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**Authors:** Wick W, Gorlia T, Bady P, Platten M, van den Bent MJ, Taphoorn MJ, Steuve J, Brandes AA, Hamou MF, Wick A, Kosch M, Weller M, Stupp R, Roth P, Golfopoulos V, Frenel JS, Campone M, Ricard D, Marosi C, Villa S, Weyerbrock A, Hopkins K, Homicsko K, Lhermitte B, Pesce G, Hegi ME

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1 **Phase II study of radiotherapy and temsirolimus versus radiochemotherapy**  
2 **with temozolomide in patients with newly diagnosed glioblastoma without**  
3 ***MGMT* promoter hypermethylation (EORTC 26082)**

4  
5 <sup>1,2</sup>Wolfgang Wick, <sup>3</sup>Thierry Gorlia, <sup>4,5</sup>Pierre Bady, <sup>1,6</sup>Michael Platten, <sup>7</sup>Martin J van den Bent,  
6 <sup>8</sup>Martin JB Taphoorn, <sup>3</sup>Jonathan Steuve, <sup>9</sup>Alba A. Brandes, <sup>5,10</sup>Marie-France Hamou, <sup>1</sup>Antje  
7 Wick, <sup>11</sup>Markus Kosch, <sup>13</sup>Michael Weller, <sup>10</sup>Roger Stupp, <sup>13</sup>Patrick Roth, <sup>3</sup>Vassilis  
8 Golfopoulos, <sup>12</sup>Jean-Sebastien Frenel, <sup>12</sup>Mario Campone, <sup>14</sup>Damien Ricard, <sup>15</sup>Christine  
9 Marosi, <sup>16</sup>Salvador Villa, <sup>17</sup>Astrid Weyerbrock, <sup>18</sup>Kirsten Hopkins, <sup>19</sup>Krisztian Homicsko,  
10 <sup>20</sup>Benoit Lhermitte, <sup>21</sup>Gianfranco Pesce, <sup>5,10</sup>Monika E Hegi

11  
12 <sup>1</sup>Neurology Clinic, University of Heidelberg and <sup>2</sup>Clinical Cooperation Unit (CCU)  
13 Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center  
14 (DKFZ), Heidelberg, Germany; <sup>3</sup>European Organisation for Research and Treatment of  
15 Cancer (EORTC); <sup>4</sup>SIB Swiss Institute of Bioinformatics, <sup>5</sup>Neuroscience Research Center,  
16 University Hospital Lausanne (CHUV), both Lausanne, Switzerland; <sup>6</sup>CCU Brain Tumor  
17 Immunology, DKFZ, Heidelberg, Germany; <sup>7</sup>Department of Neurology/Neuro-Oncology,  
18 Erasmus MC - Cancer Institute, Rotterdam; <sup>8</sup>Neuro-oncology Unit, MC Haaglanden, The  
19 Hague, both The Netherlands; <sup>9</sup>Department of Medical Oncology, Ospedale Bellaria,  
20 Bologna, Italy; <sup>10</sup>Department of Neurosurgery, CHUV, Lausanne, Switzerland; <sup>11</sup>Pfizer,  
21 Berlin, Germany; <sup>12</sup>1-Institut de Cancérologie de l'OUEST, Saint Herblain-Nantes Cedex,  
22 France; <sup>13</sup>Department of Neurology, University Hospital and University of Zurich; <sup>14</sup>AP-HP,  
23 Groupe Hospitalier Pitié-Salpêtrière, Service de Neurologie 2-Mazarin; UMR8257 MD4  
24 Cognac G, CNRS, Service de Santé des Armées, Université Paris Descartes, Paris, France;  
25 <sup>15</sup>General Hospital AKH, Medical University Vienna, Austria; <sup>16</sup>Institut Catala d'Oncologia  
26 (ICO). Hospital Germans Trias Pujol, Badalona, Barcelona, Spain; <sup>17</sup>Department of  
27 Neurosurgery, Medical Center - University of Freiburg, Germany; <sup>18</sup>University Hospitals

28 Bristol NHS Foundation Trust - Bristol Haematology and Oncology Centre, Bristol, U.K.;

29 <sup>19</sup>Department of Oncology, <sup>20</sup>Institute of Pathology, both CHUV, Lausanne, Switzerland;

30 <sup>21</sup>Department of Radio-oncology, Oncology Institute of Southern Switzerland, Bellinzona; all

31 Switzerland.

32

33 **Running Head:** Temsirolimus for newly diagnosed glioblastoma

34

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36

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39

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42

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44

45 **Address correspondence to:** Wolfgang Wick, MD

46 Neurology Clinic & National Centre for Tumour Disease

47 University of Heidelberg

48 Im Neuenheimer Feld 400, D-69120 Heidelberg, Germany

49 Tel.: +49 (0)6221/56-7075

50 Fax: +49 (0)6221/56-7554

51 E-mail: wolfgang.wick@med.uni-heidelberg.de

52

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59 R.S., M.J.B.T., M.W. and W.W. have a consultant relationship with Genentech/Roche. M.K.  
60 is an employee of Pfizer, the manufacturer of Temsirolimus.

61 M.E.H. has served on advisory boards for MSD, Genentech/Roche, and MDxHealth, and  
62 has provided services to Novocure.

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65

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69 Supplemental Information

70

71 **Statement of clinical relevance:** The prospective randomized EORTC 26082 trial  
72 assessed the tolerability and efficacy of the mechanistic target of rapamycin (mTOR)  
73 inhibitor temsirolimus in patients with newly diagnosed, *O6 methylguanine-DNA-*  
74 *methyltransferase (MGMT)* promoter unmethylated glioblastoma. Temozolomide could be  
75 omitted without detriment in the experimental arm. Efficacy of radiotherapy plus  
76 temsirolimus failed to reach the pre-specified number of patients alive at 12 months. Pre-  
77 specified assessment of activity in the mTOR pathway allows to suggest that one third of  
78 patients with phosphorylated mTOR at Ser2448 derive a robust and clinically relevant  
79 survival benefit and will be candidates for clinical development of temsirolimus as a targeted  
80 therapy in a molecularly defined subgroup.

81

82

83 **ABSTRACT**

84

85 **Purpose:** EORTC 26082 assessed the activity of temsirolimus in patients with newly  
86 diagnosed glioblastoma harboring an unmethylated O6 methylguanine-DNA-  
87 methyltransferase (*MGMT*) promoter.

88 **Patients and Methods:** Patients (n=257) fulfilling eligibility criteria underwent central *MGMT*  
89 testing. Patients with *MGMT* unmethylated glioblastoma (n=111) were randomized 1:1  
90 between standard chemo-radiotherapy with temozolomide or radiotherapy plus weekly  
91 temsirolimus (25 mg). Primary endpoint was overall survival at 12 months (OS12). A positive  
92 signal was considered >38 patients alive at 12 months in the per protocol population. A non-  
93 comparative reference arm of 54 patients evaluated the assumptions on OS12 in a standard-  
94 treated cohort of patients. Pre-specified post hoc analyses of markers reflecting target  
95 activation were performed.

96 **Results:** Both therapies were administered per protocol with a median of 13 cycles of  
97 maintenance temsirolimus. Median age was 55 and 58 years in the temsirolimus and  
98 standard arms, the WHO performance status 0 or 1 for most patients (95.5%). In the per  
99 protocol population, 38 of 54 patients treated with temsirolimus reached OS12. The actuarial  
100 1-year survival was 72.2% [95% CI (58.2-82.2)] in the temozolomide arm and 69.6% [95%  
101 CI (55.8-79.9)] in the temsirolimus arm [HR=1.16, 95% CI (0.77-1.76), p=0.47]. In  
102 multivariable prognostic analyses of clinical and molecular factors phosphorylation of  
103 mTORSer2448 in tumor tissue (HR=0.13, 95% CI (0.04-0.47), p=0.002), detected in 37.6%,  
104 was associated with benefit from temsirolimus.

105 **Conclusions:** Temsirolimus was not superior to temozolomide in patients with an  
106 unmethylated *MGMT* promoter. Phosphorylation of mTORSer2448 in the pretreatment tumor  
107 tissue may define a subgroup benefitting from mTOR inhibition.

108

109

110 **INTRODUCTION**

111

112 The serine/threonine kinase, mechanistic target of rapamycin (mTOR) serves as a hub  
113 integrating multiple intra- and extracellular cues in cancer cells (1). mTOR is involved in the  
114 formation of two multi-protein complexes, mTORC1 and mTORC2, that direct cell  
115 metabolism, growth, proliferation, survival, and angiogenesis.

116 Preclinical studies suggested an enhanced activity of mTOR inhibition in PTEN-deficient  
117 tumour models (2, 3).

118 Activation of the PI3K/AKT/mTOR pathway has been associated with reduced survival of  
119 glioma patients (4) and this signalling pathway has been subjected to a number of negative  
120 single- or multi-targeted therapies including the mTOR inhibitor rapamycin or its derivatives,  
121 the 'rapalogs' everolimus (RAD001), deforolimus (AP23573), and temsirolimus (CCI-779) (5-  
122 9).

123 The experience with temozolomide (TMZ) teaches that limited activity at recurrence (10)  
124 may still relevantly modify the disease in patients with newly diagnosed glioblastoma when  
125 combined with radiotherapy (11). Accordingly, mTOR inhibition has been considered an  
126 option for patients with treatment-naïve glioblastomas that likely lack some of the  
127 mechanisms of resistance acquired at recurrence.

128 Temsirolimus (Torisel<sup>®</sup>) has been approved for advanced renal cell carcinoma (12) and  
129 relapsed or refractory mantle cell lymphoma (13). Additive effects of temsirolimus plus  
130 radiotherapy (RT) in preclinical models demonstrate that temsirolimus could complement the  
131 genotoxic activity of RT in the treatment of newly diagnosed glioblastoma. However,  
132 combination of TMZ and temsirolimus plus RT was too toxic (14).

133 Therefore, the rationale of this study was to test the biological effects of mTOR inhibition  
134 when combined with ionizing radiation in patients in whom TMZ could be safely omitted. To  
135 this end patients with tumors with an unmethylated *O6 methyguanine-DNA-*  
136 *methytransferase (MGMT)* gene promoter were selected for the trial, as they derive little if  
137 any benefit from the addition of TMZ (15). Another aim was to identify biological factors, i.e.

138 biomarkers linked to benefit from mTOR inhibition. Temsirolimus may counteract therapy-  
139 induced angiogenesis and invasion (16, 17).  
140

141 **PATIENTS AND METHODS**

142

143 ***Clinical Trial***144 *Study design and treatment*

145 Patients for EORTC 26082 (NCT01019434) were recruited at 14 study sites in 10 countries  
146 in Europe. First, patients were registered after consenting for independent pathology review  
147 and central testing of the *MGMT* promoter methylation status by licensed laboratories of  
148 MDxHealth (Herstal, Belgium) using quantitative methylation-specific polymerase chain  
149 reaction of DNA isolated from macro-dissected formalin fixed paraffin embedded tumor  
150 sections (18). Patients were considered *MGMT* unmethylated, applying a safety margin,  
151 when the ratio of *MGMT* to the control gene *ACTB* was  $< 0.6$ , calculated as (methylated  
152 *MGMT/ACTB*) $\times 1000$ . This corresponds to the lower bound of the 95% confidence interval  
153 established in a cohort of 602 glioblastoma samples screened in the CENTRIC trial where  
154 the cut-off corresponding to the established nadir was at a ratio of 2 that separates  
155 methylated from unmethylated. (19) as visualized in **Supplementary Figure S1**. A minimum  
156 of 1,250 copies of *ACTB* were required for a valid result, unless the copy number for  
157 methylated *MGMT* was ten or more, which was scored as *MGMT* methylated.

158 Eligible patients (see **Supplementary Information**) were randomly assigned to receive  
159 either standard chemoradiotherapy (TMZ/RT $\rightarrow$ TMZ) (11), or standard fractionated RT with  
160 concomitant temsirolimus (standard dose of 25 mg i.v. weekly beginning at day -7 from the  
161 start of RT, to be continued until disease progression) (**Figure 1 and Supplement**). The  
162 study was conducted according to the Declaration of Helsinki, the International Conference  
163 on Harmonisation note for good clinical practice (Topic E6, 1996), and regulatory  
164 requirements.

165 This study was funded by a grant from Pfizer, Berlin, Germany (details on the Role of the  
166 Funding Source in the **Supplement**).

167

168 *Randomisation and masking*

169 Randomisation was performed centrally using an interactive voice response system.  
170 Patients were stratified according to age, WHO performance status and baseline steroids.  
171 As this was an open-label study, no blinding procedures were applied.

172

### 173 *Study endpoints*

174 The primary endpoint was overall survival at 12 months (OS12) to avoid issues around  
175 pseudoprogression and generate a timely signal. Secondary endpoints included  
176 progression-free survival (PFS), OS, safety and assessment of prognostic and predictive  
177 biomarkers.

