



HAL
open science

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

Johan Pallud, Gilles Huberfeld, Edouard Dezamis, Sophie Peeters, Alessandro Moiraghi, Martine Gavaret, Eléonore Guinard, Frédéric Dhermain, Pascale Varlet, Catherine Oppenheim, et al.

► To cite this version:

Johan Pallud, Gilles Huberfeld, Edouard Dezamis, Sophie Peeters, Alessandro Moiraghi, et al.. Effect of Levetiracetam use duration on overall survival of isocitrate dehydrogenase wildtype glioblastoma in adults: an observational study. *Neurology*, 2021, pp.10.1212/WNL.000000000013005. 10.1212/WNL.000000000013005 . inserm-03421138

HAL Id: inserm-03421138

<https://inserm.hal.science/inserm-03421138v1>

Submitted on 9 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Author(s):

Johan Pallud, MD, PhD^{1, 2, 3}; Gilles Huberfeld, MD, PhD^{4, 5}; Edouard Dezamis, MD, MSc^{1, 2, 3}; Sophie Peeters, MD⁶; Alessandro Moiraghi, MD^{1, 2}; Martine Gavaret, MD, PhD^{2, 3, 7}; Eléonore Guinard, MD, MSc^{2, 3, 7}; Frédéric Dhermain, MD, PhD⁸; pascale VARLET, MD, PhD^{2, 3, 9}; Catherine Oppenheim, MD, PhD^{2, 3, 10}; Fabrice Chrétien, MD, PhD^{2, 9}; Alexandre Roux, MD, PhD^{1, 2, 3}; Marc ZANELLO, MD, PhD^{1, 2, 3}

Corresponding Author:

Johan Pallud

johanpallud@hotmail.com

Affiliation Information for All Authors: 1. Department of Neurosurgery, GHU Paris - Sainte-Anne Hospital, Paris, France 2. Université de Paris, Sorbonne Paris Cité, Paris, France 3. Inserm, U1266, IMA-Brain, Institut de Psychiatrie et Neurosciences de Paris, Paris, France 4. Neurology Department, Hopital Fondation Adolphe de Rothschild, 29 rue Main, 75019 Paris, France 5. Neuroglial Interactions in Cerebral Physiopathology, Center for Interdisciplinary Research in Biology, Collège de France, CNRS UMR 7241, INSERM U1050, Université PSL Paris, France 6. Department of Neurosurgery, University of California, Los Angeles - Los Angeles, CA, USA 7. Department of Neurophysiology, GHU Paris - Sainte-Anne Hospital, Paris, France 8. Department of Radiotherapy, Gustave Roussy University Hospital, Villejuif, France 9. Department of Neuropathology, GHU Paris - Sainte-Anne Hospital, Paris, France 10. Department of Neuroradiology, GHU Paris - Sainte-Anne Hospital, Paris, France

Contributions:

Johan Pallud: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Gilles Huberfeld: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Edouard Dezamis: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Sophie Peeters: Drafting/revision of the manuscript for content, including medical writing for content

Alessandro Moiraghi: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Martine Gavaret: Drafting/revision of the manuscript for content, including medical writing for content

Eléonore Guinard: Drafting/revision of the manuscript for content, including medical writing for content

Frédéric Dhermain: Drafting/revision of the manuscript for content, including medical writing for content

pascale VARLET: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Catherine Oppenheim: Drafting/revision of the manuscript for content, including medical writing for content

Fabrice Chrétien: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

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

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.

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

4
5 Johan PALLUD MD, PhD^{1,2,3}; Gilles HUBERFELD, MD^{4,5}, PhD; Edouard DEZAMIS MD,
6 MSc^{1,2,3}; Sophie PEETERS, MD, BA⁶; Alessandro MOIRAGHI, MD^{1,2,3}; Martine
7 GAVARET, MD, PhD^{2,3,7}; Eléonore GUINARD, MD, MSc^{2,3,7}; Frédéric DHERMAIN, MD,
8 PhD⁸; Pascale VARLET, MD, PhD^{2,3,9}; Catherine OPPENHEIM, MD, PhD^{2,3,10}; Fabrice
9 CHRETIEN, MD, PhD^{2,9}; Alexandre ROUX, MD, PhD^{1,2,3*}; Marc ZANELLO, MD, PhD^{1,2,3*}

