Fotemustine in recurrent high-grade glioma: MRI neuro-radiological findings

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Abstract. The use of fotemustine (FTM) has been authorized in certain countries for the treatment of recurrent high-grade gliomas (HGG) after Stupp therapy. However, to the best of our knowledge, no studies have assessed changes in magnetic resonance imaging (MRI) during treatment with FTM monotherapy. The aim of the present study was to assess the neuroradiological findings in a cohort of patients with recurrent HGG treated with FTM monotherapy. Patients with HGG already undergoing the Stupp protocol were retrospectively included. MRIs (pre- and post-FTM treatment) were analyzed by two neuroradiologists in consensus: Volume and diffusion values of the contrast-enhanced component were measured on T1-weighted volumetric sequences after gadolinium injection and on apparent diffusion coefficient (ADC) maps, respectively. A total of 19 patients [median age, 49 years; interquartile range (IQR), 43-57 years] were included, 17 of whom had glioblastoma and 2 had astrocytoma isocitrate dehydrogenase-mutated grade 4. The median duration of FTM therapy was 4 months (IQR, 2-6 months). The median tumor volume measured on the contrast-enhanced component was 2,216 mm^3 (IQR, 768-13,169 mm^3) at baseline and 9,217 mm^3 (IQR, 3,455-16,697 mm^3) at the end of treatment, with a median change of +38% (IQR, -45-+574%). A total of seven patients showed a volume decrease. ADC value analysis of the enhancement area demonstrated no significant difference between the pre- and the post-FTM treatment periods (P=0.36); however, in three patients, the decreases in ADC levels were particularly marked. In conclusion, the present study described a series of patients with recurrent HGG treated with FTM in monotherapy, demonstrating a prevalent increase in lesion enhancement and three cases of marked restrictions on diffusion-weighted imaging. Further prospective studies are required to corroborate such preliminary results.

Introduction

High-grade gliomas (HGG) are the most common malignant primary brain tumors in adults, with an incidence of ~5/100,000 individuals per year in Europe and North America. Out of all HGGs, ~70% are glioblastomas (GBM) (1). Malignant primary brain tumors are responsible for the highest average number of years of life lost among all cancers, with an average loss of ~20 years (2). After first HGG diagnosis at adjuvant therapy, the disease within a few months recurs and the survival remains limited (3).

Standard first-line therapy includes the following: Maximal surgical resection, followed by adjuvant conformational radiotherapy with concomitant or adjuvant temozolomide (TMZ) administration in the case of GBM, and concomitant and/or exclusively adjuvant chemotherapy for anaplastic astrocytomas based on the isocitrate dehydrogenase (IDH1) status (4-6). Despite advances in understanding tumor biology and in the development of therapeutic regimens, GBM prognosis remains poor, with an overall survival of 14.6 months (4). Furthermore, virtually all cases eventually recur. Among prognostic and predictive factors, the O(6)-methylguanine-DNA methyltransferase promoter (MGMTp) enzyme methylation status has remained the strongest (4). Although MGMTp-methylated HGGs exhibited a higher response to alkylating drugs than

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Abbreviations: ADC, apparent diffusion coefficient; ADC^{10}, ADC 10th percentile; CET, contrast-enhancing tumor; DCR, disease control rate; DWI, diffusion-weighted imaging; FTM, fotemustine; GBM, glioblastoma; HGG, high-grade glioma; IDH, isocitrate dehydrogenase; IQR, interquartile range; MGMT, O(6)-methylguanine-DNA methyltransferase; MGMTp, MGMT promotor; MRI, magnetic resonance imaging; PR, partial response; PD, progressive disease; RANO, Response Assessment in Neuro-Oncology; SD, stable disease

Key words: apparent diffusion coefficient, fotemustine, high-grade glioma, glioblastoma, recurrence, MRI, diffusion-weighted imaging

unmethylated ones, the tumor often progresses. A strategy to avoid resistance to alkylating agents needs to be developed and certain studies have proposed the use of high-dose alkylating drugs (7), dose dense TMZ (8) or TMZ enhancers (9).

Treatment options for recurrent HGG are limited: As only a small proportion of patients are suitable candidates for re-irradiation or further surgery, the most common approach involves systemic treatment with chemotherapy, immunotherapy or antiangiogenic agents (10,11). However, studies have reported controversial results and a standard of care has not yet been clearly defined (12). Among the chemotherapy agents, fotemustine (FTM), a third-generation nitrosurea that has been studied as a second-line therapy for recurrent HGG, is currently approved for use in a limited number of countries (13). It is an alkylating cytotoxic agent characterized by a phosphoalanine carrier, which facilitates movement through the blood-brain barrier (14,15).

In patients with HGG, FTM can be administered according to different schedules. The standard schedule includes an induction phase dose of 100 mg/m^2 weekly for 3 consecutive weeks, followed by a 5-week rest period and a maintenance phase dose of 100 mg/m^2 every 3 weeks (16-18). FTM has also been proposed in combination with other alkylating drugs or bevacizumab (19). Furthermore, high-dose FTM has been suggested to overcome MGMT resistance (7). In 2011, Addeo *et al* (15) proposed a fractionated FTM schedule including an induction-phase dose of 80 mg/m^2 every 2 weeks for 5 consecutive weeks, followed by a 4-week rest period and a maintenance-phase dose of 80 mg/m^2 every 4 weeks. The latter schedule was also proposed for elderly patients and showed a good level of safety (20).

As there are no standard second-line treatments for recurrent HGG, an individualized approach involving either surgery, radiotherapy, systemic therapy or a combination thereof can be considered, based on different factors, such as the time interval since the first diagnosis, the location of tumor recurrence, the clinical performance of the patient and their previous response to therapy. However, often only supportive therapy is administered (21,22). Among chemotherapy agents used as second-line therapies, FTM has been reported to have a good efficacy and safety profile in patients who have adequate hematologic function (15).

