

Gemcitabine followed by radiotherapy in treatment of newly diagnosed high-grade gliomas

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A B S T R A C T

Aim: High-grade glioblastoma multiforme (GBM) has a poor median overall survival (OS). The standard treatment after surgery is temozolomide and radiotherapy (RTH). Patients with unmethylated methylguanine-methyltransferase promoter (MGMT) have no or little benefit from temozolomide and are eligible for alternative therapies. Gemcitabine is a good radiosensitizer. We aimed to evaluate the combination of gemcitabine with RTH in newly diagnosed GBM. **Methods:** The study was a prospective phase II study. Eligible patients were required to have histologically proven anaplastic astrocytoma or GBM. Patients underwent biopsies or subtotal resection. The treatment consisted of fixed-dose rate gemcitabine 175 mg/m² weekly followed after 24 h by standard cranial RTH for 6 weeks. Tumor response was evaluated by Macdonald criteria. In case of progression, patients received temozolomide (200 mg/m²/5 days every 28 days). **Results:** Thirty patients with a median age of 52 years (30-69), 73%/27% male/female, the Eastern Cooperative Oncology Group performance status 1 (range 0-2) were enrolled. Five patients had a partial-response (17%) and 13 stable-disease (43%). Median time to progression was 7.88 months (95% CI 6.1-9.69) and OS was 11.77 months (95% CI 9.97-13.56). The treatment was well tolerated with grade-3 neutropenia in 3, grade-3 anemia in 2 and impaired liver enzymes in 1 patient. **Conclusion:** Gemcitabine followed by radiotherapy is active and promising regimen in newly diagnosed GBM. Gemcitabine uptake is easy, with a long local retention of active metabolites, precluding systemic side effects of radiosensitization. In a phase III study this treatment should be evaluated in patients with unmethylated MGMT promoter who will not benefit from temozolomide.

Key words: Gemcitabine; radiation; glioblastoma multiforma; temozolomide

INTRODUCTION

Malignant gliomas grade 3 anaplastic astrocytoma (AA) or grade 4 glioblastoma multiforme (GBM) are rapidly progressing primary brain tumors, which in spite of advances in surgery, radiotherapy and chemotherapy, remain associated with high morbidity and mortality.^[1] Despite the current multimodality therapy, the overall median survival for newly diagnosed patients is 10 months for patients with GBM and 2-3 years for those with AA.^[2,3]

Standard treatment of malignant gliomas is surgery, followed by radiotherapy concomitant with temozolomide (TMZ), followed by adjuvant TMZ (Stupp *et al.*,^[2] 2005). Surgery followed by involved field radiotherapy up to total

dose of 60 Gray (Gy) significantly prolongs survival. There have been many efforts to intensify radiotherapy, including the use of radiosensitizers, brachytherapy, radioactive seeds implanted in the tumor bed, and stereotactic radiosurgery in selected cases.^[4]

Initially the routine use of chemotherapy in addition to cranial irradiation was controversial. Individual randomized, controlled studies demonstrated no significant improvement in median survival with single agent or multiple agents chemotherapy, although a significant increase in survival was noted in a meta-analysis.^[1] There was a significant

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increase in the proportion of long-term survivors with the addition of chemotherapy, from less than 5 percent to approximately 15-20 percent, regardless of the patient's performance status, the histological features of the tumor, the duration of symptoms or age (up to 65 years).^[5]

However, the combination of radiotherapy with TMZ completely changed standard therapy, since this improved the median survival and overall survival.^[2] However, not all patients benefit since an unmethylated promoter of methylguanine methyltransferase (MGMT) enables extensive repair of TMZ-adducts and these patients do not benefit from temozolomide^[6] and are eligible for development of alternative therapies. Moreover, TMZ is relatively a poor radiosensitizer compared with other cytotoxic drugs.^[7] However, the group of nucleoside analogs, including gemcitabine are excellent radiosensitizers.^[8,9] Gemcitabine has been evaluated as a radiosensitizer for several tumor types, both in model systems and patients.^[8,10] Gemcitabine, an analogue of deoxycytidine, enters the cell by the action of a nucleoside transporter^[11] and it is activated by phosphorylation in a reaction catalyzed by deoxycytidine kinase (DCK), to gemcitabine monophosphate (dFdCMP) and subsequently phosphorylated to the 5'-diphosphate (dFdCDP) and triphosphate (dFdCTP) derivatives.^[12,13] Gemcitabine exerts its cytotoxicity mainly through the irreversible incorporation of the activated triphosphate into DNA, resulting in chain termination. Due to a number of self-potentiating mechanisms, gemcitabine exhibits prolonged intracellular retention, a property likely partially responsible for gemcitabine's broad spectrum of activity.^[14,15]