- 10
11 1. Department of Neurosurgery, GHU Paris - Sainte-Anne Hospital, Paris, France
12 2. Université de Paris, Sorbonne Paris Cité, Paris, France
13 3. Inserm, U1266, IMA-Brain, Institut de Psychiatrie et Neurosciences de Paris, Paris, France
14 4. Neurology Department, Hôpital Fondation Adolphe de Rothschild, 29 rue Main, 75019
15 Paris, France
16 5. Neuroglial Interactions in Cerebral Physiopathology, Center for Interdisciplinary Research
17 in Biology, Collège de France, CNRS UMR 7241, INSERM U1050, Université PSL Paris,
18 France
19 6. Department of Neurosurgery, University of California, Los Angeles - Los Angeles, CA,
20 USA
21 7. Department of Neurophysiology, GHU Paris - Sainte-Anne Hospital, Paris, France
22 8. Department of Radiotherapy, Gustave Roussy University Hospital, Villejuif, France
23 9. Department of Neuropathology, GHU Paris - Sainte-Anne Hospital, Paris, France
24 10. Department of Neuroradiology, GHU Paris - Sainte-Anne Hospital, Paris, France

25 * These authors participated equally

26

27 **Corresponding author information**

28 Pr Johan PALLUD, MD, PhD

29 Service de Neurochirurgie

30 Hôpital Sainte-Anne

31 1, rue Cabanis

32 75674 Paris Cedex 14, France

33 Phone: 033 1 45 65 84 87

34 Fax: 033 1 45 65 74 28

35 E-mail: johanpallud@hotmail.com

36

37 **E-mails**

38 johanpallud@hotmail.com / gilles.huberfeld@college-de-france.fr / e.dezamis@ghu-paris.fr /

39 speeters@mednet.ucla.edu / alessandro.moiraghi@sfits.ch / m.gavaret@ghu-paris.fr /

40 e.guinard@ghu-paris.fr / frederic.dhermain@igr.fr / p.varlet@ghu-paris.fr /

41 c.oppenheim@ghu-paris.fr / f.chretien@ghu-paris.fr / a.roux@ghu-paris.fr / [42 \[paris.fr\]\(mailto:paris.fr\)](mailto:m.zanello@ghu-</p></div><div data-bbox=)

43

44 **Manuscript word count:** 3445

45 **Abstract word count:** 349

46 **Number of figures :** 1

47 **Number of color figures :** 0

48 **Number of tables :** 4

49 **Number of supplementary figures:** 0

50 **Number of supplementary tables: 0**

51

52 **Abbreviation list**

53 HR: Hazard Ratio

54 IDH: Isocitrate Dehydrogenase

55 MGMT: O6-Methylguanine-DNA methyltransferase

56 WHO: World Health Organization

57

58 **Funding Sources**

59 None.

60

61 **Conflicts of Interest**

62 None.

63

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

65

66 **Search Terms**

67 Glioblastoma

68 *IDH*-wildtype

69 Levetiracetam

70 O6-methylguanine-DNA methyltransferase

71 Overall survival

72 Temozolomide

73

74 **Author contribution**

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

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

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

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

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

81

82 **Author Disclosures**

83 JP reports no disclosures relevant to the manuscript.

84 GH reports no disclosures relevant to the manuscript.

85 ED reports no disclosures relevant to the manuscript.

86 SP reports no disclosures relevant to the manuscript.

87 AM reports no disclosures relevant to the manuscript.

88 MG reports no disclosures relevant to the manuscript.

89 EG reports no disclosures relevant to the manuscript.

90 FD reports no disclosures relevant to the manuscript.

91 PV reports no disclosures relevant to the manuscript.

92 CO reports no disclosures relevant to the manuscript.

93 FC reports no disclosures relevant to the manuscript.

94 AR reports no disclosures relevant to the manuscript.

95 MZ reports no disclosures relevant to the manuscript.

96

97

98 **Abstract**

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

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

115 **Results.** 460 patients were included. The median overall survival was longer in the 116
116 patients with Levetiracetam use during the whole duration of the standard chemoradiation
117 protocol (21.0 months; 95%CI, 17.2-24.0) than in the 126 patients with part time
118 Levetiracetam use (16.8 months; 95%CI, 12.4-19.0], and in the 218 patients who never
119 received Levetiracetam (16.0 months; 95%CI, 15.5-19.4; $p=0.027$). Levetiracetam use during
120 the whole duration of the standard chemoradiation protocol (adjusted Hazard Ratio (aHR)
121 0.69; 95%CI, 0.52-0.93; $p=0.014$), O6-Methylguanine-DNA methyltransferase promoter
122 methylation (aHR 0.53; 95%CI, 0.39-0.71; $p<0.001$), and gross total tumor resection (aHR

123 0.57; 95%CI, 0.44-0.74; $p < 0.001$) were independent predictors of a longer overall survival.