Despite the number of studies available in the literature on HGG treated with FTM (15-18), there are no descriptive radiological studies or studies focusing on the changes in tumor volume and cellularity on conventional magnetic resonance imaging (MRI) during FTM therapy, to the best of our knowledge. Therefore, the aim of the present study was to describe the neuroradiological changes in a cohort of patients with recurrent HGG treated with FTM as monotherapy.

Patients and methods

Study population. The present study was a single-center retrospective observational study that received approval from the Institutional Ethics Committee of Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Neurologico 'Carlo Besta' (Milan, Italy) (SEURAT study; approved on May 17, 2023). Consecutive patients with recurrent HGG who were treated at Fondazione IRCCS Istituto Neurologico 'Carlo Besta' (Milan, Italy) between September 2017 and April 2020 were enrolled. Patients who did not satisfy the following inclusion criteria were excluded: i) Adult patients with recurrent HGG who had received previous Stupp treatment; ii) patients treated with FTM therapy as monotherapy; and iii) patients who underwent MRI within 2 weeks of the start of FTM treatment and within 2 weeks from the end of the treatment. Patients underwent chemotherapy with FTM, given intravenously at a dose of 80 mg/m^2 every 2 weeks for five consecutive administrations (induction phase), and then every 4 weeks at 80 mg/m^2 as maintenance for a total of five consecutive cycles (15).

Patient evaluation and therapy response. Epidemiological, clinical and diagnostic (both radiological and histological/molecular) data were collected, as well as information on therapy, recurrence and survival. In particular, the following clinical data were obtained: date of first surgery, adjuvant therapy, therapies performed after recurrence (including radiotherapy and additional other surgeries with corresponding histological diagnosis), date of start and end of FTM therapy, reason for FTM interruption and date of death. All patients described in the present study have died at the time of the present work.

MRI acquisition. Brain MRI scans were performed using different scanners, used for clinical practice in our centre and which routinely undergo quality control according to international best practice to ensure comparability (detailed in the Supplementary material): 1.5 T Siemens Avanto Fit (Siemens AG), 1.5 T Philips Achieva or 3 T Philips Achieva Dstream (Philips Healthcare). A total of three different scanners were used because these are the ones that are available at our centre and that are used indifferently for clinical practice. As this is a retrospective study, no single scanner was used for MRI. The acquisition protocol included volumetric T1-weighted images (section thickness, 1 mm) before and after contrast medium administration (gadolinium chelates at 0.1 mmol/kg); axial or coronal T2-weighted images; axial, coronal or 3D T2-weighted fluid-attenuated inversion recovery images (slice thickness, 4 mm; 3 orthogonal directions); and diffusion-weighted images (DWI); (b=0-1,000 sec/mm^2; bicommissural acquisition) from which apparent diffusion coefficient (ADC) maps were automatically reconstructed.

Imaging analysis. The contrast-enhancing tumor portions were segmented by two neuroradiologists with 3 and 7 years of experience, respectively (APS and FMD), using 3D-T1 images after contrast medium administration. A semi-automatic open-source software, ITK-SNAP software, version 3.6.0 (23), was used for segmentation, with manual corrections of over- and under-segmentation errors. The resulting segmentations were then processed through the open-source 3D Slicer software (http://www.slicer.org; version 5.6.1) (24) in order to extract volumes of contrast-enhancing tumor (as shown in Fig. 1); the ADC values of contrast-enhancing tumor were also extracted using the software, by creating masks on ADC maps corresponding to the segmented tumor volume.