178

### 179 *Outcome measures and statistical analyses*

180 OS12 was defined as the fraction of patients alive at 12 months from randomisation; PFS  
181 was defined as duration from randomisation until first observation of PD or death from any  
182 cause or censored at last disease assessment without progression or start of second anti-  
183 cancer therapy; OS was defined as time from randomisation until death or last visit.

184 PFS was assessed locally by investigators according to the Macdonald criteria (20), in case  
185 of suspected pseudoprogression investigators were advised to continue treatment *per*  
186 *protocol* and repeat imaging after 1-2 months. If progression was confirmed, the date of first  
187 observation of tumor progress was used for the analyses.

188 Adverse events (AEs) were coded according to the Medical Dictionary for Regulatory  
189 Activities version 15.0, and their severity was graded according to National Cancer Institute  
190 Common Terminology Criteria for Adverse Events version 3.0.

191 A Fleming one-sample one-stage testing procedure was used in each arm. It was assumed  
192 that with OS12 lower or equal to 60% (P0) the therapeutic activity of temsirolimus (CCI-779)  
193 was too low(11). While a OS12 greater or equal to 80% (P1) implied that the therapeutic  
194 activity of temsirolimus (CCI-779) was adequate Type I ( $\alpha$ ) and II ( $\beta$ ) errors were both equal  
195 to 5%. Under these hypotheses, a sample size of 54 eligible patients in each arm was

196 required. The decision rule was that if >38 eligible patients were alive at 1 year, it was  
197 concluded that the therapeutic activity of temsirolimus was adequate.

198 All statistical analyses were performed on mature data (median follow-up 32 months) by  
199 Thierry Gorlia. The concept of a non-comparative control arm allows for adjustment of the  
200 initial assumptions based on contemporary control treatment. The trial would be insufficient  
201 to confirmatory declare efficacy. However, statistical comparisons are still valid and useful  
202 for hypothesis-generation and exploratory analyses.

203 The OS12 was also computed in the TMZ/RT→TMZ arm in order to assess the consistency  
204 with P0.

205

#### 206 ***Biomarker substudy***

##### 207 *Tissue Micro Array, Immunohistochemistry and FISH EGFR*

208 Tissue micro arrays (TMA) were constructed using recipient paraffin blocks with an agarose  
209 matrix (21). Immunohistochemical analyses and Fluorescent *In Situ* Hybridization (FISH)  
210 were performed in duplicate on sections from 2 replicate TMAs basically as recommended  
211 by the manufacturers (see supplemental methods for antibody description, conditions and  
212 dilutions; FISH probes). Markers for *post hoc* analyzes of the mTOR pathway were pre-  
213 specified in the protocol (phosphorylated S6 ribosomal protein, p-S6RP<sup>Ser235/236</sup>;  
214 phosphorylated AKT, p-AKT<sup>Ser473</sup>; PTEN; phosphorylated AKT1 Substrate 1 (proline-rich),  
215 p-PRAS40<sup>Thr246</sup>; phosphorylated extracellular signal-regulated kinase, ERK1/2<sup>Thr202/Tyr204</sup>) or  
216 based on a more recent study (phosphorylated p-mTOR<sup>Ser2448</sup>) (22, 23). Scoring and  
217 definition of dichotomization is detailed in the Supplemental Methods.

218

##### 219 *Multidimensional marker analysis*

220 The centered score table of the markers containing missing values was analysed by  
221 principal component analysis. Non-linear Iterative Partial Least Squares (NIPALS) algorithm  
222 (24) was used to perform singular-value decomposition with missing value and to complete

223 the data. A consensus hierarchical clustering analysis (25) based on Euclidean distance and  
224 Ward's algorithm was used to investigate the optimal number of clusters. The association  
225 among marker scores was illustrated by network representation based on Spearman  
226 correlation. Analyses and graphical representations were performed using R-3.2.0 and the R  
227 packages mixOmics, qgraphs (26) and ConsensusClusterPlus.

228

229 *Statistical analysis*

230 The scores of the P-markers were dichotomized into negative (scores 0, 1, corresponding to  
231 0 to 10%) vs positive (scores 2 to 5, >10%). Study stratification factors (age, WHO  
232 performance status, baseline steroids) and molecular markers were correlated to OS.

233 Treatment arms were compared with a log-rank test at 5 % significance. For each of them,  
234 PFS and OS were estimated using the Kaplan-Meier (KM) method. Associations of marker  
235 profiles with treatment efficacy were presented by Forest Plot and significance was  
236 assessed with the test for interaction computed from a Cox model including the treatment,  
237 the marker and their interaction term. A 5% significance was used for screening predictive  
238 markers. For each factor, univariable survival estimates were calculated using the KM  
239 technique in the TMZ and temsirolimus arms. Hazard Ratios obtained from univariable Cox  
240 models were presented with 95 % Confidence Intervals (CI) (details in the **Supplement**).

241

242 **RESULTS**

243

244 ***Patients***

245 Overall, 257 patients were registered, screened for eligibility and assessed for *MGMT*  
246 promoter methylation status, whereof 28 patients were registered after screening through the  
247 CENTRIC trial that selected *MGMT* methylated patients only (19); 190 patients were found  
248 to have glioblastoma with an unmethylated *MGMT* promoter applying the cut-off with a  
249 safety margin (Figure S1). The primary reasons for initially registered patients not to  
250 continue to randomisation were hypermethylated *MGMT* status (n=67), withdrawal of  
251 consent (n=24), and other reasons (n=55), including insufficient tumor material (n=30), and  
252 AEs after surgery (n=8) (**Figure 1**). A total of 111 patients were randomised from December  
253 2009 through September 2012 and constituted the ITT population: 56 patients were  
254 scheduled to receive weekly temsirolimus in addition to standard RT (temsirolimus arm) and  
255 55 were to receive TMZ/RT→TMZ alone (control arm). In the safety population, i.e. patients  
256 with at least one dose of drug, there were 53 patients in the temsirolimus and 51 patients in  
257 the TMZ arm.

258 Median follow-up was 33 (95% CI: 23-37) months in the temsirolimus and 32 (95% CI: 22-  
259 40) months in the TMZ arm. The median duration from operation to randomisation was 2.6  
260 weeks (range 0.4–6.1 weeks). Patient baseline and demographic characteristics were well  
261 balanced between treatment arms except for the WHO Performance status between PS0  
262 and PS1, which favored the control arm. This is explained since the stratification was PS 0-1  
263 vs PS2 (**Table 1**).

264 In the biomarker cohort (n=88), only one patient sample displayed positive staining for the  
265 IDH1-R132H mutant (1/78; 1.3%), an expected low frequency, since 75% of the few *IDH1*  
266 mutant glioblastoma are *MGMT* hypermethylated (27). The frequency of *EGFR* amplification  
267 was in the expected range (54%, 44/82). There was no difference in baseline characteristics  
268 and outcome in patients with vs without markers assessment (**Supplementary Figure S2**,

269 **Supplementary Table S1).**

270

271 ***Efficacy outcomes***

272 The median duration of radiotherapy was 6.1 weeks in both arms. Main reason for  
273 interrupting RT was technical or administrative (28%). In median, RT was interrupted 2 days.  
274 RT was completed by >90% of patients. Concomitant treatment was delivered as planned  
275 *per protocol* by >90% of patients in both arms. Patients in the temsirolimus arm received the  
276 drug for a median (95% CI) of 16 weeks post RT (4.0 – 84.3), with a mean dose intensity of  
277 21.4 (6.3 - 25) mg/week.

278 Maintenance temsirolimus was administered *per protocol* at a median of 13 weekly cycles.  
279 Median relative dose-intensity was 85.6%. Twelve patients had a reduction in dose intensity  
280 below 70%, because of dose reduction (19.1%: 6.4% for hematological toxicity, 10.6% for  
281 AE, 2.1% for other reasons), dose not given during at least one cycle (68%: 6.3% for  
282 hematological toxicity, 34% for non-hematological toxicity, 58% for other reasons) or  
283 treatment delay (58%: 2.1% for hematological toxicity, 17% for non-hematological toxicity,  
284 43% for other reasons).

285 Median OS was 14.8 (13.3-16.4) months in the temsirolimus arm and 16.0 (13.8-18.2) in the  
286 control arm (90 deaths; HR, 1.2; 95% CI, 0.8-1.8; p=0.47; **Figure 2A**). The OS12 and OS24  
287 rates did not differ between arms (70%, 72% and 15%, 16%, respectively). Median PFS as  
288 assessed by the investigator was 5.4 (95% CI, 3.7-6.1) months in the temsirolimus arm and  
289 6.0 (95% CI, 2.8-8.0) months in the control arm (54 PFS events; HR, 1.26; 95% CI, 0.86–  
290 1.86; p=0.24; **Figure 2B**). In the *per protocol* population (see **Supplementary Information**),  
291 38 patients treated with temsirolimus had survived  $\geq$  to 1 year. At least 39 patients were  
292 needed to reach the targeted drug activity.

293

294 ***Safety***

295 In the temsirolimus arm severe hematological toxicity was: neutropenia (G3: n=1, 1.9%) and  
296 lymphocytopenia (G3: n=9, 16.4%, G4: n=1, 1.8%). In the TMZ arm severe hematological

297 toxicity was: leukopenia G3 (n=2, 3.8%), neutropenia G4 (n=2, 3.8%), lymphocytopenia (G3:  
298 n=14, 26.4%, G4: n=2, 3.8%) and thrombocytopenia (G3: n=1, 1.9%, G4: n=1, 1.9%). There  
299 was no other severe (G3/4) treatment-related AE with an incidence >5% in either arm.

300

### 301 ***Molecular correlations with outcome***

302 Markers interrogated for their relevance of targeting the mTOR signaling pathway (22, 23)  
303 are visualized in the mTOR KEGG pathway (28) (**Supplementary Figure S3**).

304 Phosphorylated mTOR<sup>Ser2448</sup> was associated with prolonged OS as evidenced by the  
305 significant interaction term between treatment and p-mTOR<sup>Ser2448</sup> (p=0.047, **Figure 3**).

306 Tumors of 37.6% of the patients scored positive for p-mTOR<sup>Ser2448</sup>. There was a non-  
307 significant trend for longer OS when p-mTOR<sup>Ser2448</sup> positive patients received temsirolimus

308 as compared with controls (HR=0.62, 95% CI 0.26-1.47, p=0.27). When non-phosphorylated  
309 mTOR<sup>Ser2448</sup> patients received temsirolimus a non-significant decrease in survival was

310 observed compared with controls (HR=1.77, 95% CI 0.95-3.29, p=0.07) (**Figure 3**). The  
311 median OS in the temsirolimus group was 17.8 months (CI, 14.1-28.0) for patients with p-

312 mTOR<sup>Ser2448</sup> positive tumors and 13.1 months (CI, 9.7-15.1) in the negative subgroup  
313 (p=0.007, Figure 3A). In the RT/TMZ→TMZ control arm the median OS in the p-mTOR<sup>Ser2448</sup>

314 positive group was 14.0 months (CI, 9.6-19.6) and 16.5 months (CI, 9.5-18.8) in the p-  
315 mTOR<sup>Ser2448</sup> negative subgroup (p=0.999). For p-PRAS40<sup>Thr246</sup>, the interaction test with

316 treatment was borderline non-significant (p=0.07). The impact of all other markers on  
317 survival is illustrated in a forest plot for all other markers in **Supplementary Figure S4**.

318

319 A multi dimensional analysis used the full range of the scores of the mTOR-associated  
320 markers integrated information for the identification of clinically relevant molecular subgroups

321 and to gain further insights on pathway interactions (**Figure 4**). The two first axes obtained  
322 by PCA explained 57.8% of the total inertia. The first axis was mainly explained by p-

323 mTOR<sup>Ser2448</sup> and p-PRAS40<sup>Thr246</sup>. The p-S6RP<sup>Ser235/236</sup> mainly contributed to the construction  
324 of the second axis (**Figures 4E and F**). PTEN expression played a minor role in the

325 structure of the score table (**Figure 4F**). Subgroups were determined by consensus  
326 clustering. We kept the cluster based on two groups (k=2) by default, as no strong indication  
327 for the optimal number of clusters was obtained and the sample size is limited  
328 (**Supplementary Figure S5**). Cluster 2, highly enriched for p-mTOR<sup>Ser2448</sup>-positive cases,  
329 revealed a strong association with outcome in the temsirolimus treatment group and no  
330 difference in the TMZ/RT→TMZ group (**Figure 4**). Significant interaction was observed with  
331 treatment (p=0.009): in Cluster 2 the HR was 0.42 (95% CI 0.15-1.13, p=0.08) and in Cluster  
332 1 HR=1.77 (95% CI 0.96-3.25, p=0.06).

