

Can high-dose fotemustine reverse MGMT resistance in glioblastoma multiforme?

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Abstract Glioblastoma multiforme (GBM), the highest grade malignant glioma, is associated with a grim prognosis—median overall survival is in the range 12–15 months, despite optimum treatment. Surgery to the maximum possible extent, external beam radiotherapy, and systemic temozolomide chemotherapy are current standard treatments for newly diagnosed GBM, with intracerebral delivery of carmustine wafers (Gliadel). Unfortunately, the effectiveness of chemotherapy can be hampered by the DNA repair enzyme O6-methylguanine methyltransferase (MGMT), which confers resistance both to temozolomide and nitrosoureas, for example fotemustine and carmustine. MGMT activity can be measured by PCR and immunohistochemistry, with the former being the current validated technique. High-dose chemotherapy can deplete MGMT levels in GBM cells and has proved feasible in various trials on temozolomide, in both newly diagnosed and recurrent GBM. We here report the unique case of a GBM patient, with high MGMT expression by immunohistochemistry, who underwent an experimental, high-dose fotemustine schedule after surgery and radiotherapy. Although treatment caused two episodes of grade 3–4 thrombocytopenia, a complete response and survival of more than three years were achieved, with a 30% increase in dose intensity compared with the standard fotemustine schedule.

Keywords Glioblastoma multiforme · Fotemustine · Dose intensity · MGMT

Introduction

Glioblastoma multiforme (GBM) is the most frequent and aggressive supratentorial malignant glioma, with only 3–5% of patients surviving more than three years after diagnosis [1]. According to WHO (World Health Organization) criteria, GBM is classified as a grade IV glioma and, unlike grade III gliomas, it presents necrosis and endothelial proliferation at histologic analysis [2]. Therapy consists of surgical operation to the maximum possible extent and conformational radiotherapy (standard dose, 60 Gy) to the gross tumor volume. At the present time, intracerebral delivery of carmustine wafers (Gliadel) in the surgical resection cavity and systemic delivery of temozolomide concomitant and adjuvant to radiotherapy are also considered standard treatments for newly diagnosed GBM, because they both provided a statistically significant survival improvement of approximately two months in two separate large, phase III trials [3, 4]. The main concern about chemotherapy is that its effectiveness might be nullified by overexpression of the DNA repair enzyme O6-methylguanine methyltransferase (MGMT) gene. MGMT protein is able to rapidly reverse alkylation at the O6 position of guanine, which is essential for cytotoxicity of both temozolomide and nitrosoureas. One possibility to overcome MGMT resistance is to use high-dose regimens to deplete MGMT in the tumor, which has been attempted in several trials of temozolomide in both newly diagnosed and recurrent GBM [5]. Fotemustine is a third-generation, highly lipophilic chloroethylnitrosourea, with a phosphoalanine group with a carrier function in its chemical structure, which