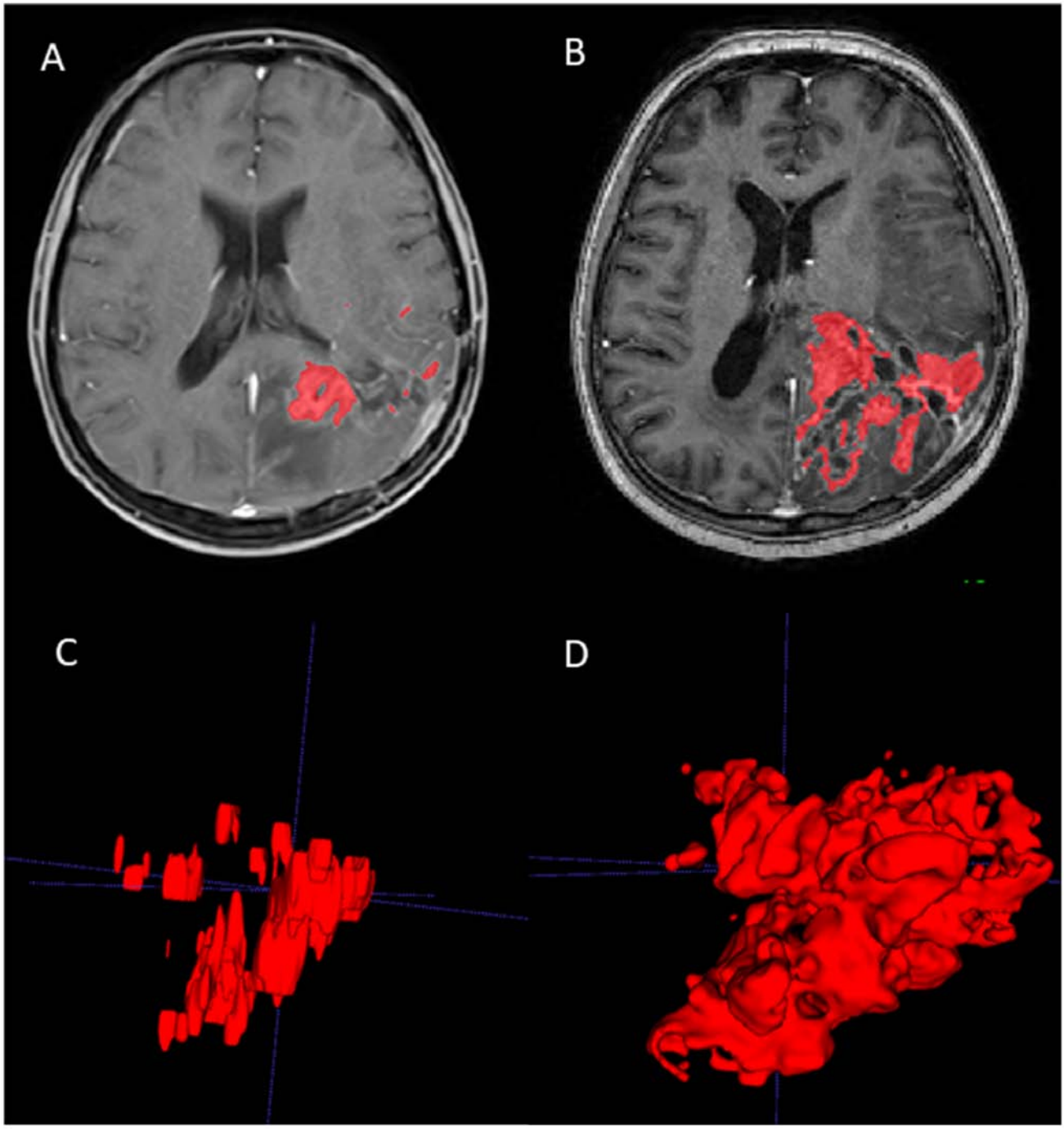

Figure 1. Example of tumor segmentation (A and B) on 3-dimensional-T1-weighted magnetic resonance imaging (A) before and (B) after FTM treatment and (C and D) 3D reconstruction (C) before and (D) after FTM treatment. FTM, fotemustine.

The ADC 10th percentile (ADC^{10}) was considered for statistical analysis. In cases of multiple brain localization, all lesions were segmented. The segmentators were blinded to both clinical and pathological data and an example of tumor segmentation is presented in Fig. 1.

Statistical analysis. The variables included in the present study were analyzed using SPSS version 20 software (IBM Corp.) and R (version 4.3.1; The R Foundation). Descriptive statistics were analyzed after checking the normality of the data distribution using the Kolmogorov-Smirnov test. Normally distributed data are presented as the mean ± standard deviation, whilst non-normally distributed data are presented as the median and interquartile range (IQR). Categorical data are presented as n (%). To assess any statistical differences in volumes and ADC^{10} values between FTM pre- and post-treatment MRI, the Wilcoxon signed-rank test was used, and to evaluate any statistical differences in volume and ADC^{10} values in patients with or without MGMTp methylation, the Mann-Whitney U-test was used. Kaplan-Meier analysis and the log-rank test were used to assess the association of survival with MGMTp methylation status and with IDH1 status. Pearson's and Spearman's correlation tests were used to assess the correlation between

FTM duration and volume, as well as between FTM duration and ADC^{10} variation. P<0.05 was considered to indicate statistical significance.

Results

Patients' characteristics. A total of 110 consecutive adult patients with recurrent HGG previously treated according to the Stupp protocol and FTM between September 2017 and April 2020 were enrolled in the present study. Of these, 91 patients did not undergo MRI within 2 weeks of the start and the end of FTM treatment and were therefore excluded. A total of 19 patients were included in the study, of which 12 (63%) were males and 7 (37%) females, with a median age of 49 years (range, 23-72 years) at the start of FTM treatment. Clinical, histomolecular and therapy characteristics of the patients are summarized in Table I. In addition, as comorbidities, high blood pressure (patients 15 and 19), osteoporosis (patient 7), breast benign nodule (patient 17), atrio-ventricular-nodal reentrant tachycardia (patient 19) and anxiety-depressive disorder (patient 19) were reported. Detailed clinical data for each patient are available in Table SI.

All patients were treated with FTM as monotherapy; in particular, anti-angiogenic drugs such as Bevacizumab were not administered throughout the duration of the study. Additional FTM schedule information is presented in Table SII.

All patients underwent ≥1 surgery prior to FTM, four patients had two surgeries prior to FTM and two patients had >2 prior to FTM. A total of 6 patients received cyber-knife treatment before MRI evaluation, in an average time interval between the two of 4 months. The reasons for the interruption of FTM therapy were disease progression in 10 cases and adverse events in five cases. The four remaining patients completed the schedule of FTM (Table I).

Pathological examination revealed IDH1-wild-type GBM [based on the 2021 World Health Organization classification for central nervous system tumors (25)] in 17 cases and IDH-mutated grade 4 astrocytoma in two cases.

Radiological characteristics and treatment response. The median tumor volume measured on the contrast-enhanced component was 2.22 cm^3 at baseline (IQR, 0.77-13.17 cm^3) and 9.27 cm^3 at the end of treatment (IQR, 3.46-16.70 cm^3), with a median change of +39% (IQR, -46 to +574%) (Table II). No significant difference in tumor volume was observed between pre- and post-FTM treatment (P=0.17). A total of 12 patients showed an increase in tumor volume, whilst seven patients showed a volume decrease. In particular, 11 patients were classified as having progressive disease (PD) according to the Response Assessment in Neuro-Oncology (RANO) criteria for evaluating the second MRI (26), four as having stable disease (SD) and four as having a partial response (PR), and thus, the disease control rate (DCR) was 42% (Table II). More detailed radiological data are available in Table SIII. After FTM treatment, ADC^{10} values of the lesion were demonstrated to be markedly decreased: The median ADC^{10} value before treatment was $1.01x10^{-3}$ mm^2/sec (IQR, $0.88\text{-}1.12x10^{-3}$ mm^2/sec), whilst after treatment, it was $0.93x10^{-3}$ mm^2/sec (IQR, $0.73\text{-}1.11x10^{-3}$ mm^2/sec), with a median change of -0.2% (IQR, -11 to +19%) (Table II). No significant

Table I. Demographic and clinical characteristics of the cohort (n=19).