The standard dose of gemcitabine (1,000 mg/m²) given over 30 min results in high gemcitabine peak levels (> 20 µmol/L), which rapidly decline below 1 µmol/L within 2 h.^[16,17] However, DCK is saturated at much lower gemcitabine levels and *in vitro* and *in vivo* sensitivity to gemcitabine is most optimal at prolonged exposure to low drug levels in the nanomolar range.^[18-20] Therefore, it was reasoned that prolonged infusion of gemcitabine, which would result in prolonged but lower plasma concentrations of gemcitabine, would be advantageous.^[16,21] The fixed dose rate of 10 mg/m²/min infusion of gemcitabine gives this pharmacodynamic advantage over the 30 min infusion,^[16] resulting in a prolonged formation and retention of gemcitabine nucleotides which favour the activity of gemcitabine. A phase II trial showed that fixed dose rate gemcitabine can improve survival in patients with pancreatic adenocarcinoma but the difference was not significant in a randomized study.^[22] Single agent studies of gemcitabine in high grade glioma did not show any benefit,^[23,24] so that development of gemcitabine for this disease was discontinued. However, gemcitabine has shown a radiosensitizing effect in a number of human glioma cell lines^[25-27] and in an animal model system.^[28]

Sigmond *et al.*^[29] demonstrated that gemcitabine could pass the blood-tumor barrier in GBM patients. In tumor samples, concentrations of gemcitabine and its active metabolite dF-dCTP were high enough to enable radio sensitization, which warrants clinical studies using gemcitabine in combination with radiation.

Weller *et al.*^[24] and Metro *et al.*^[30] indeed showed that gemcitabine combined with RTH was an active regimen, but whether it was more effective than RTH alone remained elusive. The combination gemcitabine-RTH followed by temozolomide showed promising activity.^[30] Preliminary results of another phase I study showed that gemcitabine combined with radiotherapy is efficient and tolerable in high grade glioma.^[31] The aim of our study was to evaluate the activity of gemcitabine with RTH as a treatment modality in newly diagnosed high- grade gliomas and temozolomide was administered after progression only.

METHODS

This was single center, phase II, open label, one arm non-randomized trial designed to determine the efficacy and safety of gemcitabine combined with therapy in the treatment of patients with newly diagnosed malignant glioma. The Research Ethics Board of Assiut University Hospital approved the study. All patients gave written informed consent before starting treatment.

Patients

Eligible patients had histologically proven malignant glioma (grade 3 or 4). Patients were at least 18 years of age; had the Eastern Cooperative Oncology Group (ECOG) performance status < 3, had adequate bone marrow reserves (hemoglobin > 9 g/dL, absolute granulocytes > 1.5 × 10⁹/L, platelets > 100 × 10⁹/L), and acceptable serum chemistries (serum calcium in normal range (8.8-10.2 mg/dL), serum creatinine < 1.5 × upper limit of normal, bilirubin < 1.5 × upper limits of normal and AST (aspartate transaminase) < 3 × upper limits of normal).

Patients were excluded if they were < 18 years old, had previous invasive malignancies or received previous chemotherapy or radiotherapy, had poor medical conditions, or were pregnant, nursing or not practicing effective contraception if appropriate.

Assessments and treatment plan

Pre-treatment evaluation included a history and physical and neurological examination, laboratory (complete blood picture, liver and kidney function, serum calcium level) and imaging studies (baseline CT and MRI brain) and a baseline toxicity evaluation.

After surgery of patients with malignant gliomas for either cytoreduction or a biopsy, patients received fractionated local RTH at a daily dose of 2 Gray (GY) per fraction, five days per week for six weeks (total dose of 60 GY).