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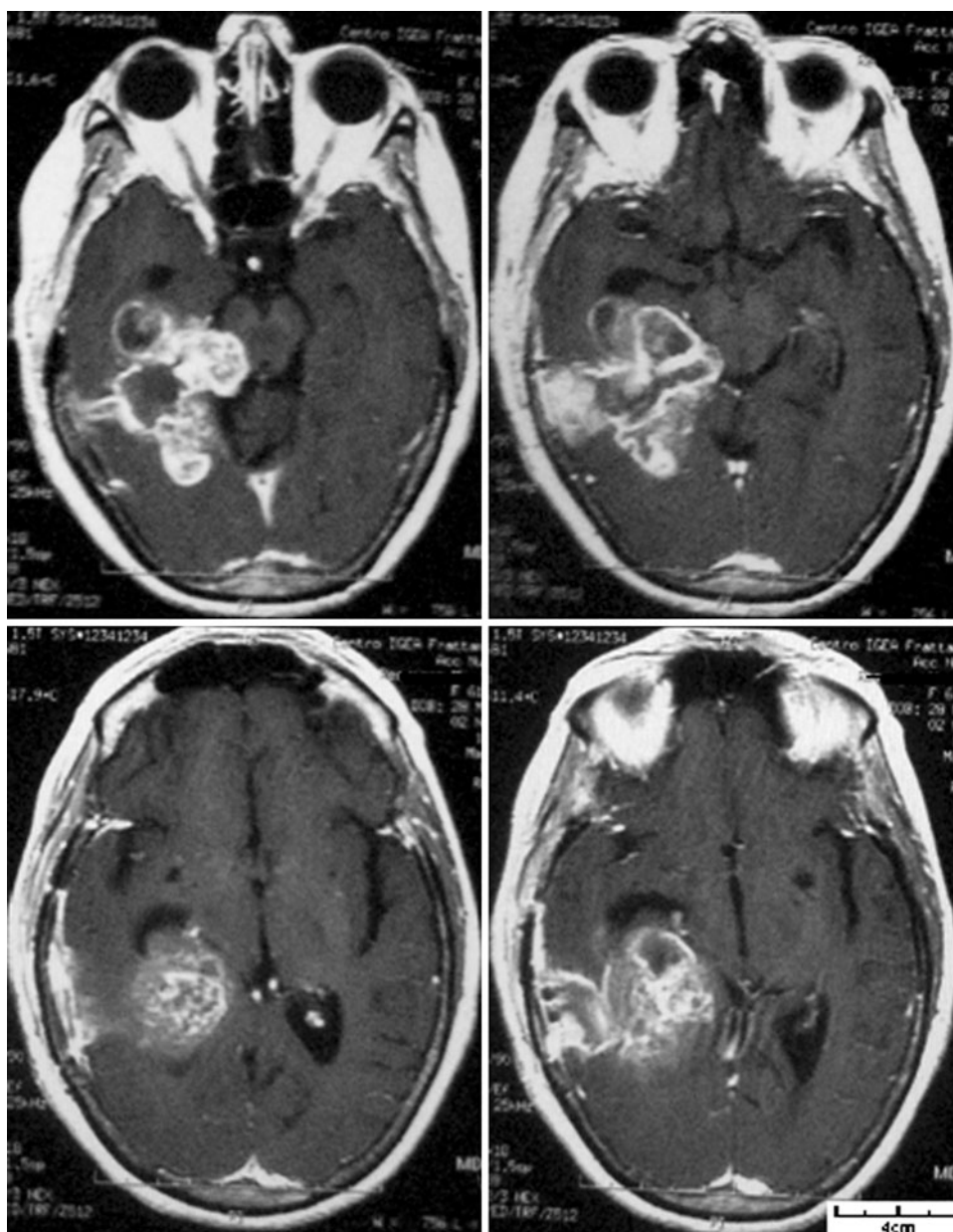
has activity against malignant gliomas [6]. Like temozolomide, fotemustine is probably associated with MGMT resistance [7]. No clinical trial has ever employed an intensified fotemustine regimen with the intention of overcoming MGMT resistance. We here report the unique case of a GBM patient with positivity to MGMT by immunohistochemistry, who showed complete and durable response to an experimental high-dose fotemustine regimen.

Case history

In September 2006, the patient, a woman aged 61, was admitted to hospital for investigation after a sudden

episode of temporary loss of consciousness. She did not present any history of major health problems, nor had she recently reported any particular symptom. A brain CT scan was urgently performed and it revealed the presence of a mass in the patient's right temporal lobe, associated with a median shift. On Gd-RMI, the ring-enhanced tumor measured approximately 4×4 cm on its largest diameters, with large cystic and necrotic areas and diffuse perilesional edema. It extended deeply from the temporal lobe to the hippocampus and parahippocampal gyrus. On pre-operative evaluation, patient seemed fully conscious, although somewhat disoriented about time and place. She also complained about short-term memory loss and ideomotor slowing. In October 2006, a right temporal craniotomy was