A, Demographic and histological characteristics

Parameter	Value
Sex	
Male	12 (63)
Female	7 (37)
Age at first surgery, years	
Median (IQR)	48 (43-57)
Range	23-71
Histology	
Grade 4 astrocytoma, IDH-mut	2 (11)
GBM	17 (89)
IDH status	
Mutated	2 (11)
Wild-type	17 (89)
MGMTp status	
Methylated	10 (53)
Unmethylated	5 (26)
Unknown	4 (21)

B, Treatment-related characteristics

Parameter	Value
Age at the start of FTM therapy, years	
Median (IQR)	49 (45-60)
Range	23-72
Duration of FTM therapy, months	
Median (IQR)	4 (2-6)
Range	1-8
Reason for FTM therapy interruption	
PD	9 (47)
AE	4 (21)
PD+AE	1 (5)
Therapy change due to no peripheral venous access	1 (5)
NA	4 (21)
Other treatments	
Further surgeries	4 (21)
CK	4 (21)
Further surgeries + CK	2 (11)
None	9 (47)

Values are expressed as n (%) unless otherwise indicated. IQR, interquartile range; GBM, glioblastoma; CK, cyber-knife; FTM, fotemustine; IDH, isocitrate dehydrogenase; MGMTp met, O^6-methylguanine-DNA methyltransferase promoter methylation; PD, progressive disease; AE, adverse event; NA, not applicable (indicating the case completed the FTM schedule as planned according to the Addeo schedule).

Table II. Radiological characteristics and treatment response.

Parameter	Value
CET volume at the start of FTM therapy[a], cm^3	2.22 (0.77-13.17), [0-30.38]
CET volume at the end of FTM therapy[a], cm^3	9.27 (3.46-16.70), [0.30-85.76]
Difference in volume before and after FTM therapy, %	+39 (-46 -+574), [-87 - +12347]
ADC^{10} at the start of FTM therapy[b], $x10^{-3}$ mm^2/sec	1.008 (0.882-1.121), [0.036-1.276]
ADC^{10} at the end of FTM therapy[b], $x10^{-3}$ mm^2/sec	0.989 (0.874-1.174), [0.264-1.412]
Difference in ADC^{10} before and after FTM therapy, %	-0.2 (-11 - +19), [-31 - +1843]
Survival from diagnosis, months	24 (20-50), [11-72]
Survival from start of FTM therapy, months	10 (5-14), [4-48]
Treatment response according to RANO criteria	
PD	11 (58)
SD	4 (21)
PR	4 (21)

[a]Difference in CET volume before and after FTM therapy was not statistically significant (P=0.17, Wilcoxon signed-rank test). [b]Difference in ADC^{10} before and after FTM therapy was not statistically significant (P=0.36, Wilcoxon signed-rank test). Values are expressed as the median (IQR), [range] or n (%). IQR, interquartile range; FTM, fotemustine; ADC^{10}, 10th percentile of apparent diffusion coefficient; CET, contrast-enhancing tumors; vol, volume; RANO, Response Assessment in Neuro-Oncology (23); PD, progressive disease; SD, stable disease; PR, partial response.

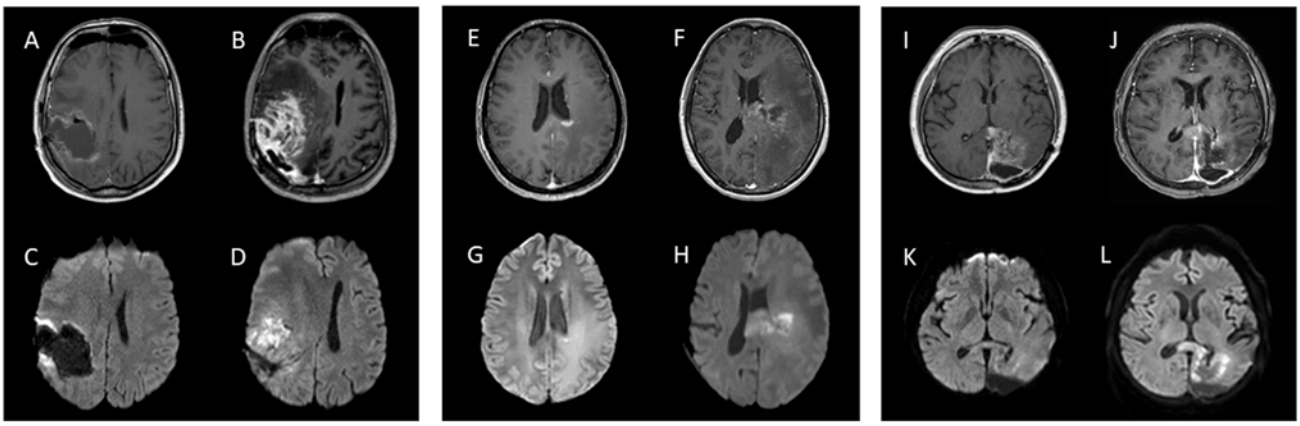

Figure 2. Representative patients of the cohort with marked diffusion restriction. (A-D) Patient 9: Post-contrast T1-WI MRI (A) before and (B) after FTM treatment; DWI b1000 maps (C) before and (D) after FTM treatment. (E-H) Patient 15: Post-contrast T1-WI MRI (E) before and (F) after FTM treatment; DWI b1000 maps (G) before and (H) after FTM treatment. (I-L) patient 16: Post-contrast T1-WI MRI (I) before and (J) after FTM treatment; DWI b1000 maps (K) before and (L) after FTM treatment. T1-WI MRI, T1-weighted magnetic resonance imaging; DWI, diffusion-weighted imaging; FTM, fotemustine.

difference was demonstrated for ADC^{10} difference before and after FTM treatment (P=0.36). Furthermore, the change in volume and ADC^{10} did not significantly correlate with FTM therapy duration (P=0.52, r=-0.169 and P=0.57, r=-0.139 respectively; Figs. S1 and S2), nor with survival (P=0.44, r=-0.199 and P=0.45, r=-0.183, respectively; Figs. S3 and S4). Of note, for certain patients (patients 9, 15 and 16), the ADC^{10} decrease was particularly marked (Fig. 2), especially in certain parts of the lesion. Representative MRI images of other patients without significant diffusion restriction are provided in Fig. 3.