333 In multivariable prognostic analyses of clinical and molecular factors (**Supplementary Table**  
334 **S1**), p-mTOR<sup>Ser2448</sup> (HR=0.13, 95% CI 0.04-0.47, p=0.002), p-PRAS40<sup>Thr246</sup> (HR=0.50, 95%  
335 CI 0.21-1.18, p=0.12), p-ERK<sup>Thr202/Tyr204</sup> (HR=2.81, 95% CI 0.97-8.09, p=0.06), but no clinical  
336 factor was associated with OS in the temsirolimus arm. The PEV was equal to 14.9% In the  
337 TMZ arm, there was a trend for decreased survival in p-AKT<sup>Ser473</sup> positive patients (HR=3.21,  
338 95% CI 0.89-11.56, p=0.07, PEV=4.5%). None of the models had a PEV larger than 20%.  
339

340 **DISCUSSION**

341

342 This randomized, open label phase II trial investigating the mTOR inhibitor temsirolimus in  
343 combination with RT for patients with low probability of benefit from the TMZ-based  
344 radiochemotherapy failed to demonstrate the targeted outcome. Neither PFS nor OS  
345 demonstrated a signal of relevant activity in the total trial population (**Figure 2**). Safety and  
346 tolerability of temsirolimus in combination with standard RT were non-concerning and the  
347 trial is an example that temozolomide can be safely omitted in patients with *MGMT*  
348 unmethylated glioblastoma. The trial proposes mTOR<sup>Ser2448</sup> phosphorylation as a biomarker  
349 for benefit from mTOR inhibition. These results need further confirmation, and a trial to  
350 prospectively assess the relevance of this putative biomarker is underway (NCT Neuro  
351 Master Match, *EudraCT 2015-002752-27*).

352 The good outcome data in both arms of the trial prompted a comparison with the  
353 EORTC26981-22981/NCIC CE3 trial. The comparison with our pivotal TMZ/RT→TMZ vs RT  
354 trial (EORTC26981-22981/NCIC CE3) (29) was favourable in all aspects supporting the  
355 principal rationale to design trials for patients with *MGMT* unmethylated glioblastoma and  
356 withhold TMZ in the experimental arm (**Supplementary Results**). Biases in favor of EORTC  
357 26082 may have been patient selection, and the lower number of patients on steroids (30).  
358 Bevacizumab was administered in about 45% of the patients in both arms of EORTC 26082.  
359 The OS of the EORTC 26082 arms is comparable to the outcome in the control arms of trials  
360 with selection of *MGMT* unmethylated patients, with 13.4 months in the CORE trial (95% CI  
361 12.2-14.3) with a bevacizumab use at recurrence of 22% (31) and 17.3 months (95%CI 14.8-  
362 20.4 months) in the GLARIUS trial with cross over to bevacizumab of 60% (32).

363 The EORTC 26082 trial aimed at not withholding TMZ from any patient with an equivocally  
364 methylated *MGMT* promoter by applying a *MGMT* cut-off with a safety margin. This  
365 prompted an adaptation also in the GLARIUS trial (32) with similar design and therefore  
366 demarcates an evolution from the S039 trial with enzastaurin (33). Two randomized phase III  
367 trials in elderly patients with newly diagnosed glioblastoma further support a strictly

368 predictive effect of the *MGMT* status for benefit from TMZ (34, 35). However, we cannot  
369 completely exclude a small baseline effect of TMZ despite the *MGMT* unmethylated state  
370 (11). Hence, withholding TMZ outside trials and elderly patients with unmethylated *MGMT*  
371 promoter is not advocated by the present data. In the temsirolimus arm 59% (n=33) of the  
372 patients received TMZ after treatment discontinuation, and 26% of TMZ patients (n=14) were  
373 re-challenged with TMZ, not being aware of the recent data from the DIRECTOR trial that re-  
374 challenge with TMZ might be relevant only for patients with a methylated *MGMT* promoter  
375 (36).

376 The choice of temsirolimus for patients with unmethylated glioblastoma was based on  
377 preclinical data already highlighting that not every tumor responds to the treatment (37) as  
378 well as a response may be only transient because of the overt feedback resistance  
379 mechanisms (22, 38).

380 Molecular analyses of prespecified principal components of the EGFR-PI3-K/mTOR/AKT  
381 pathway were performed. EORTC 26082 provides first evidence that p-mTOR<sup>Ser2448</sup> and – to  
382 a lesser extent - p-PRAS40<sup>Thr246</sup> may serve as decisive biomarkers for the treatment of  
383 patients with newly diagnosed glioblastoma with an unmethylated *MGMT* promoter.  
384 Phosphorylation of mTOR<sup>Ser2448</sup> has been shown to be targeted and blocked by rapamycin, a  
385 major metabolite of temsirolimus (39), while phosphorylated PRAS40<sup>Thr246</sup> (substrate of  
386 AKT1) relieves inhibitory function on mTORC1 (40). The survival curves may even suggest  
387 that there is a detrimental effect of temsirolimus in p-mTOR<sup>Ser2448</sup> negative tumors (**Figures 3**  
388 **and 4**). Previous trials testing temsirolimus at recurrence had focused on the PTEN status  
389 with a PTEN deficiency as a prerequisite for response (22) or on other downstream mTOR  
390 targets, e.g. p-S6RP<sup>Ser235/236</sup>, which was neither associated with outcome in biomarker  
391 analyses of patients with recurrent glioblastoma receiving temsirolimus (6, 38) nor in this  
392 study. It cannot be excluded that glioblastomas treated at recurrence may have changed  
393 mTOR pathway activity as compared to tumor specimen used for marker analyses obtained  
394 at the first resection (41). Also, “paradoxical” activation of AKT by elimination of negative  
395 feedback downregulating survival signaling has been postulated as potential resistance

396 mechanism to mTOR inhibition in previous trials, based on the analyzes of paired tumor  
397 specimen taken before and after treatment (22, 38). Interestingly, trials in other diseases did  
398 not provide predictive biomarkers (12, 13).

399 The limitations of EORTC 26082 are the relatively small sample size of this non-comparative  
400 phase II trial. For the biomarker analyses using IHC only a limited number of tumor tissue  
401 samples from the ITT cohort were available. The findings should be validated by evaluation  
402 of previous trials in particular in those treating newly diagnosed glioblastoma patients (42)  
403 and the randomized phase II study RTOG-0913. Ongoing trials using mTOR inhibitors may  
404 need to take into account a potentially detrimental effect in patients with an  
405 unphosphorylated mTOR<sup>Ser2448</sup>. Given the ongoing efforts of biomarker-driven basket trials  
406 for patients with newly diagnosed glioblastoma, the concept of mTOR inhibition using the  
407 marker predictive in this study, p-mTOR<sup>Ser2448</sup> is incorporated into the design of a future  
408 study.

409

410

411 **REFERENCES**

412

413 1. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, *et*  
414 *al.* The somatic genomic landscape of glioblastoma. *Cell* 2013;155: 462-77.

415 2. Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, *et al.* Enhanced  
416 sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci U S A*  
417 2001;98: 10314-9.

418 3. Podsypanina K, Lee RT, Politis C, Hennessy I, Crane A, Puc J, *et al.* An inhibitor of  
419 mTOR reduces neoplasia and normalizes p70/S6 kinase activity in *Pten*<sup>+/-</sup> mice. *Proc Natl*  
420 *Acad Sci U S A* 2001;98: 10320-5.

421 4. Chakravarti A, Zhai G, Suzuki Y, Sarkesh S, Black PM, Muzikansky A, *et al.* The  
422 prognostic significance of phosphatidylinositol 3-kinase pathway activation in human  
423 gliomas. *J Clin Oncol* 2004;22: 1926-33.

424 5. Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as  
425 anticancer agents. *Nat Rev Drug Discov* 2006;5: 671-88.

426 6. Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J, *et al.* Phase II  
427 trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer  
428 Treatment Group Study. *J Clin Oncol* 2005;23: 5294-304.

429 7. Doherty L, Gigas DC, Kesari S, Drappatz J, Kim R, Zimmerman J, *et al.* Pilot study of  
430 the combination of EGFR and mTOR inhibitors in recurrent malignant gliomas. *Neurology*  
431 2006;67: 156-8.

432 8. Kreisl TN, Lassman AB, Mischel PS, Rosen N, Scher HI, Teruya-Feldstein J, *et al.* A  
433 pilot study of everolimus and gefitinib in the treatment of recurrent glioblastoma (GBM). *J*  
434 *Neurooncol* 2009;92: 99-105.

435 9. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Friedman AH, Herndon  
436 JE, 2nd, *et al.* Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. *J*  
437 *Neurooncol* 2010;96: 219-30.

- 438 10. Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, *et al.* A phase II  
439 study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first  
440 relapse. *Br J Cancer* 2000;83: 588-93.
- 441 11. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, *et al.*  
442 Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*  
443 2005;352: 987-96.
- 444 12. Motzer RJ, Hudes GR, Curti BD, McDermott DF, Escudier BJ, Negrier S, *et al.* Phase  
445 I/II trial of temsirolimus combined with interferon alfa for advanced renal cell carcinoma. *J*  
446 *Clin Oncol* 2007;25: 3958-64.
- 447 13. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, *et al.*  
448 Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the  
449 treatment of relapsed or refractory mantle cell lymphoma. *J Clin Oncol* 2009;27: 3822-9.
- 450 14. Sarkaria JN, Galanis E, Wu W, Dietz AB, Kaufmann TJ, Gustafson MP, *et al.*  
451 Combination of temsirolimus (CCI-779) with chemoradiation in newly diagnosed  
452 glioblastoma multiforme (GBM) (NCCTG trial N027D) is associated with increased infectious  
453 risks. *Clin Cancer Res* 2010;16: 5573-80.
- 454 15. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, *et al.* MGMT  
455 gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005;352: 997-  
456 1003.
- 457 16. Abdollahi A, Lipson KE, Han X, Krempien R, Trinh T, Weber KJ, *et al.* SU5416 and  
458 SU6668 attenuate the angiogenic effects of radiation-induced tumor cell growth factor  
459 production and amplify the direct anti-endothelial action of radiation in vitro. *Cancer Res*  
460 2003;63: 3755-63.
- 461 17. Wild-Bode C, Weller M, Rimner A, Dichgans J, Wick W. Sublethal irradiation  
462 promotes migration and invasiveness of glioma cells: implications for radiotherapy of human  
463 glioblastoma. *Cancer Res* 2001;61: 2744-50.

- 464 18. Vlassenbroeck I, Califice S, Diserens AC, Migliavacca E, Straub J, Di Stefano I, *et al.*  
465 Validation of real-time methylation-specific PCR to determine O6-methylguanine-DNA  
466 methyltransferase gene promoter methylation in glioma. *J Mol Diagn* 2008;10: 332-7.
- 467 19. Stupp R, Hegi ME, Gorlia T, Erridge SC, Perry J, Hong YK, *et al.* Cilengitide  
468 combined with standard treatment for patients with newly diagnosed glioblastoma with  
469 methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre,  
470 randomised, open-label, phase 3 trial. *Lancet Oncol* 2014;15: 1100-8.
- 471 20. Macdonald DR, Cascino TL, Schold SC, Jr., Cairncross JG. Response criteria for  
472 phase II studies of supratentorial malignant glioma. *J Clin Oncol* 1990;8: 1277-80.
- 473 21. Yan P, Seelentag W, Bachmann A, Bosman FT. An agarose matrix facilitates  
474 sectioning of tissue microarray blocks. *J Histochem Cytochem* 2007;55: 21-4.
- 475 22. Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, *et al.* Antitumor  
476 activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient  
477 glioblastoma. *PLoS Med* 2008;5: e8.
- 478 23. Hegi ME, Diserens AC, Bady P, Kamoshima Y, Kouwenhoven MC, Delorenzi M, *et*  
479 *al.* Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR  
480 tyrosine kinase inhibitor gefitinib - A phase II trial. *Mol Cancer Ther* 2011;10: 1102-12.
- 481 24. Wold H. Estimation of principal components and related models by iterative least  
482 squares. *Multivariate Analysis: Academic Press; 1966. p. 391-420.*
- 483 25. Monti S, Tamayo P, Mesirov J, Golub T. Consensus clustering: A resampling-based  
484 method for class discovery and visualization of gene expression microarray data. *Machine*  
485 *Learning* 2003;52: 91-118.
- 486 26. Epskamp S, Cramer AOJ, Waldorp LJ, Schmittmann VD, Borsboom D. qgraph:  
487 Network visualizations of relationships in psychometric data. *J Stat Soft* 2012;48: 1-18.
- 488 27. Bady P, Sciuscio D, Diserens AC, Bloch J, van den Bent MJ, Marosi C, *et al.* MGMT  
489 methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two  
490 distinct CpG regions associated with gene silencing and outcome, yielding a prediction

- 491 model for comparisons across datasets, tumor grades, and CIMP-status. *Acta Neuropathol*  
492 2012;124: 547-60.
- 493 28. Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data  
494 integration and visualization. *Bioinformatics* 2013;29: 1830-1.
- 495 29. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, *et al.*  
496 Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy  
497 alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the  
498 EORTC-NCIC trial. *Lancet Oncol* 2009;10: 459-66.
- 499 30. Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC, *et*  
500 *al.* Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus  
501 lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled  
502 phase 2 trial. *Lancet Oncol* 2014;15: 943-53.
- 503 31. Nabors LB, Fink KL, Mikkelsen T, Grujicic D, Tarnawski R, Nam DH, *et al.* Two  
504 cilengitide regimens in combination with standard treatment for patients with newly  
505 diagnosed glioblastoma and unmethylated MGMT gene promoter: results of the open-label,  
506 controlled, randomized phase II CORE study. *Neuro Oncol* 2015;17: 708-17.
- 507 32. Herrlinger U, Schäfer N, Steinbach JP, Weyerbrock A, Hau P, Goldbrunner R, *et al.*  
508 The randomized, multicenter glioblastoma trial investigating bevacizumab/irinotecan vs standard  
509 temozolomide in newly diagnosed, mgmt-non-methylated glioblastoma patients: final survival  
510 results and quality of life. *Neuro-Oncology* 2014;16: ii23-ii4.
- 511 33. Wick W, Steinbach JP, Platten M, Hartmann C, Wenz F, von Deimling A, *et al.*  
512 Enzastaurin before and concomitant with radiation therapy, followed by enzastaurin  
513 maintenance therapy, in patients with newly diagnosed glioblastoma without MGMT  
514 promoter hypermethylation. *Neuro Oncol* 2013;15: 1405-12.
- 515 34. Wick W, Platten M, Meisner C, Felsberg J, Tabatabai G, Simon M, *et al.*  
516 Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in  
517 the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol* 2012;13: 707-15.

