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CLINICAL TRIAL REPORT

Phase II study of fixed dose rate gemcitabine as radiosensitizer for newly diagnosed glioblastoma multiforme

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Abstract
Purpose In order to evaluate the activity of gemcitabine as radiosensitizer for newly diagnosed glioblastoma multiforme (GBM), a prospective single-center phase II study was conducted.
Methods Eligible patients were required to have histologically proven GBM with evaluable and/or measurable disease after surgery. They were treated by standard cranial irradiation plus concomitant fixed dose rate gemcitabine given intravenously at 175 mg/m^2 weekly for 6 weeks. After chemo-radiotherapy, irrespective of tumor response, patients went on to receive oral temozolomide at 150–200 mg/m^2 for 5 days every 28 days.
Results Twenty-three patients were enrolled. Median age was 57 years (range 43–72) and median Karnofsky performance status was 90 (range 70–100). Seventeen patients had received subtotal resection of the tumor, while six patients had biopsied-only tumors. Four patients responded to treatment (17.5%) with additional 14 (61%) experiencing stable disease for an overall disease control rate of 78.5%. Median progression-free and overall survival were 6.8 and 10.1 months, respectively. The concomitant radiotherapy–gemcitabine combination was well tolerated and severe adverse events were rare, consisting of grade 3 neutropenia and hypertransaminasemia in two cases each. Twenty patients were assessable for methylguanine methyltransferase (MGMT) promoter methylation, 11 of which were found methylated. In the methylated and unmethylated cohorts, disease control was obtained in 10/11 patients (91%) and 7/9 patients (77.5%), respectively.
Conclusions Concomitant radiotherapy–gemcitabine is active and well tolerated in newly diagnosed glioblastoma multiforme. Activity is observed both in tumors with methylated and unmethylated MGMT promoter.

Keywords Gemcitabine · Glioblastoma multiforme · MGMT · Radiosensitizer · Radiotherapy

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Introduction

Glioblastoma multiforme (GBM) is the most common form of primary brain tumor in adults. Due to its relatively high resistance to radio- and/or chemotherapy, GBM has a very dismal prognosis. Recently, a randomized phase III study clearly demonstrated the benefits of integrating the alkylating

agent temozolomide with radiotherapy for the treatment of newly diagnosed disease, thus highlighting the potentially crucial role of chemotherapy as radiosensitizer [1]. Nevertheless, temozolomide might be a less effective radiosensitizer compared with other cytotoxics [2]. In addition, innovative cytotoxic strategies should be pursued in patients with unmethylated promoter of the O^6-methylguanine methyltransferase (MGMT) gene. In fact, in these patients the lack of epigenetic silencing of the MGMT gene allows the MGMT protein to repair more effectively the DNA damage induced by alkylation, thus resulting into impaired temozolomide activity [3].

Gemcitabine (2′,2′-difluorodeoxycytidine) is a deoxycytidine analog antimetabolite acting as an excellent radiosensitizer both in vitro and in vivo [4, 5]. The positive interaction between gemcitabine and radiotherapy is likely due to a combination of mechanisms that include deoxyadenosine triphosphate depletion, cell-cycle redistribution and inhibition of DNA synthesis and repair [5]. Gemcitabine is currently approved for use in several solid tumors such as non-small cell lung cancer, pancreatic, bladder, ovarian and breast cancer [6]. Intracellularly, gemcitabine needs phosphorylation by the enzyme deoxycytidine kinase (dCK) to become active [7]. On one hand, the active metabolite gemcitabine triphosphate (dFdCTP) competes with the normal nucleotide deoxycytidine triphosphate for incorporation into DNA during replication, which leads to termination of chain elongation. On the other, gemcitabine diphosphate (dFdCDP) inhibits subunit 1 of ribonucleotide reductase, the key enzyme for the production of deoxynucleotides which required for DNA synthesis. Interestingly, the intracellular formation of dFdCDP and dFdCTP is a dose rate-dependent mechanism. In pharmacokinetic investigations, the doses needed to optimize dFdCTP accumulation in mononuclear cells were achieved when gemcitabine was administered at a fixed dose rate (FDR) of 10 mg/m^2/min [8, 9], and a prolonged infusion regimen has been associated with promising antitumor activities in clinical studies of pancreatic and non-small cell lung cancers [10, 11].

In the present phase II study, we evaluated the activity and safety of radiosensitizing gemcitabine given at a FDR of 175 mg/m^2/weekly concurrently with radiotherapy in patients with newly diagnosed glioblastoma multiforme. The dose of gemcitabine adopted was based on the recommendation of a phase I study conducted at our Institution [12].