Information on the MGMTp methylation status was available for 15/19 patients: a total of 10 patients had a methylated MGMTp, whilst 5 patients had an unmethylated MGMTp. No significant correlation was demonstrated between the change in volume or ADC^{10} and the MGMT methylation status (P=0.62 and P=0.51, respectively). In addition, patients with a methylated MGMTp exhibited a DCR of 40% (PD, n=6; SD, n=2; and PR, n=2), and those with an unmethylated MGMTp also demonstrated a DCR of 40% (PD, n=3; SD, n=2; and PR, n=0) (Table SIV). Furthermore, in the cohort of the present study, MGMTp methylation status as well as IDH status was not significantly associated with overall survival (P>0.05; Figs. S5 and S6). Furthermore, the duration of FTM therapy was significantly correlated with survival (P=0.002, r=0.669; Fig. S7).

Discussion

FTM is an alkylating drug, which links guanine, inducing the inhibition of DNA synthesis, cell cycle arrest and finally

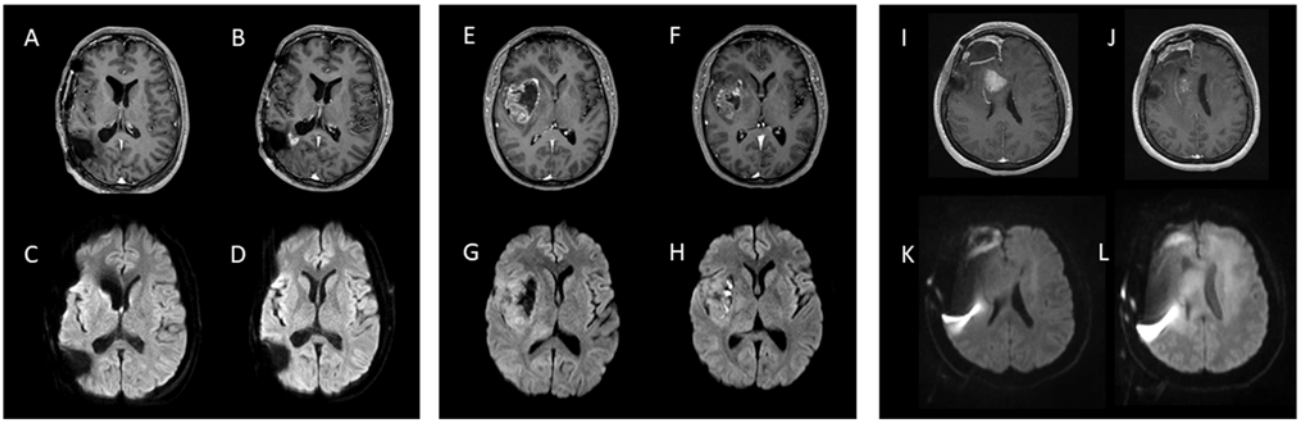

Figure 3. Patients of the cohort without marked diffusion restriction. (A-D) Patient 2: Post-contrast T1-WI MRI (A) before and (B) after FTM treatment; DWI b1000 maps (C) before and (D) after FTM treatment. (E-H) Patient 7: Post-contrast T1-WI MRI (E) before and (F) after FTM treatment; DWI b1000 maps (G) before and (H) after FTM treatment. (I-L) Patient 14: Post-contrast T1-WI MRI (I) before and (J) after FTM treatment; DWI b1000 maps (K) before and (L) after FTM treatment. T1-WI MRI, T1-weighted magnetic resonance imaging; DWI, diffusion-weighted imaging; FTM, fotemustine.

apoptosis. FTM has been used in the treatment of metastatic melanoma, hematological malignancies and brain tumors, due to its pharmacokinetic properties, such as the ability to cross the blood-brain-barrier (15,27).

Among the possible treatment alternatives for recurrent HGG, the use of FTM as a single agent in recurrent GBM has been assessed in different studies, including phase I/phase II trials (28). Previous phase II trials have reported that FTM has an activity comparable with other available therapeutic options (29), with 6-month progression-free survival ranging between 30-52%, a median overall survival of 5-10 months, as well as a relatively safe toxicity profile (2,17,28,29). Indeed, the median overall survival of the cohort in the present study after starting FTM therapy was 10 months, in line with results from the literature (2,17,30,31). FTM therapy duration was revealed to be positively associated with longer survival (20), and this could be at least partly explained by the bias that disease progression was one of the reasons for therapy suspension.