RTH was applied to the gross tumor volume with 2-3 cm margin for the clinical target volume. Radiotherapy was carried out using linear accelerator with 6-15 MV photons. Gemcitabine was administered at a fixed dose of 175 mg/m² by intravenous infusion starting 24 h prior to radiotherapy in the first week and then once weekly before RTH for the whole duration of the radiotherapy. Toxicities were graded using the NCIC-CTG expanded common toxicity criteria. Evaluation during protocol treatment included history and physical examinations (including full clinical neurologic examination), biochemical profiles; and imaging studies. Contrast-enhanced (gadolinium-DTPA) MRI of the brain was uniformly adopted for tumor assessment and evaluation of response. Baseline MRI examination was performed 24-48 h after surgery and then within 1 week prior to the start of the experimental treatment, 4 weeks after the end of chemo-radiotherapy and every 8 weeks thereafter until evidence of disease progression. Toxicity assessments were done weekly during the radiotherapy and then one month from the end of the treatment then every 2 months or when clinically indicated. Toxicity was graded according to NCI-CTC version 3.0.^[32] Response was assessed using standard Macdonald criteria,^[33] but patients were not considered to have had complete or partial responses unless clinical neurologic assessment was improved or stable. Patients were monitored until death.

Statistics

The duration of response was calculated from the first day of treatment to the date of progression for patients who achieved complete or partial response. Progression-free survival, analyzed by Kaplan-Meier method including 95% CI, was defined as the period of time elapsed from the first day of treatment to the date of disease progression, relapse or death from any cause. Overall survival was defined as the interval from the first day of study treatment to the date of patient death. The survival curves were estimated by the Kaplan-Meier product-limit method. The SPSS (11.0) statistical program was used for analysis.

RESULTS

Patients

From April 2009 to April 2011, thirty patients were enrolled. Table 1 shows the characteristics of patients entered on the study. Of the 30 patients included in the analyses, 8 were female and 22 male, with a median age of 52 years (range 30-69). Patients had an ECOG performance status range 0 to 2. There were 8 patients with anaplastic astrocytoma, and 22 with glioblastoma multiform.

Outcome

All patients received concomitant dexamethasone, while anti-convulsant treatment was given on demand. All the patients completed the chemoradiotherapy treatment. Six patients responded to the treatment (20%) and 13 patients had stable disease (43%) for an overall disease control rate

Table 1: Patient characteristics

Total patients	30
Age in years, median (range)	52 (30-69)
Gender	
Male	22 (73%)
Female	8 (27%)
ECOG performance status, median (range)	1 (0-2)
Pathology	
Anaplastic Astrocytoma	8 (27%)
Glioblastoma multiforma	22 (73%)
Surgical procedure	
Subtotal resection	10 (33%)
Biopsy	20 (67%)

ECOG: Eastern Cooperative Oncology Group

Table 2: Treatment response

Response	Patient number (%)
Complete response (CR)	1 (3)
Partial response (PR)	5 (17)
Stable disease (SD)	13(43)
Progressive disease (PD)	11 (37)
Disease control rate (CR+PR+SD)	19/30 (63)
Tumor response rate (CR+PR)	6/30 (20)

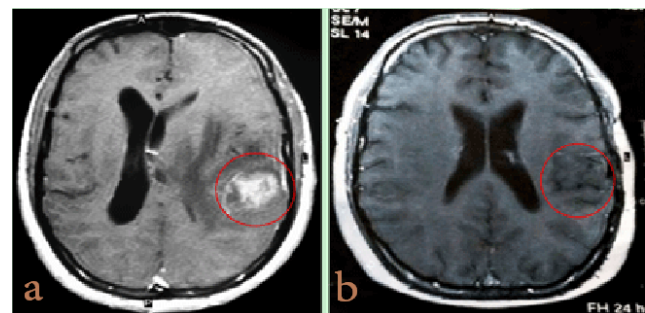


Figure 1: (a) MRI in T1 after Gd-DTPA infusion in axial plane shows an area of enhancement in GBM in the left parietal area; (b) MRI performed 1 year after the end of chemo-radiotherapy shows a dramatic response of the tumour T1 axial plane after Gd-DTPA infusion. MRI: magnetic resonance imaging; GBM: glioblastoma multiforme

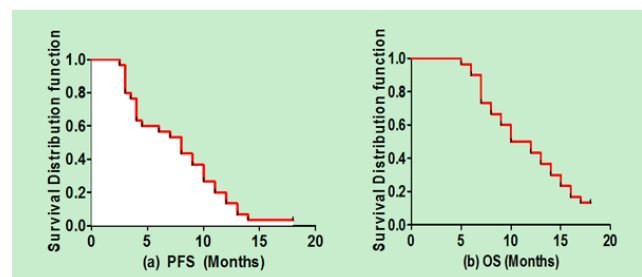


Figure 2: (a) PFS at a follow up of 18 months. The median PFS was 7.9 months (95% CI 6.1-9.7); (b) analysis of OS for 18 months, median OS was 11.8 months (95% CI 10.0-13.6). PFS: progression free survival; OS: overall survival

of 63% [Table 2] [Figures 1 and 2]. At a median follow up of 18 months median time to progression was 7.88 months (95% CI 6.1-9.69) and overall survival was 11.77 months (95% CI 9.97-13.56). According to the histology Grade 3