- 518 35. Malmstrom A, Gronberg BH, Marosi C, Stupp R, Frappaz D, Schultz H, *et al.*  
519 Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy  
520 in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial.  
521 *Lancet Oncol* 2012;13: 916-26.
- 522 36. Weller M, Tabatabai G, Kastner B, Felsberg J, Steinbach JP, Wick A, *et al.* MGMT  
523 promoter methylation is a strong prognostic biomarker for benefit from dose-intensified  
524 temozolomide rechallenge in progressive glioblastoma: The DIRECTOR trial. *Clin Cancer*  
525 *Res* 2015;21: 2057-64.
- 526 37. Weiler M, Pfenning PN, Thiebold AL, Blaes J, Jestaedt L, Gronych J, *et al.*  
527 Suppression of proinvasive RGS4 by mTOR inhibition optimizes glioma treatment.  
528 *Oncogene* 2013;32: 1099-109.
- 529 38. Wen PY, Chang SM, Lamborn KR, Kuhn JG, Norden AD, Cloughesy TF, *et al.* Phase  
530 I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas: North  
531 American Brain Tumor Consortium trial 04-02. *Neuro Oncol* 2014;16: 567-78.
- 532 39. Chiang GG, Abraham RT. Phosphorylation of mammalian target of rapamycin  
533 (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J Biol Chem* 2005;280: 25485-90.
- 534 40. Wiza C, Nascimento EB, Ouwens DM. Role of PRAS40 in Akt and mTOR signaling  
535 in health and disease. *Am J Physiol Endocrinol Metab* 2012;302: E1453-60.
- 536 41. Kim H, Zheng S, Amini SS, Virk SM, Mikkelsen T, Brat DJ, *et al.* Whole-genome and  
537 multisector exome sequencing of primary and post-treatment glioblastoma reveals patterns  
538 of tumor evolution. *Genome Res* 2015;3: 114.
- 539 42. Ma DJ, Galanis E, Anderson SK, Schiff D, Kaufmann TJ, Peller PJ, *et al.* A phase II  
540 trial of everolimus, temozolomide, and radiotherapy in patients with newly diagnosed  
541 glioblastoma: NCCTG N057K. *Neuro Oncol* 2015;17: 1261-9.
- 542
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551 This report has been presented in part as abstract 2003 at ASCO 2014 by W. Wick.

552

553 **CONTRIBUTORS**

554 The concept of the trial was developed by W.W. in collaboration with the T.G., G.P., M.E.H.,  
555 R.S. and the EORTC Brain Tumor Group. The concept of the biomarker analyses was  
556 developed by M.E.H. in collaboration with T.G, P.B. and W.W.

557 Study material: W.W., M.P., M.J.v.d.B., M.J.B.T., A.A., M.W., P.R., M.C., J.-S. F., M.W.,  
558 R.S., D.R., C.M., S.V., A.W., Ki.H., Kr.H., G.P. recruited patients to the study, were involved  
559 in data collection and provided administrative support.

560 The biomarker data were generated and evaluated by P.B., M.-F.H, B.L. and M.E.H.

561 Reference pathology was performed by B.L.

562 The statistical analyses were performed by T.G. and P.B.

563 The article was written by W.W. and M.E.H. with support from all co-authors.

564 All authors reviewed and approved the manuscript.

565

566 **FIGURE LEGENDS**

567

568 **Figure 1. Supplemented CONSORT diagram of patient disposition.**

569

570 **Figure 2. Principal efficacy outcomes per treatment.**

571

572 **Figure 3. Overall survival according to phosphorylated mTOR stratified by treatment.**

573 (A) Kaplan-Meier curves shown represent patients separated by the phosphorylation status  
574 of mTOR<sup>Ser2448</sup> (Pos, positive; Neg, negative) stratified for the two treatment arms CCI-  
575 779/RT and TMZ/RT→TMZ (TMZ). The interaction test was significant p=0.047). (B)  
576 Representative glioblastoma samples negative or positive for p-mTOR<sup>Ser2448</sup> expression.

577

578 **Figure 4. Multidimensional analysis of m-TOR associated markers.**

579 The associations among markers in the mTOR pathway are illustrated by “The network  
580 representation” based on Spearman correlations between scores (A). (B) The glioblastoma  
581 subgroups based on mTOR pathway markers are visualized in a heatmap of the score table  
582 obtained after reconstruction using Non-linear Iterative Partial Least Squares (NIPALS). The  
583 rows were ordered by the first axis of the PCA. The columns are ordered by the consensus  
584 classification (k=2; clusters 1, blue; cluster 2, red) and are annotated for absence or  
585 presence of mutated IDH1<sup>R132H</sup> (positive, red; negative, grey; unknown; white), and the  
586 *EGFR* status (amplified dark green, non-amplified, green; unknown, white). The association  
587 between OS and consensus classification for two groups (k=2) (cluster 1, blue; cluster 2,  
588 red) is illustrated by Kaplan-Meier representation for patients randomized to CCI-779 (C) and  
589 TMZ (D). The p-value is given for each KM. The patients (E) and m-TOR-associated  
590 markers (F) were projected onto the two first components of the principal component  
591 analysis (PCA). Inertia ellipses and stars visualize the separation of the patients into the two  
592 groups obtained from consensus clustering (cluster 1, blue; cluster 2, red) (E).

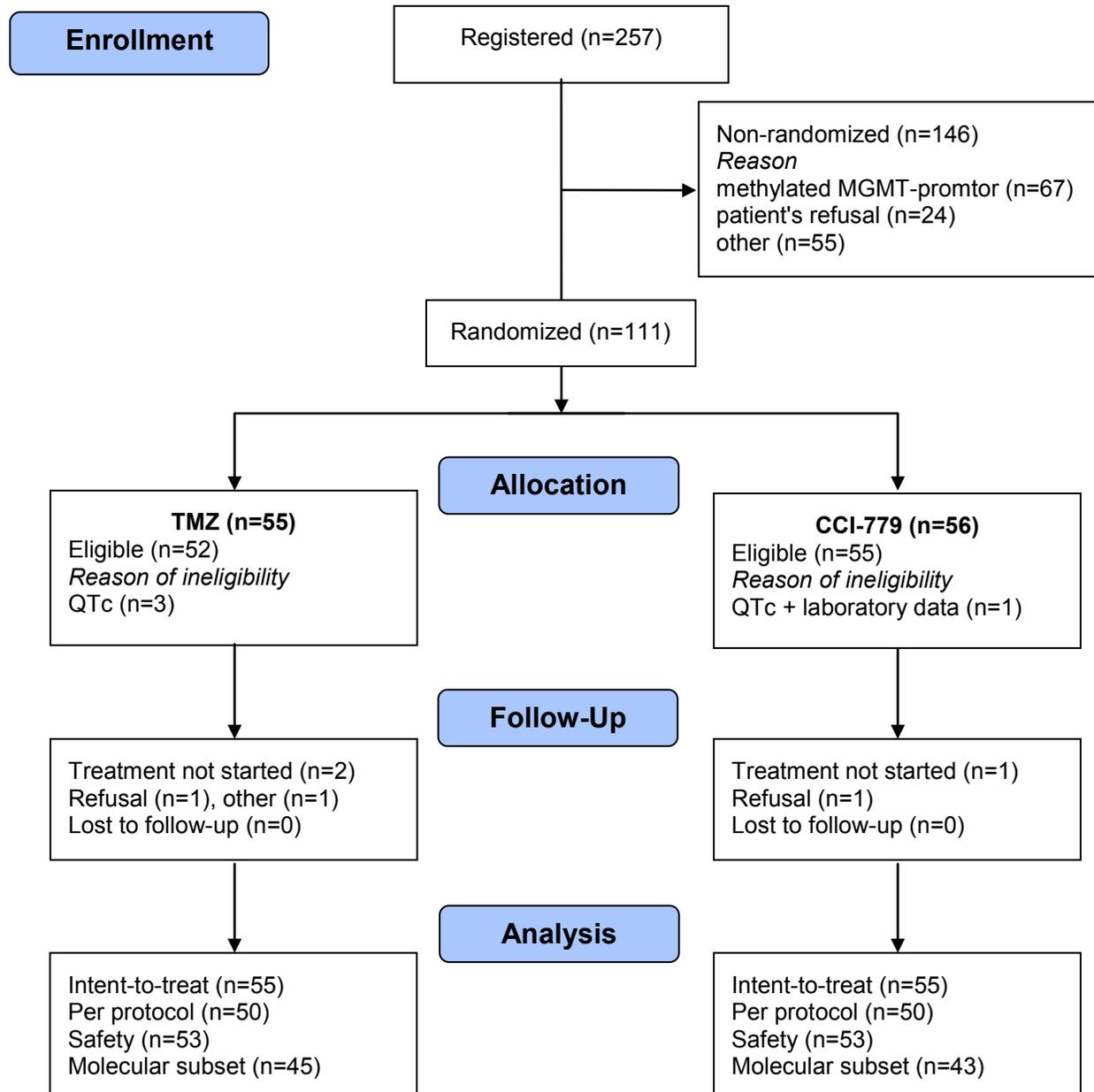
**Table Baseline characteristics**

	<b>TMZ (N=55)</b>	<b>Temsirolimus (N=56)</b>	<b>Total (N=111)</b>
	<b>N (%)</b>	<b>N (%)</b>	<b>N (%)</b>
<b>Age</b>			
<b>median</b>	57.7	54.9	55.7
<b>range</b>	24.4 - 76.0	28.2 - 74.7	24.4 - 76.0
<b>Sex</b>			
<b>male</b>	36 (65.5)	35 (62.5)	71 (64.0)
<b>female</b>	19 (34.5)	21 (37.5)	40 (36.0)
<b>Extent of resection</b>			
<b>open biopsy</b>	1 (1.8)	3 (5.4)	4 (3.6)
<b>resection</b>	54 (98.2)	53 (94.6)	107 (96.4)
<b>Corticosteroids</b>			
<b>no</b>	37 (67.3)	40 (71.4)	77 (69.4)
<b>yes</b>	18 (32.7)	16 (28.6)	33 (29.7)
<b>WHO PS (0-4)</b>			
<b>0</b>	40 (72.7)	32 (57.1)	72 (64.9)
<b>1</b>	14 (25.5)	20 (35.7)	34 (30.6)
<b>2</b>	1 (1.8)	4 (7.1)	5 (4.5)