Fig. 1 T1-weighted MRI with contrast, performed before the start of chemotherapy

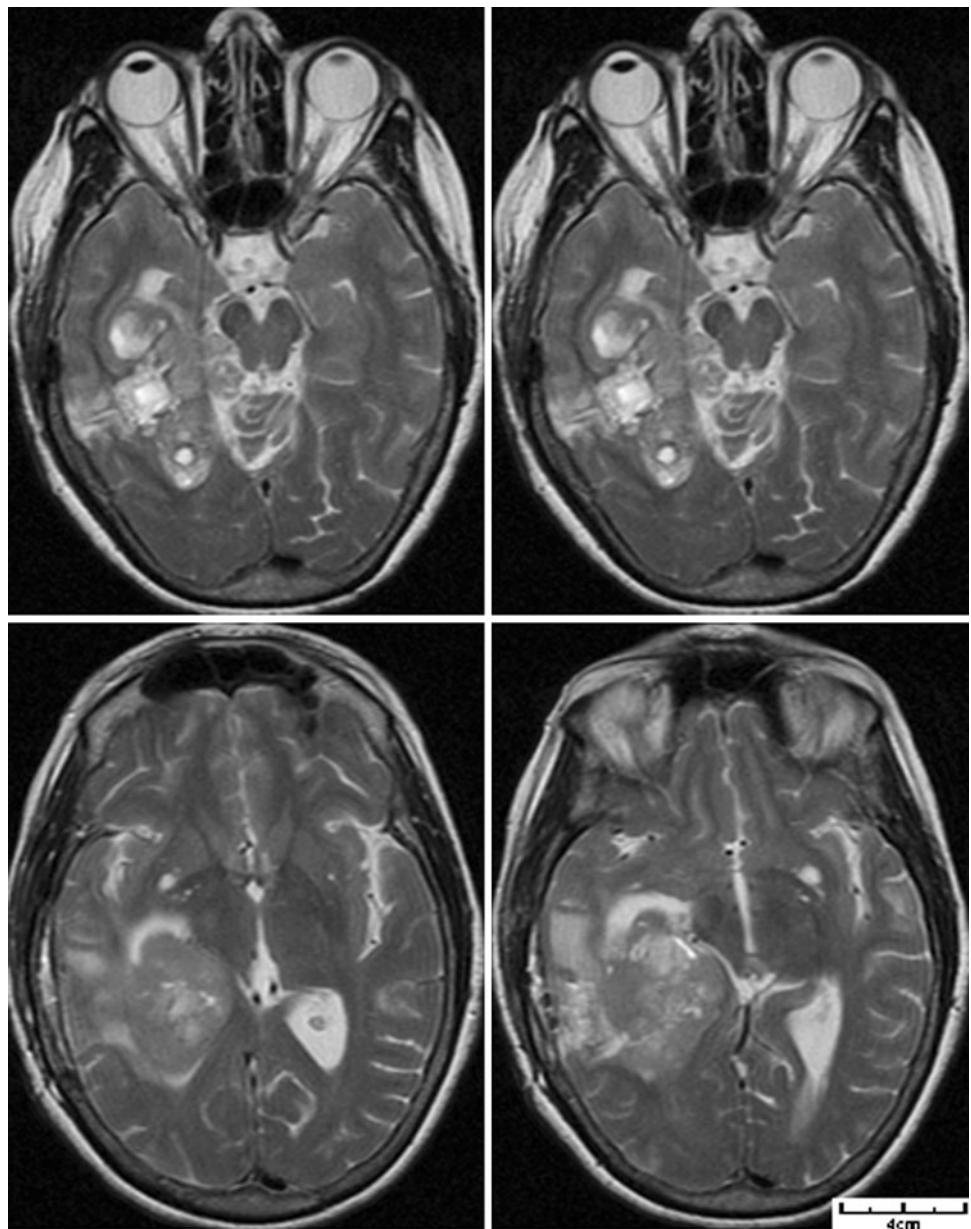


performed at the “Istituto Besta - Milano”, with sub-total tumor resection and excision of a 4 × 3 cm nodule. Operation did not cause any adjunctive neurologic deficit and the patient maintained her ECOG performance status of 0. Histology analysis showed pleomorphic, poorly differentiated glial cells, with necrosis, dense cellularity, mitotic figures and endothelial proliferation, and immunohistochemistry for both MGMT and Ki-67 expression showed diffuse staining at high-power field magnification. The final diagnosis was GBM with MGMT overexpression by immunohistochemistry. In view of this result and the absence of any recommended effective alternative treatment for GBM with MGMT overexpression, we proposed the patient underwent an experimental high-dose fotemustine-

based regimen. We explained that fotemustine had proved to be active in malignant gliomas, although it was not regarded as the standard first line of treatment. A written consent form to therapy was obtained.