Table 3: Adverse events

	Grade 1 Patient number (%)	Grade 2 Patient number (%)	Grade 3 Patient number (%)	Grade 4 Patient number (%)
Heamatological toxicity				
Neutropenia	2 (7)	2 (7)	3 (10)	0
Aneamia	2 (7)	1 (3)	2 (7)	0
Thrombocytopenia	1 (3)	0	0	0
Non hematological toxicity				
Impaired liver enzymes	2 (7)	1 (3)	1 (3)	0
Fever	2 (7)	0	0	0
Nausea/vomiting	3 (10)	1 (3)	0	0
Anorexia	2 (7)	0	0	0
Diarrhea	2 (7)	1 (3)	0	0
Fatigue	3 (10)	2 (7)	0	0
Convulsion	2 (7)	0	0	0
Headache	4 (13)	0	0	0
Insomnia	1(3)	0	0	0
Alopecia	9 (30)	4 (13)	0	0
Otitis externa	1 (3)	0	0	0
Scalp dermatitis	2 (7)	0	0	0

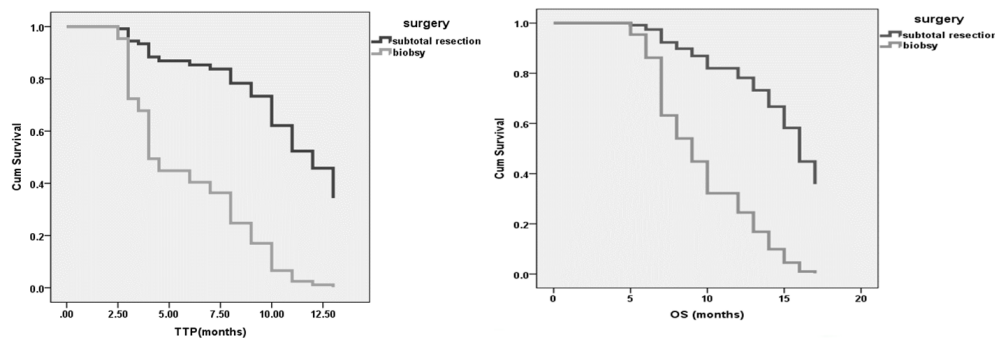


Figure 3: Subgroup analysis of PFS (depicted as Time to Progression, TTP) and OS according to surgical extension median PFS of 11 months (95% CI 8.1-13.9) for subtotal resection versus 4 months (95% CI 3.5-4.6) for a biopsied patients and median OS 15.4 months (95% CI 13.5-17.3) versus 9.5 months (95% CI 8.1-11.0) for subtotal resection versus biopsy, respectively. PFS: progression free survival; OS: overall survival

astrocytoma has more favorable PFS 11.13 months (95% CI 9.37-12.88) and OS 15.25 months (95% CI 12.54-17.96) than Glioblastoma multiform patients with PFS 6.70 months (95% CI 4.505-8.90) and OS 10.50 months (95% CI 8.41-12.59). On multivariate analysis, factors predictive of progression were performance status ($P = 0.04$) and the extent of surgery ($P = 0.02$). The latter was evaluated in a subgroup analysis, which showed that patients with a subtotal resection had a higher probability for a longer survival than those patients who were only biopsied [Figure 3].

Safety

All patients completed radiotherapy for a total dose of 60 Gy. All patients were evaluable for safety of the combination of gemcitabine and radiotherapy. Treatment-related adverse events are summarized in Table 3. Generally the treatment was well tolerated. Hematological toxicity consisted of grade 3 neutropenia in three patients (10%), while grade 3 anemia was reported in 2 patients (7%) on day 24; these patients received packed red blood cells. In the 3 cases of neutropenia, this was afebrile and occurred on day 16 in 2 cases and on day 24 in one case after the initiation of study treatment. Also, non-hematological adverse events were mostly mild (grade 1) or moderate (grade 2)

in intensity. Hypertransaminasemia was the only grade 3 non-hematological adverse event in one patient (3%), and this patient was receiving antiepileptic treatment. No treatment-related grade 4 toxicities were observed.