124 After case matching (n=54 per group), a longer overall survival was found for Levetiracetam

125 use during the whole duration of the standard chemoradiation protocol (HR=0.63; 95%CI,

126 0.42-0.94, $p=0.023$).

127 **Discussion.** Levetiracetam use during the whole standard chemoradiation protocol possibly

128 improves overall survival of *IDH*-wildtype glioblastoma patients. It should be considered in

129 the anti-tumor strategy of future multicentric trials.

130 **Classification of Evidence.** This study provides Class III evidence that in individuals with

131 *IDH*-wildtype glioblastoma, levetiracetam use throughout the duration of standard

132 chemotherapy is associated with longer median overall survival.

133 **Introduction**

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

143 The presence of epileptic seizures at diagnosis is a predictor of longer survival in patients
144 with diffuse gliomas ⁴⁻⁶. This clinical observation appears paradoxical since both epilepsy and
145 diffuse gliomas share common pathogenic mechanisms and influence each other ^{4,5,7}.
146 Furthermore, neuronal activity has been shown to promote glioma growth ^{8,9}. A first
147 hypothesis would be that less aggressive diffuse gliomas - with expected prolonged survival -
148 may be more conducive to the development of epileptogenic processes, which require
149 surviving neurons and plasticity ⁷. One alternative explanation is that drug therapies targeting
150 the shared pathogenic mechanisms may affect both seizure and tumor control. It has been
151 previously suggested that several antiepileptic drugs, mainly Sodium valproate ¹⁰⁻¹²,
152 Levetiracetam ¹³⁻¹⁵, and Perampanel ¹⁶, can have anti-tumor effects and that Temozolomide
153 chemotherapy can improve seizure control ^{17,18}. In patients with *IDH*-wildtype glioblastomas,
154 epileptic seizures are common during the course of the tumor, with incidence ranging up to
155 30-60%, and require long-term use of antiepileptic drugs ^{4,5}. Therefore, antiepileptic drugs are
156 often combined with Temozolomide during the standard combined chemoradiation protocol.
157 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.

171 **Methods**

172 **Study design**

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

177

178 **Participants**

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

187

188 **Variables and data sources**

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

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

203 The tumor volume (cm³) was calculated using manual segmentation of abnormal signal on
204 post-contrast T1-weighted sequence by one blinded investigator (AR) for every tumor. The
205 extent of resection was quantified using an early postoperative MRI (within 48 hours) and
206 performing manual segmentation of residual enhancing tumor by the same blinded
207 investigator. A gross total resection corresponded to 100% resection of the enhancing signal
208 on post-contrast T1-weighted sequence. All other cases were considered partial resections.

209 Overall survival was measured from the date of surgery to the date of death from any cause.

210 Surviving patients were censored at the date of last follow-up.

211

212 **Statistical analyses**

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

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

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

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

242

243 **Standard protocol approvals, registrations and patient consents**

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

247

248 **Data availability**

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

251 **Results**

252 **Patient and tumor characteristics**

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

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

259

260 **Epilepsy and anti-epileptic drug therapy**

261 One hundred and sixty-two patients (35.2%) had epileptic seizures at the time of diagnosis.

262 One hundred and seventy-one patients were taking at least one antiepileptic drug at the time
263 of diagnosis (37.2%), consisting in one drug for 150 patients, two drugs for 20 patients, and
264 three drugs for one patient. Of the 162 patients who had experienced epileptic seizures at
265 diagnosis, 155 (95.7%) were on antiepileptic drug therapy, and of the 298 patients who had
266 not experienced seizures, 16 (5.4%) were on antiepileptic drug therapy. One hundred and
267 twenty-nine patients were taking Levetiracetam (representing 75.4% of the treated patients) at
268 the beginning of the standard combined chemoradiation protocol. Levetiracetam was
269 discontinued in 13 patients and introduced in 113 patients during the standard combined
270 chemoradiation protocol. One hundred and sixteen patients (25.2%) received Levetiracetam
271 during the whole duration of the standard combined chemoradiation protocol, 145 (31.5%)
272 received Levetiracetam part time during the standard combined chemoradiation protocol, and
273 199 (43.3%) never received Levetiracetam during the standard combined chemoradiation
274 protocol.