After standard conformational radiotherapy (60 Gy to gross tumor volume, with a 2–3 cm margin for the clinical target volume), in December 2006 the patient was started on fotemustine at the Department of Molecular and Clinical Endocrinology and Oncology. According to our intensified schedule, fotemustine was administered at 130 mg/m² (instead of 100 mg/m² of the conventional schedule) on days 1, 8, and 15, with a five-week pause (induction phase), and then every two weeks (instead of every three weeks of the conventional schedule) at 130 mg/m² (instead of

Fig. 2 T2-weighted MRI, performed before the start of chemotherapy



100 mg/m² of the conventional schedule), until progression or unacceptable toxicity. Blood count was tested every week, while liver and kidney function was tested every three weeks. An MRI was performed before the start of chemotherapy (Figs. 1, 2) and then every 6–8 weeks during therapy. After the induction phase, patient experienced grade 3 thrombocytopenia, which resolved spontaneously. A Gd-MRI was performed eight weeks after the start of treatment and this showed a 25% reduction in the size of the enhancing tumor, with improvement of neurologic deficits and no change in steroid use (desametasone 8 mg per day). Maintenance phase was started with biweekly administration. After three biweekly cycles at 130 mg/m², the patient experienced grade 4 thrombocytopenia, which required

platelet transfusion. As shown in Fig. 3, after a total of six cycles, Gd-MRI indicated a partial response according to MacDonald's criteria, with more than 50% reduction in tumor size in comparison with baseline MRI, reduced steroid use (desametasone 4 mg per day), and improved neurologic function. On the basis of this result, we decided to continue fotemustine administration with the same regimen and the same dose, despite previous grade 3–4 thrombocytopenia. In June 2007, after undergoing four additional biweekly fotemustine cycles with no grade 3–4 hematologic toxicity, the patient showed complete response on MRI, with disappearance of the contrast enhanced lesion, suspension of desametasone consumption, and almost complete neurologic recovery. Ten fotemustine cycles were administered,