DISCUSSION

In this study we demonstrated that gemcitabine followed by RTH is an active regimen for treatment of high grade newly diagnosed GBM. Our study met the primary activity objective, producing a response rate of 20% and disease control rate of 63%, which was in line with earlier gemcitabine/RTH data of 17.5% and 75%, respectively.^[30] The results of these gemcitabine/RTH studies compare favorably with corresponding values for activity and disease control of 15.5% and 57.5%, respectively, obtained with nitrosurea given concurrently with radiotherapy.^[34] Furthermore, the promising values of PFS of 7.88 months and OS 11.77 months are in the same range as that observed for temozolomide plus radiotherapy 6.9 months for PFS and of 14.6 months for OS.^[2]

However, it is difficult to compare PFS and OS of the present study with those obtained in studies of radiotherapy-temozolomide with or without adjuvant temozolomide,

where 17.5-34.5% of patients had received complete tumor resection prior to study entry.^[2,35] All patients in our study had residual disease after surgery, while 67% of the patients were biopsied-only. A subgroup analysis of TMZ plus radiotherapy showed that the 93 patients who underwent only biopsy had no significant improvement in median overall survival.^[2]

Although PFS was the secondary objective in the present study, our results are not likely to be influenced by sequential temozolomide as it was only given after progression of the patients. In contrast, OS may be affected by the TMZ administered at disease progression. In another study,^[30] this might not have been the case.

The combination of gemcitabine plus radiotherapy was well tolerated. No treatment-related grade 4 toxicities were observed, while there were only 6 cases of grade 3 adverse events, including 5 patients with hematological toxicity and one with hypertransaminasemia. This was in line with toxicity reported for gemcitabine with RTH in high grade glioma patients with weekly schedule.^[30] On the whole, the treatment-related morbidity did not differ significantly from that observed with nitrosourea or temozolomide given concurrently with radiotherapy.^[2,34,35]

The standard treatment of high grade glioma patients has changed considerably since the introduction of radiotherapy combined with temozolomide.^[2,36] However, the benefit is only achieved in a subgroup of patients, who have a methylated MGMT promoter in the tumor.^[6] The patients with a hypomethylated MGMT promoter have an active MGMT enzyme, which will repair the DNA damage. Moreover, even in cells with a methylated MGMT promoter TMZ is a relatively poor radiosensitizer.^[7] Therefore these patients are eligible for an alternative treatment. Although gemcitabine as a single drug does not have an antitumor activity against GBM,^[23,24] this is not due to a poor passage of the blood-brain barrier. Normal brain depends on preformed nucleosides to enable nucleotide synthesis in the brain.^[37] Since gemcitabine resembles normal deoxynucleosides it is not unexpected that it is taken up efficiently into the brain and that the blood-brain barrier does not preclude gemcitabine's passage.^[29] Although the low dose of gemcitabine would preclude an antitumor effect by the drug itself, this dose results in sufficiently high concentrations in the tumor for radiosensitization can be reached. Since gemcitabine is an excellent radiosensitizer, low concentrations are sufficient,^[25-27,38] while it is also important to have an adequate time-period between the drug and radiation,^[8,9,39] since the active metabolite, dFdCTP, is retained for at least 24 h in tumors this will allow to maintain sufficiently high levels.^[19] This delay will also prevent cumulative toxicity. In addition to the radiosensitizing effect of gemcitabine, also its main catabolite, difluorodeoxyuridine, has been shown to be a good radiosensitizer,^[40,41] while the catabolite is

maintained at micromolar levels for days, including brain. This catabolite was recently shown to be able to inhibit thymidylate synthase.^[42] This inhibition, leading to an accumulation of deoxyuridine triphosphate (dUTP), might be basis for an additional radiosensitizing effect.^[9] Hence a dual radiosensitization might be achieved in glioma.

In conclusion, this study shows that fixed dose rate infusion of gemcitabine given before radiotherapy is clinically effective as a radiosensitizer for newly diagnosed GBM. Gemcitabine has a better cost effectiveness compared to the financial cost temozolomide. Further investigation of chemo-radiotherapy is needed and a Phase 3 trial with a higher number of patients will be initiated, to determine whether the gemcitabine radiosensitizing effect can be achieved irrespective of the methylation status of the MGMT promoter.^[30]

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Conflicts of interest

There are no conflicts of interest.

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