Abbreviations: TMZ, temozolomide; WHO PS, World Health Organization

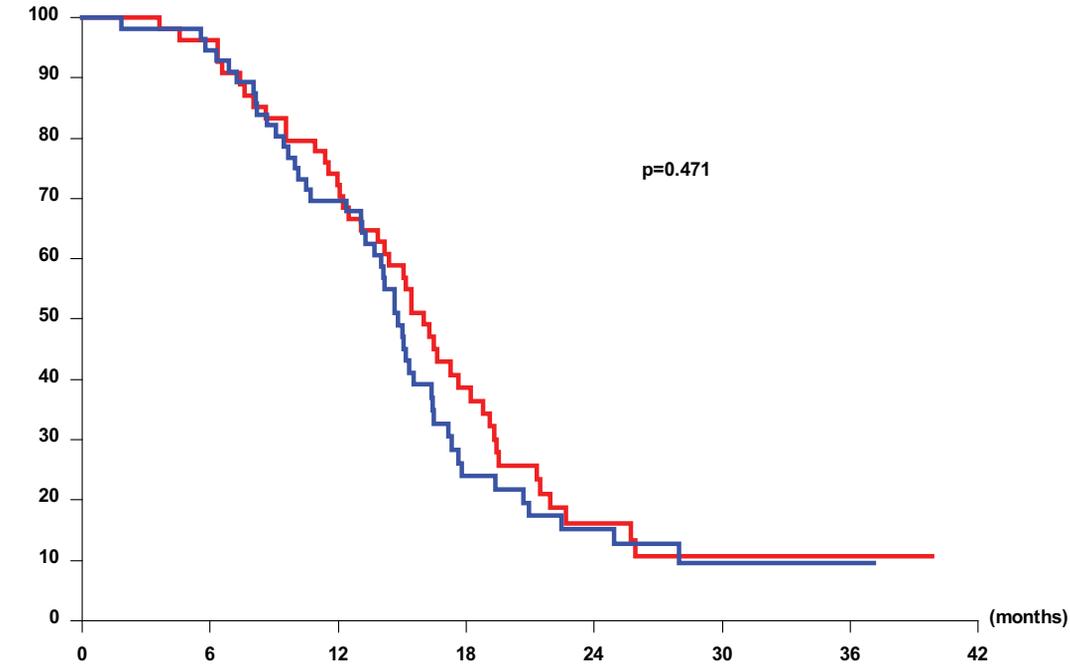
Performance Status

Figure 1



A

## Overall Survival



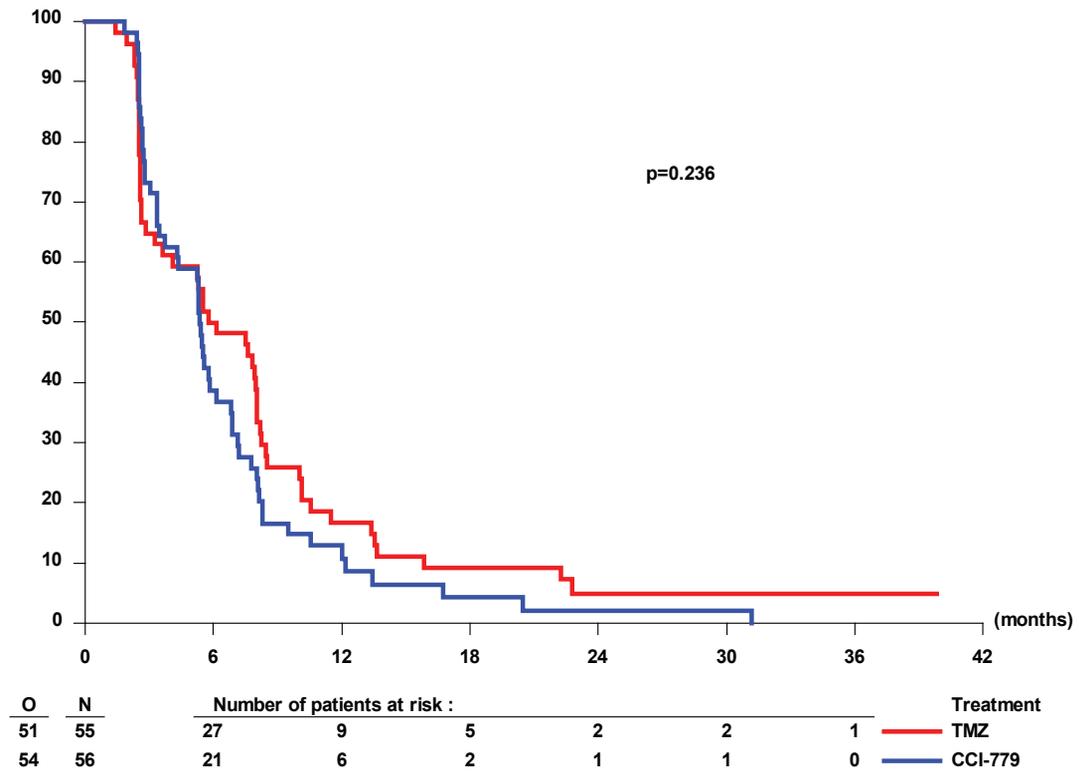
O	N	Number of patients at risk :						Treatment
44	55	52	39	18	6	3	1	TMZ
46	56	53	39	11	6	3	1	CCI-779

## Survival Time

Treatment	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	P-Value (Log-Rank)	Median (95% CI) (Months)	% at 1 Year (95% CI)
TMZ	55	44	1.00	0.4708	16.03 (13.83, 18.20)	72.22 (58.22, 82.22)
CCI-779	56	46	1.16 (0.77, 1.76)		14.78 (13.27, 16.39)	69.64 (55.79, 79.91)

B

### Progression Free Survival

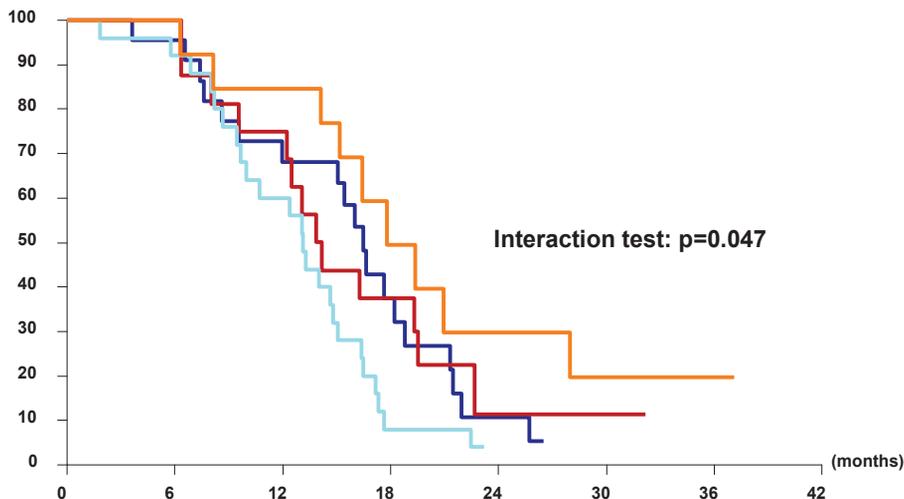


### Survival Time

Treatment	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	P-Value (Log-Rank)	Median (95% CI) (Months)	% at 0.5 Year(s) (95% CI)
TMZ	55	51	1.00	0.2358	5.95 (3.25, 8.02)	50.00 (36.12, 62.39)
CCI-779	56	54	1.26 (0.86, 1.86)		5.36 (3.71, 6.14)	38.67 (25.96, 51.20)

A

### Overall Survival

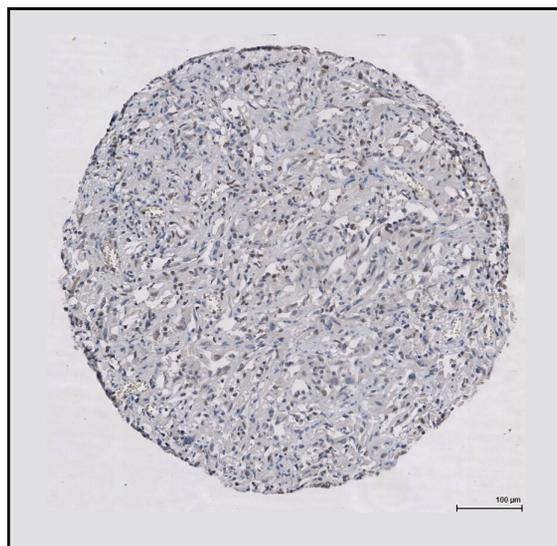
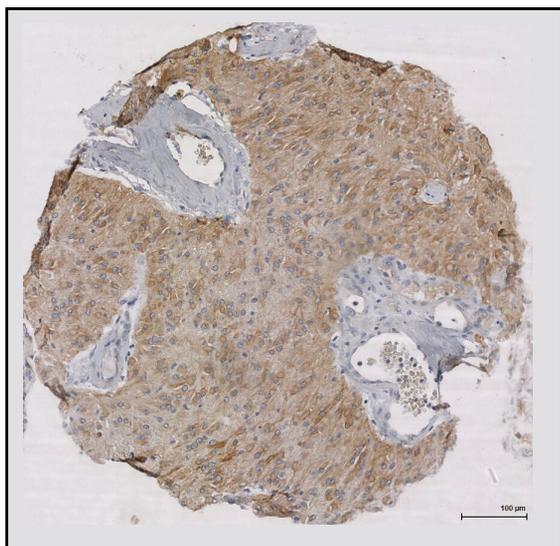


O	N	Number of patients at risk :						Trt/p-mTOR
19	23	21	15	7	2	0	0	— TMZ/Neg
13	16	16	12	5	1	1	0	— TMZ/Pos
24	25	23	15	2	0	0	0	— CCI-779/Neg
9	13	13	11	5	3	2	1	— CCI-779/Pos

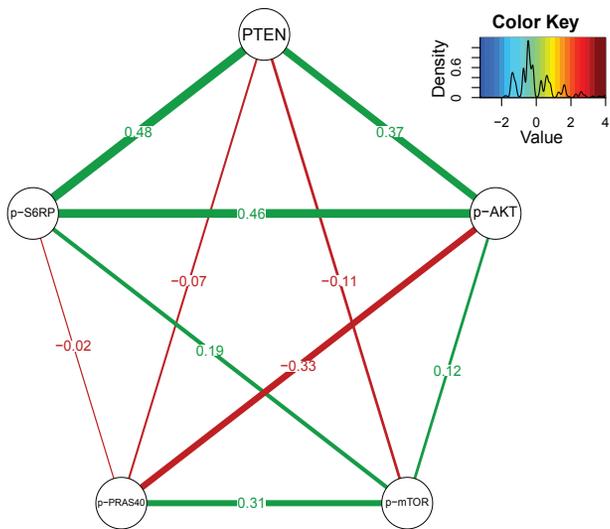
treatment/p-mtor	Survival Time		Non-parametric		Cox model	
	Patients (N)	Observed Events (O)	Median (95% CI) (Months)	% at 2 Year(s) (95% CI)	Hazard Ratio (95% CI)	P-Value (Score test)
TMZ/p-mTOR Neg	23	19	16.46 (9.53, 18.79)	10.7 (1.8, 28.7)	1.00	0.042 (df=3)
TMZ/p-mTOR Pos	16	13	14.01 (9.56, 19.55)	11.3 (0.9, 36.4)	0.99 (0.49, 2.01)	
CCI-779/p-mTOR Neg	25	24	13.11 (9.66, 15.08)	4.0 (0.3, 17.0)	1.71 (0.93, 3.14)	
CCI-779/p-mTOR Pos	13	9	17.77 (14.09, 27.99)	29.7 (7.4, 56.8)	0.59 (0.26, 1.32)	

Log-rank test: p-value=0.041

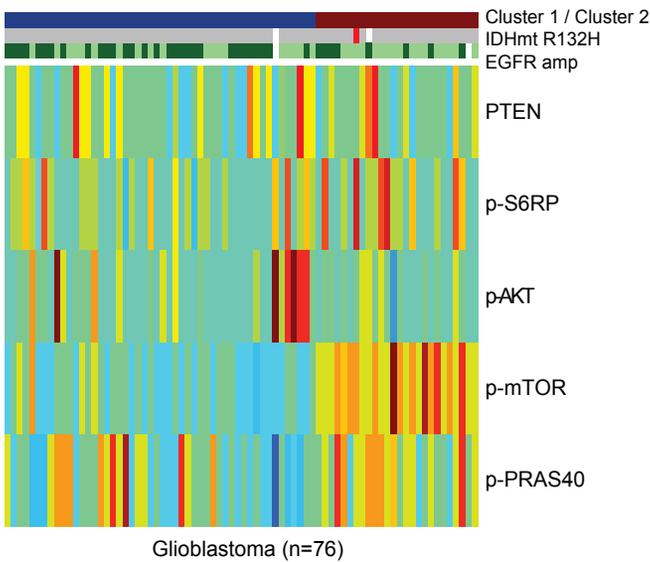
B



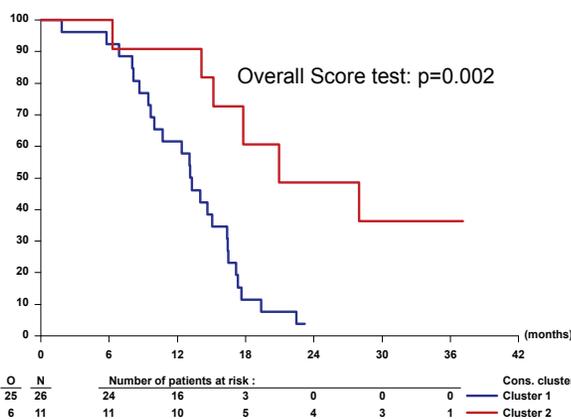
**A** Marker Correlations  
(Spearman correlation)