275

276 Survival Analysis

277 The median duration of follow-up was 16.5 months [95% CI, 3.1-44.1]. Three hundred and
278 sixty-five patients (79.3%) died over the follow-up period. The median overall survival was
279 17.4 months [95% CI, 16.3-19.0] for the whole population.

280 Unadjusted Hazard Ratio's (HR) for overall survival in the whole series are detailed in Table
281 2. In a univariate analysis, Levetiracetam use during the whole duration of the standard
282 combined chemoradiation protocol (excluding the part time or never subgroups), together
283 with younger age (<60 years old), absence of neurological deficit, Radiation Therapy
284 Oncology Group Recursive Partitioning Analysis classes 3-4, Karnofsky Performance Status
285 score ≥ 70 , *MGMT* promoter methylation, and total tumor resection were associated with
286 longer overall survival. The median overall survival was longer in the 116 patients with
287 Levetiracetam use during the whole duration of the standard combined chemoradiation
288 protocol (21.0 months; 95% CI, 17.2-24.0) than in the 145 patients with Levetiracetam use
289 part time during the standard combined chemoradiation protocol (16.8 months; 95% CI, 12.4-
290 19.0], and the 199 patients who never received Levetiracetam (16.0 months; 95% CI, 15.5-
291 19.4; $p=0.027$). Kaplan-Meier survival curves are shown in Figure 1. Adjusted HRs for
292 overall survival in the whole series are detailed in Table 2. After multiple adjustments using
293 Cox models, Levetiracetam use during the whole duration of the standard combined
294 chemoradiation protocol (aHR 0.69; 95%CI, 0.52-0.93; $p=0.014$), *MGMT* promoter
295 methylation (aHR 0.53; 95%CI, 0.39-0.71; $p<0.001$), partial tumor resection (aHR 0.61;
296 95%CI, 0.40-0.90; $p=0.0145$), and gross total tumor resection (aHR 0.57; 95%CI, 0.44-0.74;
297 $p<0.001$) were independent predictors of longer overall survival.

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

301 subgroups), together with Karnofsky Performance Status score ≥ 70 , *MGMT* promoter
302 methylation, and gross total tumor resection were associated with longer overall survival. The
303 median overall survival was longer in the 108 patients with Levetiracetam use during the
304 whole duration of the standard combined chemoradiation protocol (21.0 months; 95% CI,
305 17.6-24.9) than in the 19 patients with Levetiracetam use part time during the standard
306 combined chemoradiation protocol (19.2 months; 95% CI, 10.0-34.0], and the 35 patients
307 who never received Levetiracetam (16.3 months; 95% CI, 13.5-18.3; $p=0.019$). Adjusted HRs
308 for overall survival in the subgroup of patients with epileptic seizures at diagnosis are detailed
309 in Table 3. After multiple adjustments using Cox models, Levetiracetam use during the whole
310 duration of the standard combined chemoradiation protocol (aHR 0.45; 95%CI, 0.27-0.73;
311 $p=0.001$), Levetiracetam use part time during the standard combined chemoradiation protocol
312 (aHR 0.56; 95%CI, 0.27-0.72; $p=0.022$), *MGMT* promoter methylation (aHR 0.53; 95%CI,
313 0.37-0.76; $p<0.001$) and gross total tumor resection (aHR 0.45; 95%CI, 0.31-0.66; $p<0.001$)
314 were independent predictors of a longer overall survival.