Several studies focusing on the evaluation of the clinical feasibility and effectiveness of second-line FTM for the treatment of recurrent GBM have reported radiological response rates during and after therapy. A study by Scoccianti *et al* (31), which enrolled 27 patients, reported that 29.6% had PR and 18.5% had SD, with a DCR of 48.1%. A previous study by Fabrini *et al* (17) including 50 patients reported CR in 2% of cases, PR in 16%, SD in 44% and PD in 38% after FTM induction, with a DCR of 62%. Another study by De Felice *et al* (18) comprising 15 patients with recurrent GBM reported no CR, PR in 26% and SD in 33% after FTM induction, with a DCR of 60%, while Prelaj *et al* (30) assessed 40 patients with recurrent GBM treated with fractioned FTM therapy and reported that 13% had PR and 47% had SD, with a DCR of 60%. However, none of the above-mentioned studies addressed the change in tumoral volume and ADC values, neither did the study by Lombardi *et al* (19), which provided a review of FTM treatment in recurrent HGG, and the case report by Gallo *et al* (7), which analysed the case of a patient with a MGMT-unmethylated GBM which responded to high-dose FTM therapy. In particular, to the best of our knowledge, no study has performed a quantitative analysis of the changes in tumor volume and in ADC values on conventional brain MRI before and after monotherapy with FTM. In the present study, ADC^{10} values in brain MRI were determined using different MRI machines, based on the consistency of the values across different MRI machines when standardized imaging protocols were applied. In our center, care is taken to perform standardization of protocols, calibration and application of quality control measures, which contribute to minimizing variability. While minor differences may exist, ADC^{10} values are reliable for clinical and research purposes, facilitating meaningful comparisons within and across MRI machines and institutions (32).

In the present study, the volumetric change of the contrast-enhancing part of the lesions was analyzed and a median increase in volume of +39% after treatment was determined. However, our results were heterogeneous and no statistically significant difference in volume before and after treatment was found. Furthermore, the volume change was not correlated with survival after initiating FTM therapy nor with the duration of FTM therapy. This latter point may be influenced by the fact that FTM treatment was suspended in numerous cases for several reasons, most commonly due to disease progression (n=8) and for adverse effects (n=4).

In addition, all patients included in the present study underwent at least one surgery, after which they were referred to our hospital for tumor recurrence and were then treated with FTM with no further surgery in this time period. Therefore, tissue loss due to surgery occurred several weeks before the timepoints considered in the study and was already present at baseline, thus it should not be regarded as a possible bias in the present analysis.

Furthermore, a fixed time-point may have been necessary to assess the effect of the chemotherapeutic drug FTM, but the retrospective nature of the study and the great inhomogeneity of the resonance time-points collected did not allow for homogenization. In the retrospective cohort of the present study, FTM was administered as a third-line chemotherapy to patients who had already undergone multiple rounds of treatment, including chemotherapy and radiotherapy. Treatment cessation is often prompted by tumor progression or the emergence of side effects.

Most patients analyzed (91 out of 110) did not undergo imaging at the time of treatment cessation, and were thus not included. To obtain a timely understanding of the tumor's status in relation to chemotherapy, only patients who received FTM treatment and underwent a concurrent MRI scan were included in the present analysis. This selection aimed to provide a direct assessment of the tumor's response to chemotherapy at the time of treatment cessation. Furthermore, in the cohort of the present study, ADC^{10} values decreased after therapy but without a statistically significant difference. However, it was demonstrated that three patients showed areas with marked restricted diffusion after therapy. DWI refers to a routinely used sequence widely known for its role in the evaluation of stroke. However, together with the derived ADC maps, it has shown value in the evaluation of brain tumors due to its relationship with tumor cellularity (32). Lower ADC values have been reported in higher-grade gliomas compared with those in lower-grade gliomas and lower ADC values have been reported to have a poorer prognosis independent of tumor grade (33). Furthermore, a decrease in ADC values has been previously demonstrated after second-line chemotherapy with anti-angiogenic agents in pediatric low-grade gliomas (decrease of median ADC values by 14%) (34). In adult patients with recurrent HGG treated with anti-angiogenic therapy, a decrease in ADC values after treatment was reported to be predictive of a shorter overall survival (35) and a poorer outcome (36). Compared with these studies, the three patients in the present study showed a marked ADC^{10} reduction (range, 0.76-0.93x10^{-3} mm^2/sec), lower than the values described in the aforementioned studies. Furthermore, Pope *et al* (36) reported ADC^{10} values of ~1.2x10^{-3} mm^2/sec. Indeed, the values obtained in the present study were much lower than the usual ADC values associated with tumor hypercellularity and they were more in agreement with areas of ischemic changes (4,37,38). It may be hypothesized that this could be in relation to a particular chemotoxic mechanism induced by FTM. Of note, these three patients showed early progression of disease and FTM was suspended after 1 month.

The correlation between MGMTp methylation status and radiological changes during FTM therapy was also assessed. The MGMT gene encodes for a DNA enzyme able to reverse alkylation at the O6 position of guanine, which is one of the targets of TMZ and nitrosureas (4). It is known that MGMT overexpression can confer resistance to TMZ, whilst methylation of this gene is associated with an improved response to TMZ (39). Certain studies have hypothesized that the same may also be the case for FTM: Fabi *et al* (40) analyzed 19 patients with known MGMT methylation status and reported a DCR of 66.5% in patients with MGMT methylation, whilst all patients with an unmethylated MGMTp had progressive disease. However, they did not find a significant difference. Furthermore, Gallo *et al* (7) published a report on a case in which a patient with recurrent GBM with an unmethylated MGMTp was successfully treated with a high-dose FTM regimen to overcome resistance.

In the cohort of the present study, there was no significant difference in terms of overall survival, volume or ADC^{10} values between MGMTp-methylated and -unmethylated tumors. The results, combined with that from the study by Fabi *et al* (40), support the absence of a correlation between MGMTp methylation and the efficacy of FTM, although both cohorts are limited in terms of the number of patients. Further studies are needed to support the use of FTM in MGMTp-unmethylated GBM.

Of note, the present study has certain limitations. First, the small sample size hinders the generalizability of the present findings and may have led to reduced statistical power of the study; however, since FTM is used only in a small number of countries in the world and the number of studies on its radiological effects is therefore limited, the present study may be of value despite its limitations for clinicians performing radiological follow-up in patients with similar characteristics as those of the present cohort. Studies with larger sample sizes are however needed.