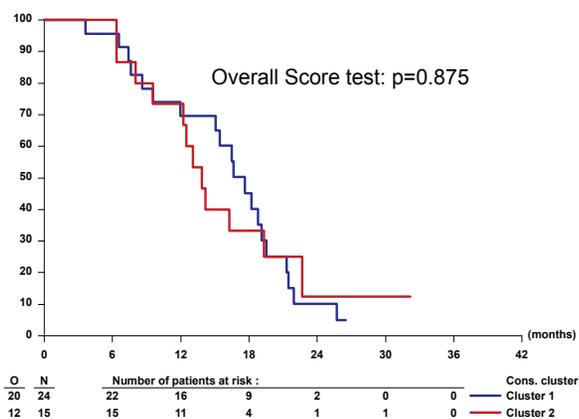
**B** Consensus Clustering (k=2)



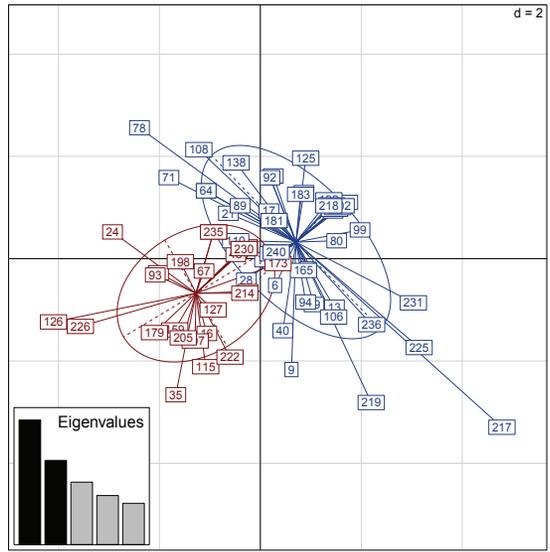
**C** Overall Survival (CCI-779)



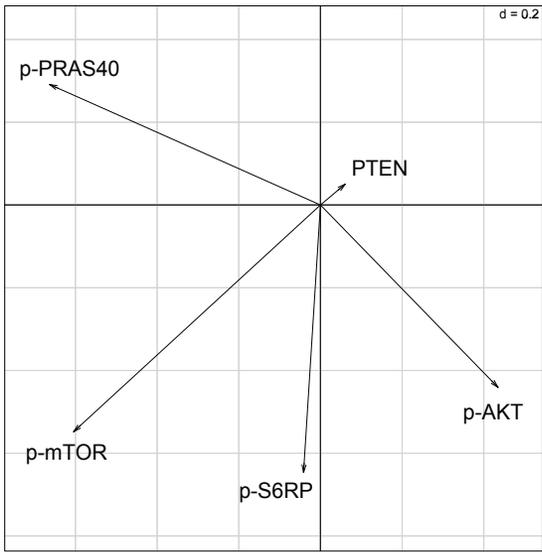
**D** Overall Survival (TMZ)



**E** Patient Representation (PCA, F1-F2)



**F** Variable Representation (PCA, F1-F2)



**SUPPLEMENTARY INFORMATION TO****Phase II study of radiotherapy and temsirolimus versus radiochemotherapy with temozolomide in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation (EORTC 26082)**

<sup>1,2</sup>Wolfgang Wick, <sup>3</sup>Thierry Gorlia, <sup>4,5</sup>Pierre Bady, <sup>1,6</sup>Michael Platten, <sup>7</sup>Martin J van den Bent, <sup>8</sup>Martin JB Taphoorn, <sup>3</sup>Jonathan Steuve, <sup>9</sup>Alba A. Brandes, <sup>5,10</sup>Marie-France Hamou, <sup>1</sup>Antje Wick, <sup>11</sup>Markus Kosch, <sup>13</sup>Michael Weller, <sup>10</sup>Roger Stupp, <sup>13</sup>Patrick Roth, <sup>3</sup>Vassilis Gofinopoulos, <sup>12</sup>Jean-Sebastien Frenel, <sup>12</sup>Mario Campone, <sup>14</sup>Damien Ricard, <sup>15</sup>Christine Marosi, <sup>16</sup>Salvador Villa, <sup>17</sup>Astrid Weyerbrock, <sup>18</sup>Kirsten Hopkins, <sup>10</sup>Krisztian Homicsko, <sup>19</sup>Benoit Lhermitte, <sup>20</sup>Gianfranco Pesce, <sup>5,10</sup>Monika E Hegi

<sup>1</sup>Neurology Clinic, University of Heidelberg and <sup>2</sup>Clinical Cooperation Unit (CCU) Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany; <sup>3</sup>European Organisation for Research and Treatment of Cancer (EORTC); <sup>4</sup>Swiss Institute of Bioinformatics, <sup>5</sup>Neuroscience Research Center, University Hospital Lausanne (CHUV), both Lausanne, Switzerland; <sup>6</sup>CCU Brain Tumor Immunology, DKFZ, Heidelberg, Germany; <sup>7</sup>Department of Neurology/Neuro-Oncology, Erasmus MC - Cancer Institute, Rotterdam; <sup>8</sup>Neuro-oncology Unit, MC Haaglanden, The Hague, both The Netherlands; <sup>9</sup>Department of Medical Oncology, Ospedale Bellaria, Bologna, Italy; <sup>10</sup>Departments of Neurosurgery and Oncology, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland; <sup>11</sup>Pfizer, Berlin, Germany; <sup>12</sup>1-Institut de Cancérologie de l'OUEST, Saint Herblain-Nantes Cedex, France; <sup>13</sup>Department of Neurology, University Hospital and University of Zurich; <sup>14</sup>Hopitaux de Paris - La Pitié Salpêtrière, Paris, France; <sup>15</sup>General Hospital AKH, Medical University Vienna, Austria; <sup>16</sup>Institut Catala d'Oncologia (ICO). Hospital Germans Trias Pujol, Badalona,

Barcelona, Spain; <sup>17</sup>Neurosurgery Clinic, University Medical Center, Freiburg, Germany;

<sup>18</sup>University Hospitals Bristol NHS Foundation Trust - Bristol Haematology and Oncology

Centre, Bristol, U.K.; <sup>19</sup>Institute of Pathology, CHUV, Lausanne; <sup>20</sup>Department of Radio-

oncology, Oncology Institute of Southern Switzerland, Bellinzona; all Switzerland

**Supplementary Patients and Methods***MGMT Testing*

In brief, DNA was isolated from formalin-fixed, paraffin-embedded tumour samples using macro-dissected sections; DNA was modified with sodium bisulfite and subjected to quantitative methylation-specific PCR using  $\beta$ -actin as a reference gene (*ACTB*).<sup>1</sup>

*Key eligibility criteria*

Patients aged  $\geq 18$  years with newly diagnosed, histologically confirmed supratentorial glioblastoma (WHO Grade IV), centrally determined unmethylated *MGMT* status, and with an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 were eligible. Additional inclusion criteria were: written informed consent; available tumour tissue from surgery or open biopsy (stereotactic biopsy was not allowed) for *MGMT* promoter methylation status analysis and central pathology review; gadolinium-enhanced (Gd) MRI performed within 48 hours post surgery, or alternatively, Gd-MRI performed before randomisation; stable or decreasing steroid doses for  $\geq 5$  days prior to randomisation; and adequate haematological, renal, and liver function. Key exclusion criteria were prior chemotherapy within the last 5 years, prior RT of the head, treatment with other investigational agents 30 days before first dose of temsirolimus, and prior systemic antiangiogenic therapy; history of coagulation disorder associated with bleeding or recurrent thromboembolic events; presence of QTc prolongation  $>450/470$  msec (males/females); placement of Gliadel® wafers at surgery; history of malignancy within the last 5 years (except curatively treated cervical carcinoma *in situ* or basal cell carcinoma of the skin); clinically manifest cardiovascular insufficiency (NYHA III, IV) or myocardial infarction during the past 6 months, and uncontrolled arterial hypertension.

Patients randomized into the trial constituted the intention-to-treat population (n=55 control arm; n=56 temsirolimus arm).

Patients having received at least one trial-specific treatment and fulfilling the basic eligibility criteria constituted the per-protocol-population (n=50 control arm; n=54 temsirolimus arm). Reasons for exclusion from the per-protocol population were no treatment (n=3), and QTc or laboratory value deviations in the baseline criteria that should have prevented inclusion into the trial (n=5). One patient fulfilled two reasons not to be counted for the per-protocol-population.

The safety population excluded only patients that never received any study-specific therapy (n=3) and resulted in 53 patients in the control arm and 55 patients in the temsirolimus arm.

### *Treatment*

Each treatment with temsirolimus was to be preceded by supportive medication with a histamine H<sub>2</sub>-receptor antagonist. RT consisted of 3D conformal radiotherapy and was given at 2 Gy per fraction, 5 days/week, for up to 6 weeks and to a total dose of 60 Gy; TMZ 75 mg/m<sup>2</sup> was administered orally 7 days/week throughout RT, thereafter, starting 4 weeks after the end of RT (week 11) TMZ 150–200 mg/m<sup>2</sup> was administered for 5 consecutive days every 4 weeks for 6 cycles. Temsirolimus was to be continued until disease progression (PD) or unacceptable toxicity. Crossover from the control to the temsirolimus arm was not allowed. Temsirolimus was administered as 30-minute infusion starting 2 hours before RT; TMZ was given orally at least 1 hour before RT.

### *Biomarker substudy*

Immunohistochemistry was performed basically as recommended by the manufacturers using a heat antigen retrieval procedure (citrate buffer) using the following antibodies and respective dilutions: Phospho-S6 Ribosomal Protein (Ser235/236; 1:400; #2211; Cell Signaling Technology [CST]), Phospho-AKT (Ser473; 1:50; D9E, #4060, CST), Phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204; 1:600; #4370, CST), Phospho-mTOR (Ser2448; 1:100; 49F9, #2976, CST), Phospho-PRAS40 (Thr246; 1:25, #2997, CST), PTEN (1:50, 138G6, #9559, CST), EGFR (1:50; DAKO M7239), and IDH1<sup>R132H</sup> (1:25; clone H14; Dianova,

Hamburg, Germany). The scoring was performed blinded to outcome data. Percentage of tumor cells with any level of positive staining were scored as follows: p-S6RP, p-AKT, p-ERK: invalid, absent or inappropriate tissue, 0 = no positive cells, 1 = 1 - 10%, 2 = 11% - 30%, 3 = 31% - 50%, 4 = 51% - 80%, and 5 = 81% - 100%; p-mTOR, p-PRAS40, PTEN: invalid, absent or inappropriate tissue, 0 = no positive cells, 1 = 1% - 10%, 2 = 11% - 50%, 3 = 51 - 80%, 4 = 81% - 90%, 5 = 91% - 100%. For PTEN presence of vascular staining was used as internal control. For marker analyses the scores were dichotomized into negative (scores 0, 1, corresponding to 0 to 10%) versus positive (scores 2 to 5, >10%). EGFR was evaluated according to the Hirsch score, and IDH1<sup>R132H</sup> was considered positive when cytoplasmic expression was detected.<sup>3,4</sup> FISH for EGFR amplification was performed using Vysis LSI EGFR SpectrumOrange /CEP7 SpectrumGreen Probes (Abbott Molecular, Des Plaines, IL, USA). Tumors with a ratio >2 of the Average EGFR/Average CEP7 were classified as amplified.<sup>3</sup>

#### *Role of the funding source*

This study was funded by an academic grant from Pfizer, Berlin, Germany. Study design, data analysis, and data interpretation were performed collaboratively by the principal investigator, the study team and EORTC. The Steering Committee of the EORTC Brain Tumor Group oversaw the study. The principal investigator (WW) had full access to and reviewed all data, and had final responsibility for the decision to submit for publication. Data collection was performed by the investigators with monitoring performed by the EORTC; the database remained blinded to primary outcome variables for all parties including molecular marker analyses until final analysis.

#### *Statistical considerations*

For multivariable prognostic analysis, Cox models including the three clinical stratification factors, the P-markers and EGFR amplification were computed in each treatment arm. Forward stepwise method was used to select the most significant factors. Because of limited

sample size, this screening was done at a relaxed 15% significance level. Results are interpreted taking this limitation into account. To assess model goodness of fit, the Schemper Percentage of Explained Survival Variation (PEV) was calculated. A PEV of at least 20% was considered a minimum requirement for sufficiently precise predictions. Primary OS12 analysis was performed in the *per protocol* population (i.e. eligible patients who started randomized treatment). All outcome analyses were performed on the intention-to-treat (ITT) population. For multivariable analyses, only samples with all molecular markers assessed were used. Safety was assessed on patients who started randomized treatment.

SAS version 9.4 (SAS Institute Inc., Cary, NC, United States of America [USA]) was used for all analyses. The percentage of explained survival variation (PEV) was computed using the SAS macro RELIMPCR.

