Methylated
24	M	59	Yes	3 4	Frontal	**< median**	Total	Unmethylated	M	64	Yes	3 4	Frontal	**> median**	Total	Unmethylated
25	M	45	**No**	3 4	Temporal	< median	Total	Unmethylated	M	40	**Yes**	3 4	Temporal	< median	Total	Unmethylated
26	M	58	**No**	3 4	Temporal	< median	Total	Unmethylated	M	61	**Yes**	3 4	Temporal	< median	Total	Unmethylated
27	M	66	**No**	3 4	Temporal	< median	Total	Methylated	M	71	**Yes**	3 4	Temporal	< median	Total	Methylated
28	M	34	**No**	3 4	Temporal	< median	Total	Methylated	M	29	**Yes**	3 4	Temporal	< median	Total	Methylated
29	M	**37**	Yes	3 4	Parietal	> median	Total	Methylated	M	**59**	Yes	3 4	Parietal	> median	Total	Methylated
30	M	**35**	Yes	3 4	Frontal	< median	Total	Methylated	M	**59**	Yes	3 4	Frontal	< median	Total	Methylated
31	M	**37**	Yes	3 4	Temporal	< median	Total	Methylated	M	**50**	Yes	3 4	Temporal	< median	Total	Methylated
32	M	59	**No**	3 4	Temporal	> median	Partial	Methylated	M	58	**Yes**	3 4	Temporal	> median	Partial	Methylated
33	M	**50**	No	5 6	Frontal	> median	Total	Methylated	M	**68**	No	5 6	Frontal	> median	Total	Methylated
34	M	32	**No**	3 4	Frontal	> median	Total	Unmethylated	M	26	**Yes**	3 4	Frontal	> median	Total	Unmethylated
Incomplete match in 2/8 criteria *																
35	M	75	**No**	3 4	Temporal	< median	**Total**	Unmethylated	M	79	**Yes**	3 4	Temporal	< median	**Partial**	Unmethylated
36	F	77	**No**	5 6	Parietal	< median	Partial	**Methylated**	F	76	**Yes**	5 6	Parietal	< median	Partial	**Unmethylated**
37	M	36	**No**	3 4	Temporal	< median	**Total**	Unmethylated	M	41	**Yes**	3 4	Temporal	< median	**Partial**	Unmethylated
38	F	**47**	Yes	3 4	Temporal	< median	Partial	**Unmethylated**	F	**72**	Yes	3 4	Temporal	< median	Partial	**Methylated**
39	F	**54**	**No**	3 4	Frontal	> median	Total	Methylated	F	**48**	**Yes**	3 4	Frontal	> median	Total	Methylated
40	F	56	**No**	3 4	Frontal	**> median**	**Total**	Methylated	F	57	**Yes**	3 4	Frontal	**< median**	**Partial**	Methylated
41	F	49	**No**	3 4	**Temporal**	> median	Total	Unmethylated	F	45	**Yes**	3 4	**Occipital**	> median	Total	Unmethylated
42	M	71	**No**	3 4	Frontal	**> median**	Total	Unmethylated	M	71	**Yes**	3 4	Frontal	**< median**	Total	Unmethylated
43	M	55	**No**	5 6	Frontal	**> median**	Total	Methylated	M	59	**Yes**	5 6	Frontal	**< median**	Total	Methylated
44	M	**64**	**No**	5 6	Temporal	< median	Partial	Unmethylated	M	**50**	**Yes**	5 6	Temporal	< median	Partial	Unmethylated
45	M	50	Yes	5 6	**Frontal**	**< median**	Total	Unmethylated	M	54	Yes	5 6	**Temporal**	**> median**	Total	Unmethylated
46	M	54	**No**	5 6	**Frontal**	> median	Total	Unmethylated	M	54	**Yes**	5 6	**Temporal**	> median	Total	Unmethylated
47	M	48	Yes	3 4	**Insular**	< median	**Total**	Unmethylated	M	49	Yes	3 4	**Parietal**	< median	**Partial**	Unmethylated
Incomplete match in 3/8 criteria *																
48	M	**64**	No	3 4	**Parietal**	**> median**	Partial	Methylated	M	**70**	No	3 4	**Occipital**	**< median**	Partial	Methylated
49	M	58	**No**	3 4	**Frontal**	**> median**	Partial	Unmethylated	M	59	**Yes**	3 4	**Occipital**	**< median**	Partial	Unmethylated
50	M	65	**No**	5 6	**Frontal**	**> median**	Total	Unmethylated	M	68	**Yes**	5 6	**Temporal**	**< median**	Total	Unmethylated
51	M	**74**	**No**	5 6	**Parietal**	> median	Partial	Unmethylated	M	**67**	**Yes**	5 6	**Temporal**	> median	Partial	Unmethylated
52	M	**67**	**No**	**5 6**	Parietal	< median	Partial	Methylated	M	**60**	**Yes**	**3 4**	Parietal	< median	Partial	Methylated
53	M	**73**	**No**	5 6	Temporal	**> median**	Total	Methylated	M	**51**	**Yes**	5 6	Temporal	**< median**	Total	Methylated
54	M	41	**No**	3 4	**Temporal**	**> median**	Partial	Unmethylated	M	42	**Yes**	3 4	**Parietal**	**< median**	Partial	Unmethylated

* Imperfectly matched criteria are shown in bold type.
F: Female; M: Male; RTOG: Radiation Therapy Oncology Group; RPA: Recursive Partitioning Analysis.

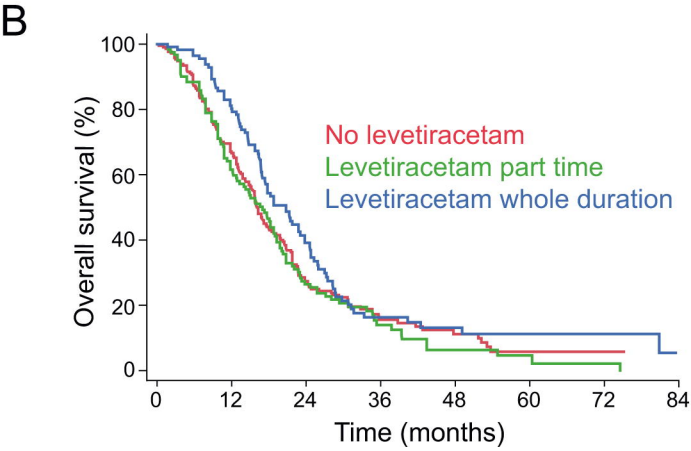
A



Number at risk

Whole series	460	309	117	43	21	9	6	2

B



Number at risk (events)

No LEV	199	144	50	21	10	3	2	0
LEV part time	145	74	30	10	4	2	1	0
LEV whole duration	116	91	37	12	7	4	3	2