Due to the retrospective nature of the study, besides the first MRI, which was performed within 2 weeks from the start of FTM, further follow-up MRIs were not performed at a standardized time. Therefore, different time-points were available for different patients, and in most cases, only one follow-up MRI was available. The lack of additional early follow-up MRIs at a fixed timepoint for all patients limited the present analysis, e.g. it was not possible to investigate the role of ADC value reduction as an early marker of progression. This may be the subject of further studies. Finally, only conventional MRI with DWI could be used, since advanced sequences such as perfusion MRI were not available in all cases.

In conclusion, the present study was the first to specifically address the radiological evolution besides the most traditional bidimensional sizes used according to the RANO criteria (26) in patients with recurrent HGG treated with FTM. In the small cohort of patients with recurrent HGGs, radiological progression was demonstrated as a dimensional increase in post-contrast enhancements. Furthermore, in certain patients, a marked restriction of diffusivity was also described; however, it was not related to the duration of therapy.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Conceptualization: FMD, EA; Methodology: FMD, DA; Formal analysis and investigation: APS, EA, DA, FMD, MG, MM, AS, BP, CV and RP; Writing-original draft preparation: APS, EA, FMD, MG; Writing-review and editing: MM, APS, AS, BP, CV, RP, MG; Supervision: MG, FMD. FMD and EA checked and confirmed the authenticity of the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of Fondazione IRCCS Istituto Neurologico 'Carlo Besta' (Milan, Italy) (SEURAT study; approved on May 17,

2023). Informed consent to participate was waived due to the retrospective nature of this study.

Patient consent for publication

All patient data, including MRI images, have been fully anonymized; therefore, obtaining individual patient consent for publication was not required.

Competing interests

The authors declare that they have no competing interests.

References

1. Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C and Barnholtz-Sloan JS: CBTRUS statistical report: Primary brain and other central nervous system Tumors Diagnosed in the United States in 2012-2016. Neuro Oncol 21 (Suppl 5): v1-v100, 2019.
2. Burnet NG, Jefferies SJ, Benson RJ, Hunt DP and Treasure FP: Years of life lost (YLL) from cancer is an important measure of population burden- and should be considered when allocating research funds. Br J Cancer 92: 241-245, 2005.
3. Majewska P, Ioannidis S, Raza MH, Tanna N, Bulbeck H and Williams M: Postprogression survival in patients with glioblastoma treated with concurrent chemoradiotherapy: A routine care cohort study. CNS Oncol 6: 307-313, 2017.
4. Stupp R, Mason WP, Van Den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, *et al*: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987-996, 2005.
5. van den Bent MJ, Tesileanu CMS, Wick W, Sanson M, Brandes AA, Clement PM, Erridge S, Vogelbaum MA, Nowak AK, Baurain JF, *et al*: Adjuvant and concurrent temozolomide for 1p/19q non-co-deleted anaplastic glioma (CATNON; EORTC study 26053-22054): Second interim analysis of a randomised, open-label, phase 3 study. Lancet Oncol 22: 813-823, 2021.
6. Central Nervous System Cancers-NCCN Guidelines. https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf. Accessed 12 Apr 2023.
7. Gallo C, Buonerba C, Di Lorenzo G, Romeo V, De Placido S and Marinelli A: Can high-dose fotemustine reverse MGMT resistance in glioblastoma multiforme?. J Neurooncol 100: 311-319, 2010.
8. Wei W, Chen X, Ma X, Wang D and Guo Z: The efficacy and safety of various dose-dense regimens of temozolomide for recurrent high-grade glioma: A systematic review with meta-analysis. J Neurooncol 125: 339-349, 2015.
9. Jiapaer S, Furuta T, Tanaka S, Kitabayashi T and Nakada M: Potential strategies overcoming the temozolomide resistance for glioblastoma. Neurol Med Chir (Tokyo) 58: 405-421, 2018.
10. Malkki H: Trial Watch: Glioblastoma vaccine therapy disappointment in Phase III trial. Nat Rev Neurol 12: 190, 2016.
11. Wang Y, Xing D, Zhao M, Wang J and Yang Y: The role of a single angiogenesis inhibitor in the treatment of recurrent Glioblastoma multiforme: A Meta-analysis and systematic review. PLoS One 11: e0152170, 2016.
12. Birzu C, French P, Caccese M, Cerretti G, Idbaih A, Zagonel V and Lombardi G: Recurrent Glioblastoma: From molecular landscape to new treatment perspectives. Cancers (Basel) 13: 47, 2020.
13. Beauchesne P: Fotemustine: A third-generation nitrosourea for the treatment of recurrent malignant gliomas. Cancers (Basel) 4: 77-87, 2012.
14. Addeo R, De Santi MS, Del Prete S and Caraglia M: Fotemustine and recurrent glioblastoma: Possible new opportunities for an old drug. Cancer Chemother Pharmacol 64: 863-866, 2009.
15. Addeo R, Caraglia M, De Santi MS, Montella L, Abbruzzese A, Parlato C, Vincenzi B, Carraturo M, Faiola V, Genovese M, *et al*: A new schedule of fotemustine in temozolomide-pretreated patients with relapsing glioblastoma. J Neurooncol 102: 417-424, 2011.
16. Frenay M, Giroux B, Khoury S, Derlon JM and Namer M: Phase II study of fotemustine in recurrent supratentorial malignant gliomas. Eur J Cancer 27: 852-856, 1991.
17. Fabrini MG, Silvano G, Lolli I, Perrone F, Marsella A, Scotti V and Cionini L: A multi-institutional phase II study on second-line Fotemustine chemotherapy in recurrent glioblastoma. J Neurooncol 92: 79-86, 2009.
18. De Felice F, Bulzonetti N, Musio D, D'Elia A, Salvati M and Tombolini V: Low-dose fotemustine as second-line chemotherapy for recurrent glioblastoma multiforme. Anticancer Res 33: 4013-4016, 2013.
19. Lombardi G, Farina P, Della Puppa A, Cecchin D, Pambuku A, Bellu L and Zagonel V: An overview of fotemustine in high-grade gliomas: From single agent to association with bevacizumab. Biomed Res Int 2014: 698542, 2014.
20. Addeo R, Lamberti G, Simonetti G, Iodice P, Marinelli A, Montella L, Cappabianca S, Gaviani P, Caraglia M, Prete SD and Silvani A: Biweekly fotemustine schedule for recurrent glioblastoma in the elderly: Activity and toxicity assessment of a multicenter study. CNS Oncol 8: CNS32, 2019.
21. Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, Cohen-Jonathan-Moyal E, Frappaz D, Henriksson R, Balana C, *et al*: EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. Lancet Oncol 15: e395-e403, 2014.
22. Leone A, Colamaria A, Fochi NP, Sacco M, Landriscina M, Parbonetti G, de Notaris M, Coppola G, De Santis E, Giordano G and Carbone F: Recurrent Glioblastoma treatment: State of the art and future perspectives in the precision medicine era. Biomedicines 10: 1927, 2022.
23. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC and Gerig G: User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. Neuroimage 31: 1116-1128, 2006.
24. Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin JC, Pujol S, Bauer C, Jennings D, Fennessy F, Sonka M, *et al*: 3D slicer as an image computing platform for the quantitative imaging network. Magn Reson Imaging 30: 1323-1341, 2012.
25. WHO Classification of Tumours Editorial Board: World Health Organization classification of tumours of the central nervous system, 5th edition. International Agency for Research on Cancer, Lyon, 2021.
26. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, Degroot J, Wick W, Gilbert MR, Lassman AB, *et al*: Updated response assessment criteria for high-grade gliomas: Response assessment in Neuro-oncology working group. J Clin Oncol 28: 1963-1972, 2010.
27. De Rossi A, Rossi L, Laudisi A, Sini V, Toppo L, Marchesi F, Tortorelli G, Leti M, Turriziani M, Aquino A, *et al*: Focus on fotemustine. J Exp Clin Cancer Res 25: 461-468, 2006.
28. Marinelli A, Lamberti G, Cerbone L, Cordua N, Buonerba C, Peluso G, Di Lorenzo G and De Placido S: High-dose fotemustine in temozolomide-pretreated glioblastoma multiforme patients. Medicine (Baltimore) 97: e11254, 2018.
29. Pérez-Segura P, Manneh R, Ceballos I, García A, Benavides M, Fuster J, Vaz MA, Cano JM, Berros JP, Covela M, *et al*: GEINOFOTE: Efficacy and safety of fotemustine in patients with high-grade recurrent gliomas and poor performance status. Clin Transl Oncol 18: 805-812, 2016.
30. Prelaj A, Rebuzzi S, Grassi M, Salvati M, D'Elia A, Buttarelli F, Ferrara C, Tomao S and Bianco V: Non-conventional fotemustine schedule as second-line treatment in recurrent malignant gliomas: Survival across disease and treatment subgroup analysis and review of the literature. Mol Clin Oncol 10: 58-66, 2018.
31. Scoccianti S, Detti B, Sardaro A, Iannalfi A, Meattini I, Leonulli BG, Borghesi S, Martinelli F, Bordi L, Ammannati F and Biti G: Second-line chemotherapy with fotemustine in temozolomide-pretreated patients with relapsing glioblastoma: A single institution experience. Anticancer Drugs 19: 613-620, 2008.
32. Kono K, Inoue Y, Nakayama K, Shakudo M, Morino M, Ohata K, Wakasa K and Yamada R: The role of diffusion-weighted imaging in patients with brain tumors. AJNR Am J Neuroradiol 22: 1081-1088, 2001.
33. Leao DJ, Craig PG, Godoy LF, Leite CC and Policeni B: Response assessment in Neuro-oncology criteria for gliomas: Practical approach using conventional and advanced techniques. Am J Neuroradiol 41: 10-20, 2020.
34. Hsu CH, Lober RM, Li MD, Partap S, Murphy PA, Barnes PD, Fisher PG and Yeom KW: Decreased tumor apparent diffusion coefficient correlates with objective response of pediatric low-grade glioma to bevacizumab. J Neurooncol 122: 491-496, 2015.