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

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

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

336 **Discussion**

337 **Key results**

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

345

346 **Interpretation**

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

361 gliomas, induces synchronized calcium releases that ultimately promotes glioma progression
362 ^{32,33}. Since Levetiracetam displays anti-AMPA effects ³⁴, its long-term use may have
363 neuromodulating effects on these neuroglial synapses, contributing to inhibition of glioma
364 progression ¹³. Lastly, chloride dynamics also play a role in regulating glioma cell growth,
365 with intracellular chloride accumulation favoring tumor progression. The chloride equilibrium
366 may be disrupted by chloride permeable GABA_A receptor activation ⁴. Levetiracetam has
367 been shown to affect both intracerebral GABA concentration ³⁵ and GABA_A receptors, ³⁶
368 likely affecting glioma cell dynamics. Similarly, a case report illustrating continuous
369 regression of a glioblastoma in a patient who received Levetiracetam and Dexamethasone
370 without Temozolomide or other cancer-targeted therapy, suggested that Levetiracetam could
371 have a direct anti-tumor effect ³⁷.

372 The present results confirm findings from previous studies, including series of 103, 332, and
373 418 patients with glioblastomas, respectively, who received standard combined
374 chemoradiation protocol with Temozolomide, which all suggested that Levetiracetam use
375 provided a survival benefit ¹³⁻¹⁵. These observations conflict with those from two large studies
376 where Levetiracetam use did not provide any significant survival benefit. The study by
377 Happold et al., which aggregated 1869 patients with a newly diagnosed glioblastoma from
378 four randomized clinical trials between 2000-2011 ²⁴, included patients with varying
379 oncological treatments (radiotherapy alone, standard combined chemoradiation protocol,
380 Cilengitide, Bevacizumab), did not detail the *IDH1/2* mutation status, and did not provide the
381 duration of Levetiracetam use. The study by Knudsen-Baas et al., which registered 1263
382 glioblastomas diagnosed in Norway between 2004-2010 ²⁵, included patients with varying
383 oncological treatments, did not detail the *IDH1/2* mutation and the *MGMT* promoter
384 methylation statuses, and did not provide the duration of Levetiracetam use. In those two
385 studies, the possible inclusion of *IDH*-mutant glioblastomas with better prognosis, of patients

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

390 The present study reports, for the first time, a link between the overall survival and the use of
391 Levetiracetam during the whole duration of the standard combined chemoradiation protocol
392 with Temozolomide. This suggests Levetiracetam might display anti-tumor properties, in
393 adjunct to standard combined chemoradiation protocol for newly diagnosed supratentorial
394 *IDH*-wildtype glioblastomas in adults. On a practical basis, the present results suggest that: 1)
395 the choice of the antiepileptic drug in patients with an *IDH*-wildtype glioblastoma should be
396 carefully considered because it may affect overall survival; 2) Levetiracetam could be
397 proposed as the first antiepileptic drug in patients presenting with epileptic seizures at
398 diagnosis in the setting of a newly diagnosed supratentorial *IDH*-wildtype glioblastoma, due
399 first to its efficacy, second to its good safety profile, third to its lack of interference with
400 chemotherapy and fourth to its possible favorable effects on survival. In addition, the present
401 results open the door for future questions that remain to be solved: 1) the introduction of
402 Levetiracetam could be discussed at the time of diagnosis, eventually even in patients without
403 epileptic seizures, while carefully weighing the risks of adverse effects related to
404 Levetiracetam, including neuropsychiatric ones⁴⁰; 2) Levetiracetam could be pursued during
405 the whole duration of the standard combined chemoradiation protocol with Temozolomide,
406 including the concomitant chemoradiotherapy and adjuvant chemotherapy periods, even in
407 seizure-controlled patients.

408

409 **Generalizability**

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

425

426 **Limitations**

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

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

443

444 **Conclusion**

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

451 **Fundings**

452 This work was supported by grants from the European Research Council (consolidator grant
453 #865592 to GH) and from the Cancéropole Ile-de-France (grant EMERG-2 2016 to JP).