Fig. 3 T1-weighted MRI with contrast, performed after six cycles of fotemustine

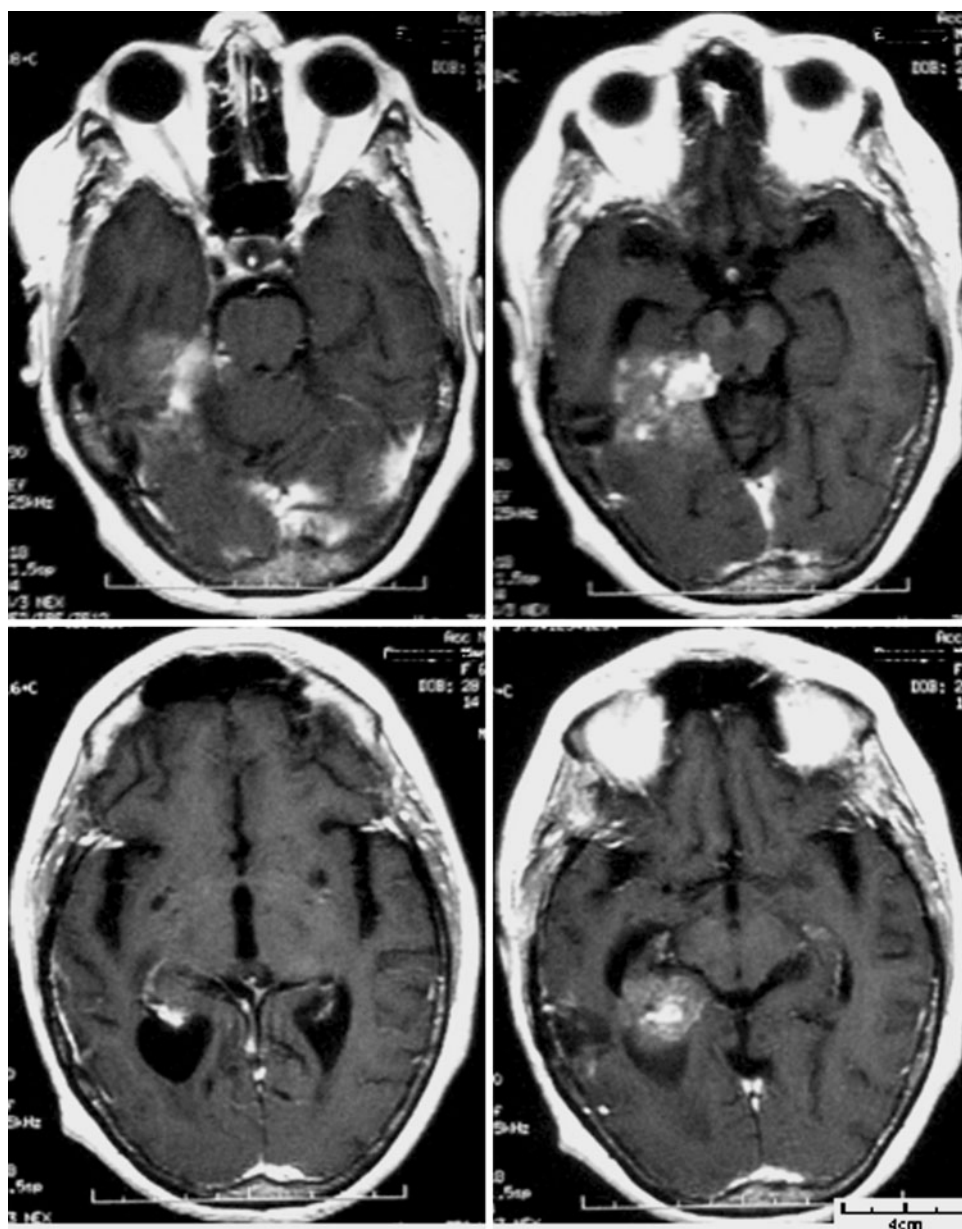
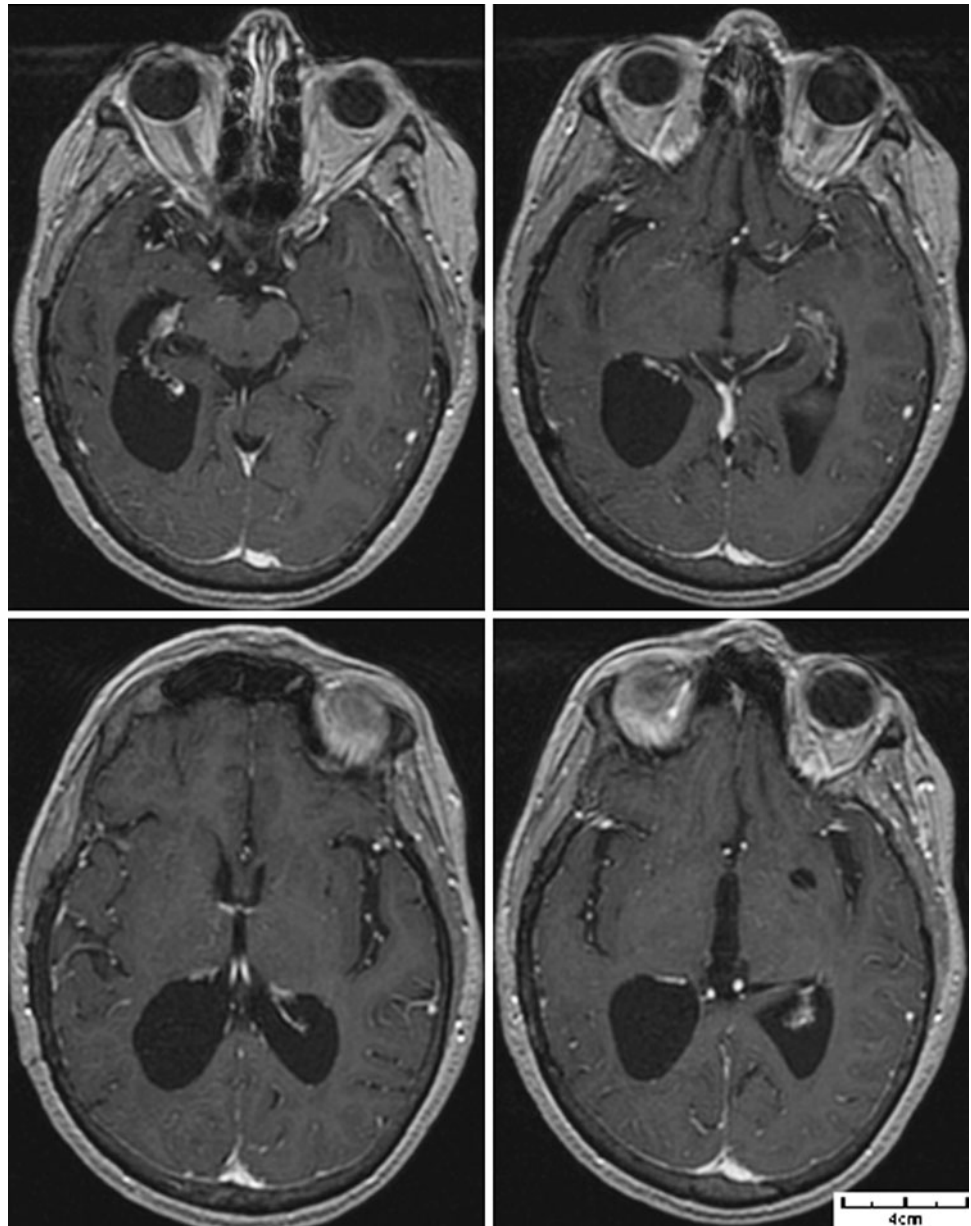