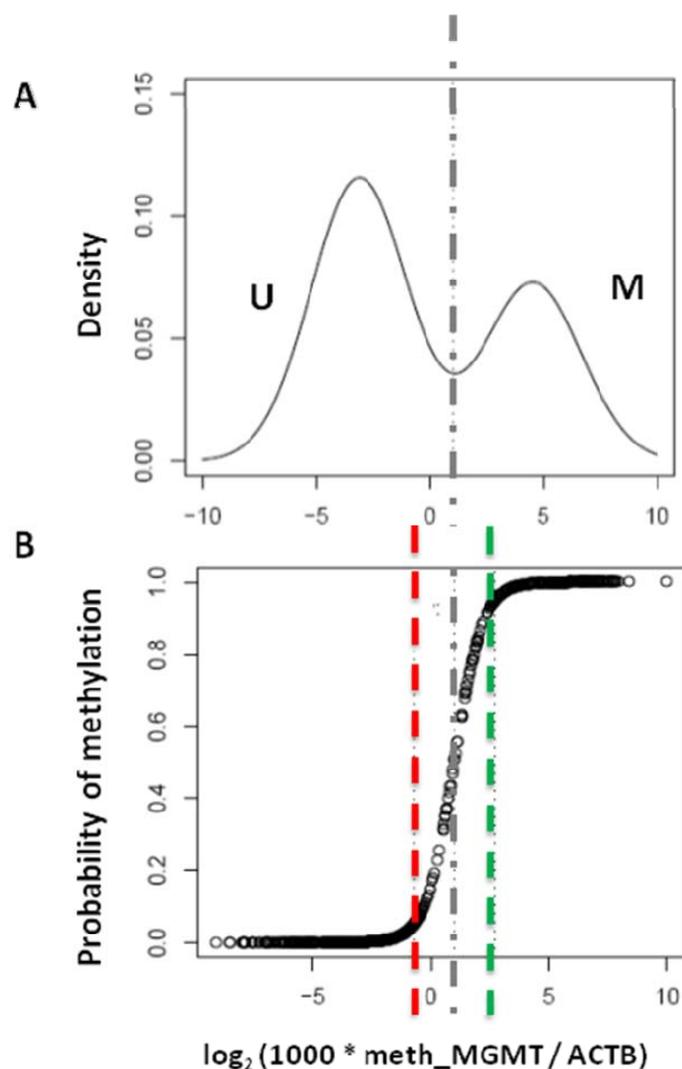
## Supplementary Results

The median OS of 14·8 and 16·0 months observed in the temsirolimus and the TMZ arms, respectively, prompted us to investigate, how the OS in EORTC 26082 compared to the *MGMT* unmethylated EORTC 26981 subpopulation. This is relevant as one of the *caveats* of trials restricted to patients with *MGMT* unmethylated glioblastoma is potential undertreatment by leaving out TMZ in the experimental group. Consistent with reports on enzastaurin<sup>28</sup> or bevacizumab<sup>29</sup>, this was not the case in EORTC 26082. Looking at comparable trial populations (**Supplementary Table 2**), PFS showed no difference for any comparison between arms of EORTC 26082 and 26981. OS shows a significant improvement in the comparison of either arm of EORTC 26082 with the control arm of EORTC 26981 with a HR= 0·45 (0·30-0·67,  $p<0\cdot0001$ ) for RT/TMZ→TMZ and HR= 0·53 (0·36-0·79,  $p=0\cdot0015$ ) for RT/temsirolimus. However, there was only a trend in the comparison between either arm of EORTC 26082 and the RT/TMZ→TMZ arm of the EORTC 26981 trial (data not shown).

**Supplementary References**

1. Vlassenbroeck I, Califice S, Diserens AC, et al: Validation of real-time methylation-specific PCR to determine O6-methylguanine-DNA methyltransferase gene promoter methylation in glioma. *J Mol Diagn* 10:332-7, 2008
2. Stupp R, Hegi ME, Gorlia T, et al: Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* 15:1100-8, 2014
3. Coulibaly B, Nanni I, Quilichini B, et al: Epidermal growth factor receptor in glioblastomas: correlation between gene copy number and protein expression. *Hum Pathol* 41:815-23, 2010
4. Capper D, Weissert S, Balss J, et al: Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. *Brain Pathol* 20:245-54, 2009
5. Luo W, Brouwer C: Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics* 29:1830-1, 2013
6. Chiang GG, Abraham RT: Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J Biol Chem* 280:25485-90, 2005
7. Wiza C, Nascimento EB, Ouwens DM: Role of PRAS40 in Akt and mTOR signaling in health and disease. *Am J Physiol Endocrinol Metab* 302:E1453-60, 2012
8. Wen PY, Chang SM, Lamborn KR, et al: Phase I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas: North American Brain Tumor Consortium trial 04-02. *Neuro Oncol* 16:567-78, 2014

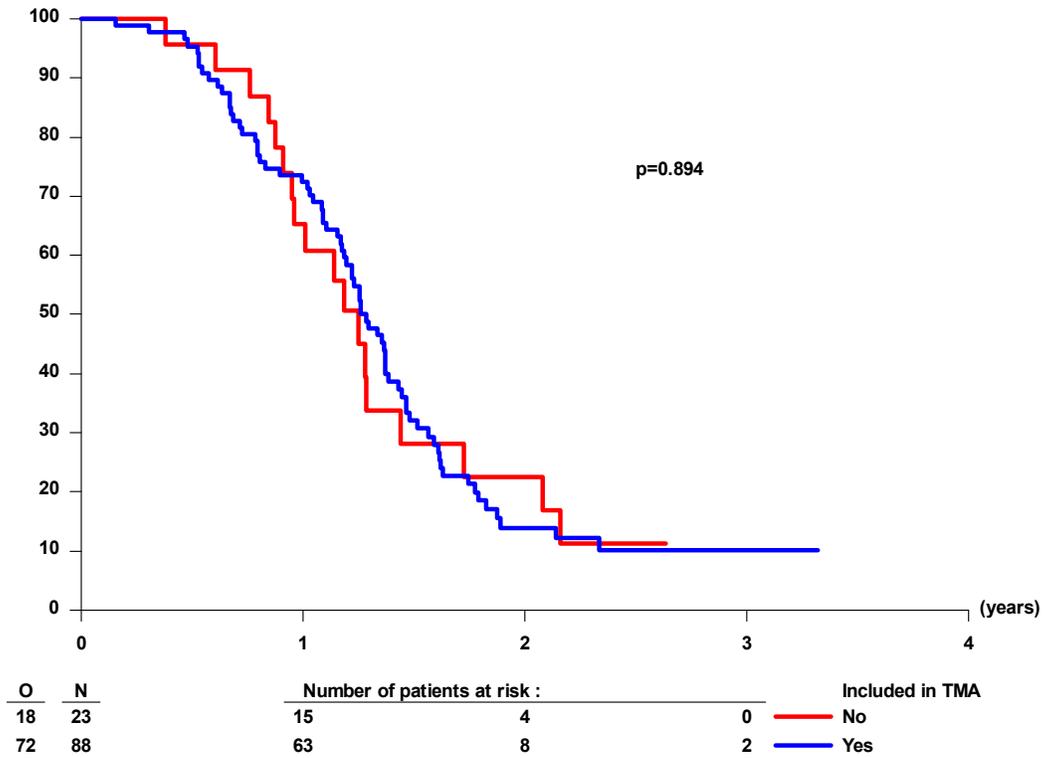
## Supplementary Figures



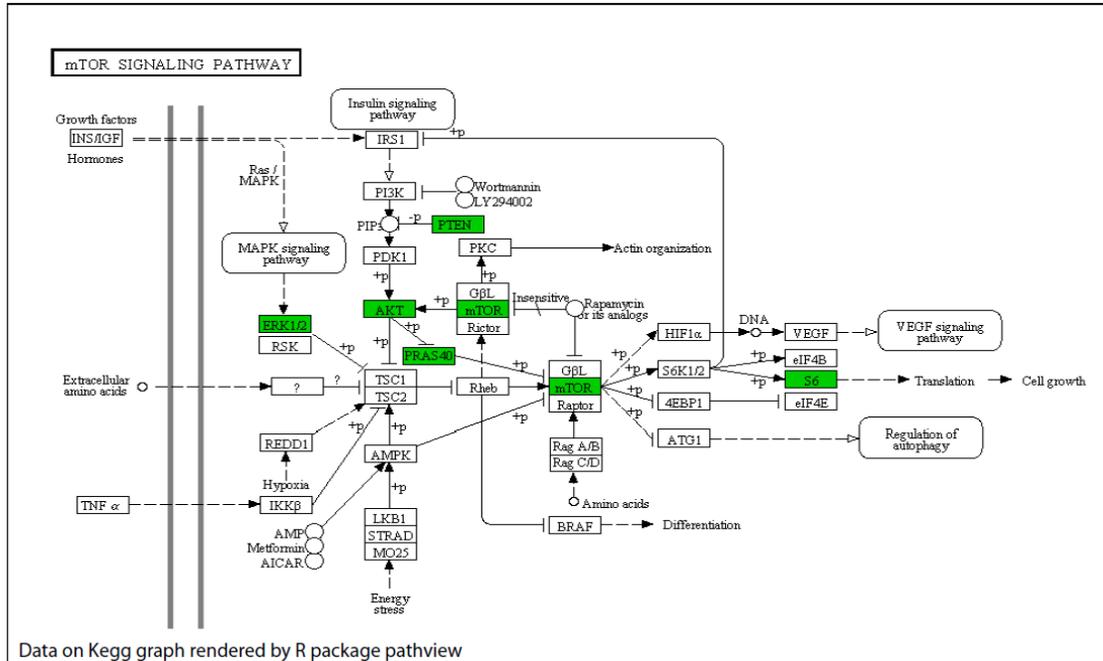
**Supplementary Figure S1.** Definition of MGMT cut-off with a safety margin. Density plot (A), and posterior probability plot (B) for the classification into *MGMT* promoter methylated (M) or unmethylated (U) tumors obtained by fitting a mixture model to the average  $\log_2(1000 * \text{meth\_MGMT}/\text{ACTB})$  for 602 glioblastoma samples. A gray **dashed line** represents the optimal cut-off according to the selected model ( $\log_2$  ratio= 1) corresponds to a **ratio value of 2**. The thresholds for lower bound of the 95% posterior probability for class U, indicated by a red dashed line ( $\log_2$  ratio= -0.75) corresponds to a **ratio value of 0.6**, which has been defined as the **cut-off with a safety margin**. The upper bound 95% posterior probability for class M, is indicated by a green dashed line ( $\log_2$  ratio= 2.72) corresponds to a **ratio value**

**of 6.59.** The region between is often referred to as “gray zone”, since it is associated with higher uncertainty.<sup>1</sup>