454

455 **Competing interests**

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

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

464 **References**

- 465 1. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and
466 adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987–996.
- 467 2. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and
468 adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a
469 randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncology*.
470 Elsevier Ltd; 2009;10:459–466.
- 471 3. Hegi ME, Diserens A-C, Gorlia T, et al. MGMT gene silencing and benefit from
472 temozolomide in glioblastoma. *N Engl J Med*. 2005;352:997–1003.
- 473 4. Huberfeld G, Vecht CJ. Seizures and gliomas — towards a single therapeutic approach.
474 *Nat Rev Neurol*. 2016;12:204–216.
- 475 5. Pallud J, Capelle L, Huberfeld G. Tumoral epileptogenicity: How does it happen?
476 *Epilepsia*. 2013;54:29–33.
- 477 6. Pallud J, Audureau E, Blonski M, et al. Epileptic seizures in diffuse low-grade gliomas in
478 adults. *Brain*. 2014;137:449–462.
- 479 7. Pallud J, Le Van Quyen M, Bielle F, et al. Cortical GABAergic excitation contributes to
480 epileptic activities around human glioma. *Science Translational Medicine*.
481 2014;6:244ra89–244ra89.
- 482 8. Venkatesh HS, Tam LT, Woo PJ, et al. Targeting neuronal activity-regulated neuroligin-
483 3 dependency in high-grade glioma. *Nature*. Nature Publishing Group; 2017;549:533–
484 537.
- 485 9. Tantillo E, Vannini E, Cerri C, et al. Differential roles of pyramidal and fast-spiking,
486 GABAergic neurons in the control of glioma cell proliferation. *Neurobiol Dis*.
487 2020;141:104942.

- 488 10. Jaeckle KA, Ballman K, Furth A, Buckner JC. Correlation of enzyme-inducing
489 anticonvulsant use with outcome of patients with glioblastoma. *Neurology*.
490 2009;73:1207–1213.
- 491 11. Oberndorfer S, Piribauer M, Marosi C, Lahrmann H, Hitzenberger P, Grisold W. P450
492 enzyme inducing and non-enzyme inducing antiepileptics in glioblastoma patients treated
493 with standard chemotherapy. *J Neurooncol*. 2005;72:255–260.
- 494 12. Weller MM, Gorlia TT, Cairncross JGJ, et al. Prolonged survival with valproic acid use
495 in the EORTC/NCIC temozolomide trial for glioblastoma. *Neurology*. 2011;77:1156–
496 1164.
- 497 13. Roh TH, Moon JH, Park HH, et al. Association between survival and levetiracetam use in
498 glioblastoma patients treated with temozolomide chemoradiotherapy. *Scientific Reports*.
499 Nature Publishing Group; 2020;10:10783–10787.
- 500 14. Kim Y-H, Kim T, Joo J-D, et al. Survival benefit of levetiracetam in patients treated with
501 concomitant chemoradiotherapy and adjuvant chemotherapy with temozolomide for
502 glioblastoma multiforme. *Wiley-Blackwell*; 2015;121:2926–2932.
- 503 15. Ryu JY, Min KL, Chang MJ. Effect of anti-epileptic drugs on the survival of patients
504 with glioblastoma multiforme: A retrospective, single-center study. *PLoS ONE*.
505 2019;14:e0225599.
- 506 16. Izumoto S, Miyauchi M, Tasaki T, et al. Seizures and Tumor Progression in Glioma
507 Patients with Uncontrollable Epilepsy Treated with Perampanel. *Anticancer Res*.
508 International Institute of Anticancer Research; 2018;38:4361–4366.
- 509 17. Ruda R, Bello L, Duffau H, Soffietti R. Seizures in low-grade gliomas: natural history,
510 pathogenesis, and outcome after treatments. *Neuro-Oncology*. 2012;14:iv55–iv64.