Fig. 4 SLAB 3D MRI with contrast, performed three years after diagnosis



for a total delivered dose of 1.3 g/m^2 . During follow-up, the patient underwent Gd-MRI scans every six weeks for the first six months, then every three months for the following year, and every six months until the present time. No recurrence has been detected. An MRI performed in January 2010 is shown in Fig. 4. The patient is currently disease free and fully functional, with an excellent quality of life and no neurological disability.

Discussion

In 2005, analysis on 206 patients enrolled in the Stupp trial showed that temozolomide concomitant and adjuvant

chemotherapy provided a statistically significant survival advantage in the subgroup of 92 patients with methylated MGMT by PCR (21.7 months in the therapy group vs. 15.3 months in the control group, $P = 0.007$), whereas there was no statistically significant survival extension in the subgroup of 114 patients with unmethylated MGMT on PCR (12.7 months in the therapy group vs. 11.8 months in the control group, $P = 0.06$) [8]. It must be underlined that in this retrospective analysis, conducted on a large, representative sample of the study population, MGMT activity was measured by PCR, which distinguishes a methylated MGMT gene (MGMT positive, favorable prognostic factor) from an unmethylated MGMT gene (MGMT negative, unfavorable prognostic factor). Although several previous

trials had correlated MGMT activity by immunohistochemistry with outcome [9–11], it is now clear that MGMT activity must be assessed by PCR, because of major limitations of immunohistochemistry, for example interobserver variability, lack of standardization, and, most importantly, its unconfirmed prognostic value [12].

Unfortunately, there is currently no established treatment for MGMT-negative patients, so routine MGMT evaluation cannot be recommended. Based on the rationale that suicide enzyme MGMT must be continuously resynthesized after exposure to alkylating agents, several trials have used intensified temozolomide schedules in both newly diagnosed and recurrent GBM. These regimens did not cause dramatic hematologic toxicity, but their increased effectiveness with regard to the standard temozolomide regimen in patients with unmethylated MGMT on PCR is yet to be definitely established [5]. The currently ongoing trial RTOG 0525 randomized 1153 patients with newly diagnosed GBM to adjuvant intensified (75–100 mg/m² on days 1–21 of a 28-day cycle) or standard (150–200 mg/m² on days 1–5 of a 28-day cycle) chemotherapy, after chemoradiotherapy (30 × daily 2 Gy plus daily 75 mg/m² temozolomide). Unlike the 2005 Stupp trial, patients were stratified by MGMT promoter methylation status before randomization, so the results of this large phase III trial will probably provide conclusive evidence about the effectiveness of high-dose temozolomide in overcoming MGMT resistance [13].

Fotemustine has gained popularity as a second-line treatment for patients with recurrent or progressive GBM. As shown in Table 1, its activity has been investigated in several phase II trials, with progression free survival and response ranging from 1.7 to 6.1 months and from 7.1 to 29.6%, respectively, in patients with recurrent disease. MGMT is very likely to confer resistance to fotemustine, because of its mechanism of action. In fact, like temozolomide, fotemustine cytotoxic activity relies on O6 alkylation of guanine, which suicide enzyme MGMT can rapidly reverse by transferring the alkyl adduct from DNA to a reactive cystein residue of MGMT itself [7]. In the study conducted by Brandes et al. on second-line fotemustine in 43 recurrent GBM patients, disease control was significantly better for patients with methylated MGMT than for patients with unmethylated MGMT (75 vs. 34.6%, $P = 0.044$). Nevertheless, no significant difference was reported for progression-free survival and survival between MGMT-positive and MGMT-negative patients, which might be interpreted as a consequence of either a change in MGMT status at progression or simply the small sample of the study [15].