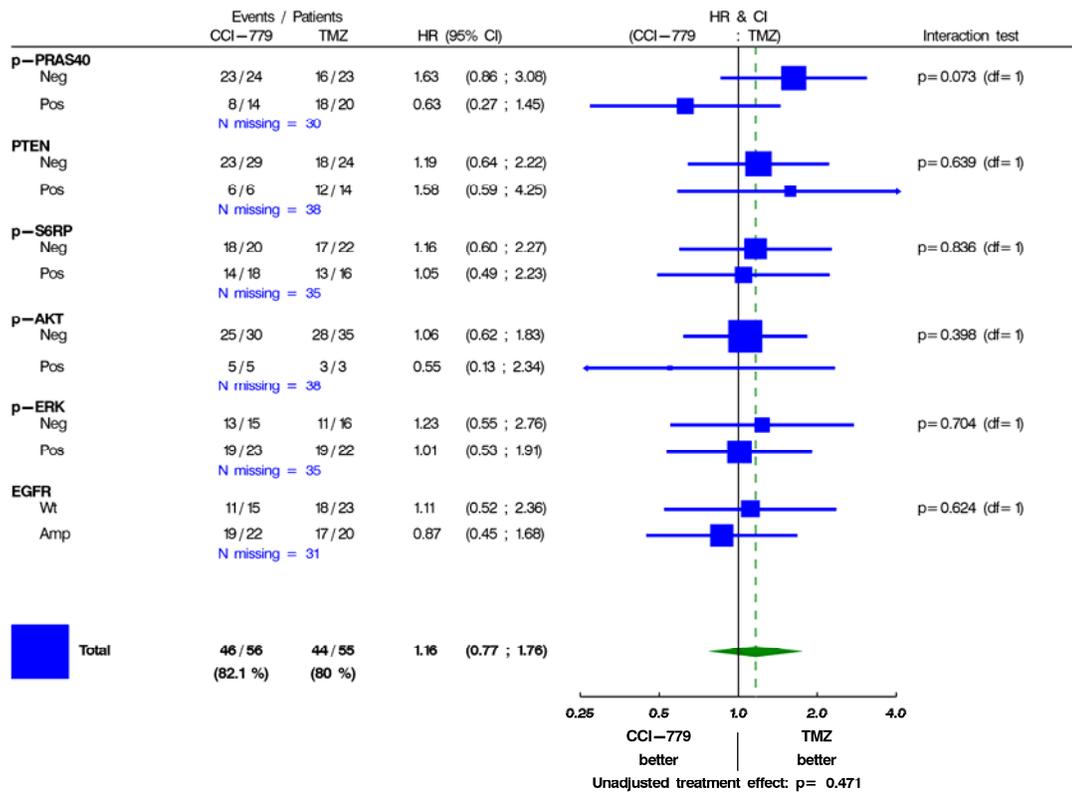
Overall Survival



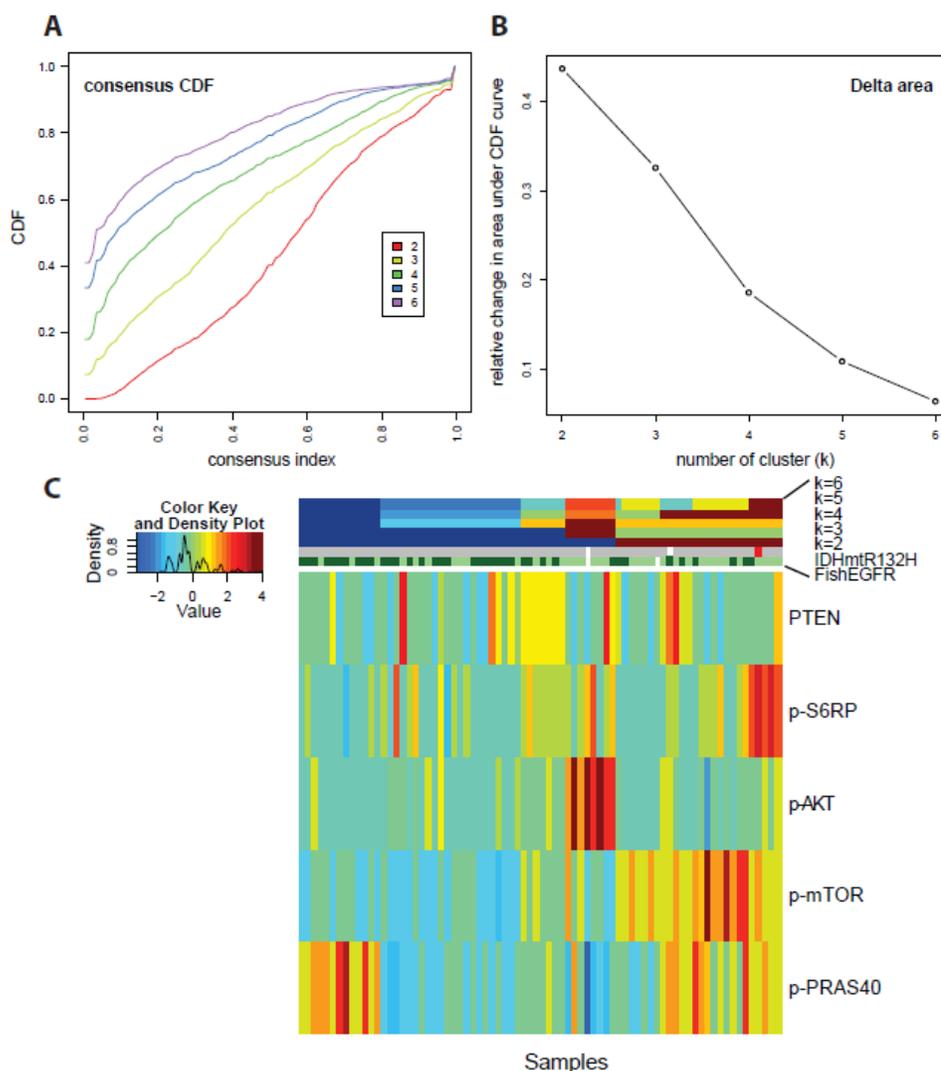
**Supplementary Figure S2.** Comparison of Overall Survival in patients with vs without markers assessments.



**Supplementary Figure S3.** Visualization of markers analyzed in the mTOR signaling pathway from KEGG. The markers are identified by green boxes and the representation was obtained using the R package pathview from the Bioconductor project.<sup>5</sup> We determined phosphorylation of mTOR at serine 2448 (p-mTOR<sup>Ser2448</sup>) which has been shown to be targeted and blocked by rapamycin, a major metabolite of temsirolimus.<sup>6</sup> Furthermore, phosphorylated S6 ribosomal protein (S6RP<sup>Ser235/236</sup>), a direct target of the mTOR effector S6 kinase 1, phosphorylation of AKT<sup>Ser473</sup>, expression of PTEN, and phosphorylation of AKT1 Substrate 1 (Proline-Rich) at Thr246 (p-PRAS40<sup>Thr246</sup>) were assessed. PRAS40<sup>Thr246</sup> is phosphorylated by AKT1. The latter relieves inhibitory function on mTORC1.<sup>7</sup> In addition the *EGFR* amplification status (not indicated) and phosphorylation of ERK1/2<sup>Thr202/Tyr204</sup> that have been postulated as potential markers for resistance to inhibition of the PI3K/AKT/mTOR pathway were determined.<sup>8</sup>



Supplementary Figure S4. Forest plot molecular markers

**Supplementary Figure S5.**

Complete Graphical summary of consensus cluster analysis based on the matrix obtained by the reconstitution of the data in using Non-linear Iterative Partial Least Squares (NIPALS) algorithm. The two first graphics (A et B) were used to determine the optimal cluster number. **(A)** displays the cumulative distribution functions (CDF) of the consensus for each number of clusters ( $k=2, \dots, 6$ ). The Delta Area plot **(B)** represents the relative change in the area under the CDF curve comparing  $k$  and  $k-1$ . Because no strong rupture was detected in this graphic, we kept the cluster based on two groups ( $k=2$ ) by default. **(C)** Display of the heatmap of the score table obtained after NIPALS reconstruction. The rows were ordered by the first axis of the PCA. The consensus classifications, and status of expression of the mutant IDH1<sup>R132H</sup> and amplification of *EGFR* were added as supplementary information. Abbreviations: mTOR

phosphorylated at serine 2448 (p-mTOR<sup>Ser2448</sup>); S6 ribosomal protein phosphorylated at serine 235 and 236, p-S6RP<sup>Ser235/236</sup>; AKT phosphorylated at serine 473, p-AKT<sup>Ser473</sup>; phosphatase and tensin homologue, PTEN; of AKT1 Substrate 1 (Proline-Rich) phosphorylated at threonin 246 (p-PRAS40<sup>Thr246</sup>)

## Supplementary Table S1

<b>Patient's characteristics of biomarker cohort</b>			
<b>Biomarker cohort, (No/Yes)</b>	<b>Included on TMA</b>		<b>Total (N=111) N (%)</b>
	<b>No (N=23) N (%)</b>	<b>Yes (N=88) N (%)</b>	
	<b>Age</b>		
<b>Median</b>	58.3	55.4	55.7
<b>Range</b>	24.4 - 73.6	27.4 - 76.0	24.4 - 76.0
<b>Age (class)</b>			
<50yrs	5 (21.7)	24 (27.3)	29 (26.1)
>=50yrs	18 (78.3)	64 (72.7)	82 (73.9)
<b>Sex</b>			
male	16 (69.6)	55 (62.5)	71 (64.0)
female	7 (30.4)	33 (37.5)	40 (36.0)
<b>Last method</b>			
open brain biopsy	0 (0.0)	4 (4.5)	4 (3.6)
resection	23 (100.0)	84 (95.5)	107 (96.4)
<b>Patient taking anti-epileptic drug</b>			
no	9 (39.1)	29 (33.0)	38 (34.2)
yes, non-EIAED only	12 (52.2)	56 (63.6)	68 (61.3)
yes, EIAED switched	2 (8.7)	3 (3.4)	5 (4.5)
<b>Currently on corticosteroids</b>			
no	16 (69.6)	61 (69.3)	77 (69.4)
yes, stable/decreasing dose	7 (30.4)	26 (29.5)	33 (29.7)
yes, increasing dose	0 (0.0)	1 (1.1)	1 (0.9)
<b>WHO performance status (0-4)</b>			
0	17 (73.9)	55 (62.5)	72 (64.9)
1	4 (17.4)	30 (34.1)	34 (30.6)
2	2 (8.7)	3 (3.4)	5 (4.5)

## Supplementary Table S2

Baseline characteristics at randomization					
	EORTC 26981 (MGMT unmethylated only)		EORTC 26082		Total (N=111)
	RT (N=58)	TMZ (N=65)	TMZ** (N=55)	CCI-779** (N=56)	
	N (%)	N (%)	N (%)	N (%)	N (%)
<b>Age</b>					
<b>Median</b>	54.5	53.0	57.7	54.9	55.7
<b>Range</b>	30.0 - 69.0	22.0 - 70.0	24.4 - 76.0	28.2 - 74.7	24.4 - 76.0
<b>N obs</b>	58	65	55	56	111
<b>Sex</b>					
<b>male</b>	37 (63.8)	38 (58.5)	36 (65.5)	35 (62.5)	71 (64.0)
<b>female</b>	21 (36.2)	27 (41.5)	19 (34.5)	21 (37.5)	40 (36.0)
<b>Extent of resection</b>					
<b>open brain biopsy</b>	2 (3.4)	3 (4.6)	1 (1.8)	3 (5.4)	4 (3.6)
<b>resection</b>	56(96.6)	62(95.4)	54 (98.2)	53 (94.6)	107 (96.4)
<b>currently on corticosteroids</b>					
<b>no</b>	17 (29.3)	20 (30.8)	37 (67.3)	40 (71.4)	77 (69.4)
<b>yes</b>	41 (70.7)	45 (69.2)	18 (32.7)	16 (28.6)	33 (29.7)
<b>WHO performance status (0-4)</b>					
<b>0</b>	17 (29.3)	28 (43.1)	40 (72.7)	32 (57.1)	72 (64.9)
<b>1</b>	35 (60.3)	33 (50.8)	14 (25.5)	20 (35.7)	34 (30.6)
<b>2</b>	6 (10.3)	4 (6.2)	1 (1.8)	4 (7.1)	5 (4.5)

\*\* there is an imbalance between arms for WHO PS.

Stratification by WHO PS (0,1 vs 2) did not work properly. WHO PS 2 accounts for less than 5%.

**Participating Investigators EORTC**Austria:

- Christine Marosi, Matthias Preusser, General Hospital AKH, Medical University Vienna, Austria

Belgium:

- Paul Clement, Jo Costermans, U.Z. Leuven - Campus Gasthuisberg, Belgium

France:

- Mario Campone, Jean-Sebastien Frenel, Gwénaelle Lewandowski, Institut de Cancerologie de l'Ouest (ICO) - Centre Rene Gauducheau, Nantes, France
- Damien Ricard, Karima Mokhtari, Assistance Publique - Hopitaux de Paris - La Pitié Salpêtrière, Paris, France

Germany:

- Astrid Weyerbrock, Sylvia Blust-Maciej, University Medical Center Freiburg, Freiburg, Germany.
- Wolfgang Wick, Michael Platten, Antje Wick, Frank Winkler, David Capper, Andreas von Deimling, Universitätsklinikum Heidelberg - Head Hospital, Heidelberg, Germany

Italy:

- Alba A. Brandes, Enrico Franceschi, Alicia Tosoni, AUSL-IRCCS Scienze Neurologiche, Bologna, Italy

Netherlands:

- Martin van den Bent, Jacqueline Bromberg, Johan M. Kros, Irene van Heuvel, Erasmus MC Cancer Institute - Location Daniel den Hoed, Rotterdam, The Netherlands
- Martin J.B. Taphoorn, Charles Vecht, Anja Kleinendorst, Inga Bottinga, Medisch Centrum Haaglanden – Westeinde, Den Haag, The Netherlands

Spain:

- Salvador Villa, Cristina Carrato, Institut Catala d'Oncologia (ICO). Hospital Germans Trias Pujol, Badalona, Barcelona, Spain

Switzerland:

- Gianfranco Pesce, Oncology Institute of Southern Switzerland - Ospedale Regionale Bellinzona e Valli, Bellinzona, Switzerland
- Patrick Roth, Caroline Happold, Dorothee Gramatzki, Michael Weller, Henning Leske, Universitätsspital und Universität Zürich, Switzerland
- Roger Stupp, Andreas Hottinger, Krisztian Homicsko, Benoit Lhermitte, University Hospital Lausanne, Lausanne, Switzerland

United Kingdom:

- Sara C. Erridge, Antonia Torgersen, Western General Hospital, Edinburgh, U.K.
- Kirsten Hopkins, Helen Andrew, University Hospitals Bristol NHS Foundation Trust - Bristol Haematology and Oncology Centre, Bristol, U.K.