- 511 18. Climans SA, Brandes AA, Cairncross JG, et al. Temozolomide and seizure outcomes in a
512 randomized clinical trial of elderly glioblastoma patients. *J Neurooncol.* Springer US;
513 2020;149:65–71.
- 514 19. van Breemen MSM, Wilms EB, Vecht CJ. Epilepsy in patients with brain tumours:
515 epidemiology, mechanisms, and management. *Lancet neurology.* 2007;6:421–430.
- 516 20. de Groot M, Douw L, Sizoo EM, et al. Levetiracetam improves verbal memory in high-
517 grade glioma patients. *Neuro-Oncology.* 2013;15:216–223.
- 518 21. Lee JW, Bromfield EB, Kesari S. Antiemetic properties of the antiepileptic drug
519 levetiracetam. *N Engl J Med.* 2008;359:1853–1853.
- 520 22. Vecht C, Royer-Perron L, Houillier C, Huberfeld G. Seizures and Anticonvulsants in
521 Brain Tumours: Frequency, Mechanisms and Anti-Epileptic Management. *Curr Pharm*
522 *Des.* 2017;23:6464–6487.
- 523 23. Chassoux F, Landré E. Prevention and management of postoperative seizures in neuro-
524 oncology. *Neuro-Chirurgie.* 2017;63:197–203.
- 525 24. Happold C, Gorlia T, Chinot O, et al. Does Valproic Acid or Levetiracetam Improve
526 Survival in Glioblastoma? A Pooled Analysis of Prospective Clinical Trials in Newly
527 Diagnosed Glioblastoma. *Journal of Clinical Oncology.* 2016;34:731–739.
- 528 25. Knudsen-Baas KM, Engeland A, Gilhus NE, Storstein AM, Owe JF. Does the choice of
529 antiepileptic drug affect survival in glioblastoma patients? *J Neurooncol.* Springer US;
530 2016;129:461–469.
- 531 26. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classi-
532 cation of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* Springer
533 Berlin Heidelberg; 2016;131:803–820.
- 534 27. Brat DJ, Aldape K, Colman H, et al. cIMPACT-NOW update 3: recommended diagnostic
535 criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of

- 536 glioblastoma, WHO grade IV". *Acta Neuropathol.* Springer Berlin Heidelberg;
537 2018;136:805–810.
- 538 28. Mirimanoff R-O, Gorlia T, Mason W, et al. Radiotherapy and temozolomide for newly
539 diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-
540 NCIC CE3 phase III randomized trial. *J Clin Oncol.* 2006;24:2563–2569.
- 541 29. Ni X-R, Guo C-C, Yu Y-J, et al. Combination of levetiracetam and IFN- α increased
542 temozolomide efficacy in MGMT-positive glioma. *Cancer Chemother Pharmacol.*
543 Springer Berlin Heidelberg; 2020;86:773–782.
- 544 30. Bobustuc GC, Baker CH, Limaye A, et al. Levetiracetam enhances p53-mediated MGMT
545 inhibition and sensitizes glioblastoma cells to temozolomide. *Neuro-Oncology.*
546 2010;12:917–927.
- 547 31. Scicchitano BM, Sorrentino S, Proietti G, et al. Levetiracetam enhances the
548 temozolomide effect on glioblastoma stem cell proliferation and apoptosis. *Cancer Cell*
549 *Int. BioMed Central*; 2018;18:136–18.
- 550 32. Venkatesh HS, Morishita W, Geraghty AC, et al. Electrical and synaptic integration of
551 glioma into neural circuits. *Nature.* Nature Publishing Group; 2019;573:539–545.
- 552 33. Venkataramani V, Tanev DI, Strahle C, et al. Glutamatergic synaptic input to glioma
553 cells drives brain tumour progression. *Nature.* Nature Publishing Group; 2019;573:532–
554 538.
- 555 34. Carunchio I, Pieri M, Ciotti MT, Albo F, Zona C. Modulation of AMPA receptors in
556 cultured cortical neurons induced by the antiepileptic drug levetiracetam. *Epilepsia.* John
557 Wiley & Sons, Ltd; 2007;48:654–662.
- 558 35. Doelken MT, Doelken MT, Hammen T, et al. Alterations of intracerebral γ -aminobutyric
559 acid (GABA) levels by titration with levetiracetam in patients with focal epilepsies.
560 *Epilepsia.* John Wiley & Sons, Ltd; 2010;51:1477–1482.

- 561 36. Rigo J-M, Hans G, Nguyen L, et al. The anti-epileptic drug levetiracetam reverses the
562 inhibition by negative allosteric modulators of neuronal GABA- and glycine-gated
563 currents. *Br J Pharmacol*. John Wiley & Sons, Ltd; 2002;136:659–672.
- 564 37. Peddi P, Ajit NE, Burton GV, El-Osta H. Regression of a glioblastoma multiforme:
565 spontaneous versus a potential antineoplastic effect of dexamethasone and levetiracetam.
566 *BMJ Case Reports*. BMJ Publishing Group; 2016;2016:bcr2016217393.
- 567 38. Dewan MC, Thompson RC, Kalkanis SN, Barker FG, Hadjipanayis CG. Prophylactic
568 antiepileptic drug administration following brain tumor resection: results of a recent
569 AANS/CNS Section on Tumors survey. *J Neurosurg*. American Association of
570 Neurological Surgeons; 2017;126:1772–1778.
- 571 39. Glantz MJ, Cole BF, Forsyth PA, et al. Practice parameter: Anticonvulsant prophylaxis in
572 patients with newly diagnosed brain tumors Report of the Quality Standards
573 Subcommittee of the American Academy of Neurology. *Neurology*. AAN Enterprises;
574 2000;54:1886–1893.
- 575 40. Bedetti C, Romoli M, Maschio M, et al. Neuropsychiatric adverse events of antiepileptic
576 drugs in brain tumour-related epilepsy: an Italian multicentre prospective observational
577 study. *Eur J Neurol*; 2017;24(10):1283-1289.