The rationale of our choice to deliver a high-dose fotemustine regimen was based on the absence of any established, effective alternative treatment for MGMT-

negative GBM patients, fotemustine activity in malignant gliomas, and the fact that, unlike patients in the Frenay study, our patient had not received prior chemotherapy, which constitutes a risk factor for fotemustine toxicity [19]. A number of reasons induced us to prefer fotemustine to carmustine or lomustine, which are more commonly prescribed nitrosoureas for GBM. Its biochemical structure, which consists of a phosphoalanine group bound to a chloroethylnitrosourea molecule, makes fotemustine highly lipophilic, with improved penetration through the blood–brain barrier compared with classic nitrosoureas, and an increased penetration coefficient into malignant cells, which are more permeable to amino acids than normal cells. Preclinical data also indicate that fotemustine is more active than carmustine in mouse and human cell lines, which can possibly be related to its lower carbamoylating and higher pro-apoptotic effect [7]. Furthermore, in a large, phase III trial [20], survival of only 10.1 months, with an incidence of grade 3–4 leukopenia of 30% and an incidence of grade 3–4 thrombocytopenia of 42%, was reported in 201 chemotherapy naïve, newly diagnosed GBM patients treated with radiotherapy and adjuvant single agent carmustine (200 mg/m²/day on day 1, every eight weeks for six cycles), while in the phase II Frenay study on fotemustine, an incidence of grade 3–4 leukopenia of 17.2% and an incidence of grade 3–4 thrombocytopenia of 23% were reported in 38 pretreated recurrent malignant glioma patients. These data seem to preclude any intensified or dose-dense carmustine-based adjuvant regimen and to suggest that an intensified or dose-dense fotemustine regimen could be feasible.

Several interesting results were obtained with our patient. First, our experimental schedule proved to be feasible, with two episodes of grade 3–4 thrombocytopenia, which we did not judge to be life-threatening. Second, despite these manageable episodes of severe toxicity, we succeeded in achieving an approximately 30% increase in dose intensity after a 26-week treatment (Fig. 5), compared with the standard regimen at full doses employed by Scoccianti et al. [16] and Fabrini et al. [17], and even greater augmentation compared with the standard regimen at 25% reduced doses, employed by Brandes et al. [15]. Third, a complete radiographic response and survival of more than three years were obtained, despite pretreatment negative prognostic factors, for example MGMT status itself and radiographic signs associated with poor prognosis [21], for example the presence of perilesional edema, which extended more than 1 cm from the margin of the tumor in one area in the right temporal lobe, and the absence of non-enhancing tumor (Figs. 1, 2). It must be emphasized that from January 2006 to December 2009, most GBM patients have been referred to our work-group for second-line treatment and that this case was the only