35. Galla N, Chiang G, Chakraborty S, Singh R, John Tsiouris A, Boockvar J and Kovanlikaya I: Apparent diffusion coefficient changes predict survival after intra-arterial bevacizumab treatment in recurrent glioblastoma. Neuroradiology 59: 499-505, 2017.
36. Pope WB, Qiao XJ, Kim HJ, Lai A, Nghiemphu P, Xue X, Ellingson BM, Schiff D, Aregawi D, Cha S, *et al*: Apparent diffusion coefficient histogram analysis stratifies progression-free and overall survival in patients with recurrent GBM treated with bevacizumab: A multi-center study. J Neurooncol 108: 491-498, 2012.
37. Sener RN: Diffusion MRI: Apparent diffusion coefficient (ADC) values in the normal brain and a classification of brain disorders based on ADC values. Comput Med Imaging Graph 25: 299-326, 2001.
38. Kamali A, Gandhi A, Nunez LC, Lugo AE, Arevalo-Espejo O, Zhu JJ, Esquenazi-Levy Y, Zhang X and Riascos RF: The role of apparent diffusion coefficient values in Glioblastoma: Differentiating tumor progression versus treatment-related changes. J Comput Assist Tomogr 46: 923-928, 2022.
39. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, *et al*: MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352: 997-1003, 2005.
40. Fabi A, Metro G, Russillo M, Vidiri A, Carapella CM, Maschio M, Cognetti F, Jandolo B, Mirri MA, Sperduti I, *et al*: Treatment of recurrent malignant gliomas with fotemustine monotherapy: Impact of dose and correlation with MGMT promoter methylation. BMC Cancer 9: 101, 2009.