578 **Table and Figure legends**

579

580 **Table 1.**

581 Characteristics of the population (n=460).

582

583 **Table 2.**

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

585

586 **Table 3.**

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

589

590 **Table 4.**

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

594

595 **Figure 1. Kaplan-Meier estimates of overall survival.**

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

597 B. Kaplan-Meier estimates of overall survival, according to Levetiracetam use during the
598 combined standard chemoradiation protocol (whole duration of the combined standard
599 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									0.016
	Male	190	41.3	35	30.2	67	46.2	88	44.2	
		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
604
605
606

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
* Quantitative assessment based on preoperative tumor volume using contrast-enhanced three-dimensional T1-weighted sequence.
‡ Quantitative assessment based on preoperative tumor volume and postoperative (MRI performed within 48h) tumor volume using contrast-enhanced three-dimensional T1-weighted sequence.

607 **Table 2. Univariate and multivariate predictors of overall survival in the whole series**
 608 **(n=460).**
 609

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)				
	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)				
	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)				
	Yes	20.0	0.77	0.62 - 0.96	0.023	0.84	0.66 - 1.07 0.154

610 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			

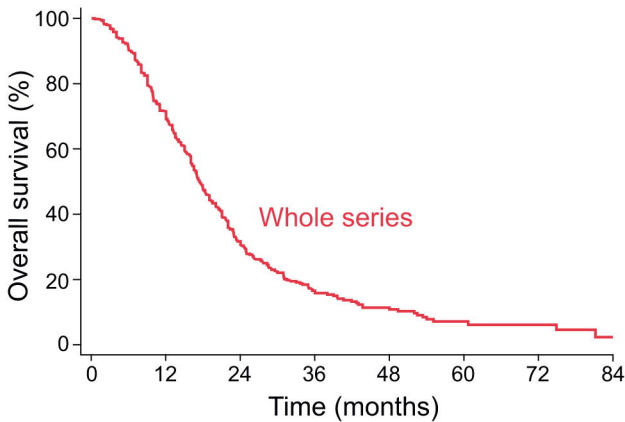
916 Confidence Interval; HR: Hazard Ratio; KPS: Karnofsky Performance Status; MGMT: O6-methylguanine-DNA methyltransferase; OS: Overall Survival; RTOG: Radiation Therapy Oncology
 916 Group; RPA: Recursive Partitioning Analysis

617 **Table 4. Levetiracetam use subgroups during standard combined chemoradiation**
 618 **protocol with Temozolomide (whole duration versus part time or never) paired by**
 619 **matching criteria (n=54 pairs).**
 620

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

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

A

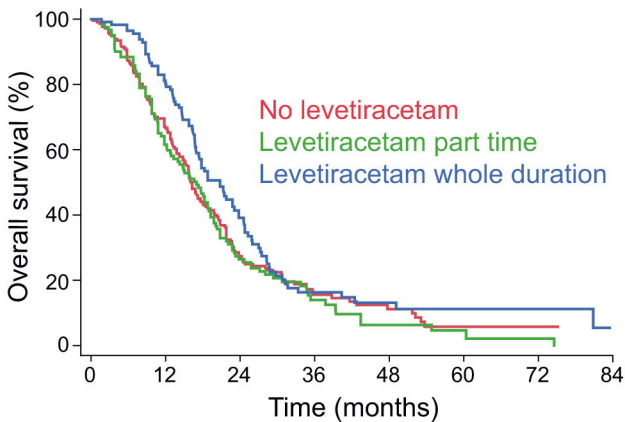


Number at risk

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