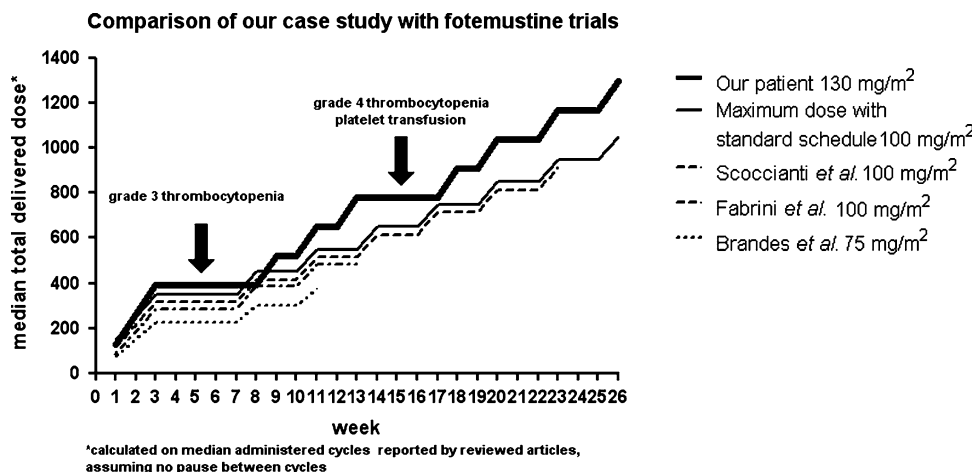
Table 1 Fotemustine in MG patients

Author year of publication reference	Main inclusion criteria and sample size	Treatment	CT schedule	Grade 3–4 HT	Median PFS	RR (%)	Survival after treatment
Beauchesne 2009 [14]	22 Newly diagnosed MG (16 GBM)	RT with concomitant and adjuvant CT	CRT: daily 10 mg/m ² FTMS and 2 Gy RT (5 days per week for 6 weeks) Adjuvant CT: weekly 100 mg/m ² for 3 weeks), then 100 mg/m ² perfusion every 28 days after a 4-week break	13.6%T	6 months	15.5	9.9 months
Brandes 2009 [15]	43 recurrent GBM	CT	Standard schedule ^a at 75–100 mg/m ²	IP: 20.9%T 16.3%L MP: 0%T 14.3%L	1.7 months	7.1	6 months (95% CI: 5–7)
Scoccianti 2008 [16]	27 recurrent GBM	CT	Standard schedule ^a at 100 mg/m ²	11.1%T 3.7%L	5.8 months	29.6	9.1 months (range: 3.3–29.4)
Fabrizi 2009 [17]	50 recurrent GBM	CT	Standard schedule ^a at 100 mg/m ²	8%T 2%L 2%A	6.1 months	18	1 year survival after treatment: 40%
Silvani 2008 [18]	54 recurrent GBM	CT	PC: fixed dose 450 mg on days 1–2 and fixed dose 300 mg on day 3 FTMS: 110 mg/m ² on day 3 every 5 week	1.3%T 2.6%L	4.5 months	11.2	Patients at first recurrence: 7.1 months, (95% CI, 3.5–8.7). Patients at second recurrence 6.2 months (95% CI, 4.0–6.9)
Frenay 1991 [6]	38 recurrent MG (21 GBM)	CT	Standard schedule ^a at 100 mg/m ²	23%T 17.2%L 5%A	Patients with PR: 7.63 months. Patients with NC: 4.9 months	26	Patients with PR: 9.3 months Patients with NC: 9.9 months Patients with PD: 3.5 months

^a Standard schedule consists of induction phase(IP): three weekly FTMS cycles at 100 mg/m, then five-week break, then maintenance phase (MP): FTMS cycles at 100 mg/m every three weeks

GBM, glioblastoma multiforme; MG, malignant glioma; FTMS, fotemustine; PC, procarbazine; CT, chemotherapy; RT, radiotherapy; CRT, chemoradiotherapy; CI, confidence interval; T, thrombocytopenia; L, leukopenia; A, anemia; IP, induction phase; MP, maintenance phase; RR, response rate; PFS, progression-free survival; PR, partial response; NC, no change; PD, progressive disease

Fig. 5 A temporal analysis of total fotemustine dose delivered to our patient. A comparison with phase II fotemustine studies in GBM is illustrated



one we treated with this experimental protocol. We are well aware of the limitation of qualitative MGMT assessment on immunohistochemistry, but it must be considered that PCR was unavailable at the time we treated our patient and that its superiority to immunohistochemistry has not been definitely established until recently. Valuable prognostic information could also be added by sequencing of the isocitrate dehydrogenase (IDH1) gene, because the presence of a mutated IDH1 gene rather than the wild-type gene has been linked to improved survival in glioma patients (27.4 vs. 14 months in grade 4 gliomas, $P < .01$) and might contribute to explaining the long-term survival achieved, if it were mutated in our patient. Nevertheless, it must be considered that, similarly to the role of MGMT assessment on PCR, the importance of IDH1 gene mutation has only recently emerged and that a small proportion of primary GBM patients (6% of 183 GBM specimens) presents a mutated IDH1 gene [22].

Conclusions

This case shows that a high-dose fotemustine regimen might be effective in overcoming MGMT resistance, without causing dramatic toxicity, especially in chemotherapy-naïve patients. In light of the rareness of long-term survival in GBM patients (especially in cases of unmethylated MGMT [1]), our high-dose fotemustine regimen might have interesting potential which could be investigated in a phase I/II trial.

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Conflict of interest None.

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