Materials and methods

Study objectives

The primary objective of this phase II study was to evaluate the activity in terms of response rate of gemcitabine as radiosensitizer for newly diagnosed glioblastoma multiforme. Secondary objectives were to assess treatment-related toxicity, progression-free survival (PFS), overall survival (OS) and the activity of chemo-radiotherapy in relation to the MGMT promoter methylation status.

Study population

Patients with newly diagnosed histologically proven supratentorial GBM (WHO grade IV) within 6 weeks from surgery (either stereotactic biopsy or subtotal resection) were eligible for the study. Age between 18 and 75 years, Karnofsky Performance Status (KPS) $\geq$70, life expectancy $\geq$3 months, presence of evaluable and/or measurable disease after surgical procedure and adequate hematological, hepatic and renal function were among inclusion criteria. Other inclusion/exclusion criteria were the same as those of the previous phase I study [12].

All enrolled patients were amenable to compliance with testing and were informed of the investigational nature of the study. The study was approved by the local Ethics Committee and a signed informed consent was obtained from all patients before study entry.

Treatment plan

Radiotherapy consisted of fractionated focal irradiation at a dose of 2.0 Gy per fraction given once daily, 5 days/week (from Monday to Friday), over a period of 6 weeks, for a total of 60 Gy. Radiotherapy was administered to the gross tumor volume with a 2–3 cm margin for the clinical target volume. Irradiation was carried out using linear accelerators with 6–15 MV photons with a highly conformal technique (3D-RT).

From 24 to 72 h before the first day of radiotherapy, patients started concomitant FDR gemcitabine given intravenously at the dose of 175 mg/m^2 (infusion duration = 17.30 min) weekly for 6 weeks, covering the whole period of radiotherapy. Antiemetic prophylaxis with i.v. dexamethasone 4 mg and i.v. metoclopramide 10 mg i.v. were recommended before each gemcitabine administration.

No later than 6 weeks after the end of the experimental treatment of chemo-radiotherapy, irrespective of tumor response, patients were treated with oral temozolomide 150–200 mg/m^2 for 5 days every 28 days until disease progression or unacceptable toxicity [1].

Response and toxicity assessment

Contrast-enhanced (gadolinium-DTPA 0.2 mmol/kg) 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. Response to treatment was assessed according to the Macdonald criteria [13].

Patients were assessed weekly during radiotherapy for tolerability. Toxicity was graded according to NCI-CTC version 3.0 [14]. Neurological examination including Barthel index (BI) and Mini-Mental-Status score (MMS) was performed at baseline as well as at the end of chemo-radiotherapy and every 2 months during the follow-up period or whenever clinically indicated.

Gemcitabine administration was omitted in case of grade 3–4 neutropenia and/or thrombocytopenia, febrile neutropenia and grade 3–4 non-haematological toxicity except for nausea/vomiting. At recovery, treatment was resumed with a 25% dose reduction. Anti-epileptics and anticoagulant drugs were given as deemed necessary. Glucocorticoids were given for neurologic stability and any modifications of steroidal therapy were taken into account for response evaluation according the criteria of Macdonald et al. [13].

Analysis of the MGMT promoter methylation

Genomic DNA was isolated from one paraffin section of GBM tissue collected at the time of diagnosis (Ex-Wax DNA Extraction Kit S4530, Chemicon), proteinase digestion lasting a maximum of 6 h. DNA was denatured with sodium hydroxide in a volume of 35 µl and subjected to bisulfite treatment in a volume of 350 µl (4.4 M sodium bisulfite and 20 mM hydroquinone) for 5 h at 55°C and then purified. Unmethylated cytosine, but not its methylated counterpart, is modified into uracil by the treatment. The methylation-specific PCR was performed in a two-step approach. The results were confirmed in an independent experiment, starting with reisolation of DNA from the tumor. The PCR products were separated on 4% agarose gels.

Statistical analysis

The study was designed according to the single stage phase II study proposed by A'Hern [15]. A sample size of 23 patients was considered sufficient to give a 90% probability of rejecting a baseline response rate of 5% with a significance level of 10% when the true response rate is 25%. If at least three responses would be observed, the study was considered positive. The response rate with a confidence interval (CI) of 95% calculated by exact binomial procedure is reported. Differences in assessment before and after treatment were calculated by Wilcoxon test. The duration of response was calculated from the first day of study 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

From January 2006 to October 2008, 23 patients with newly diagnosed GBM were enrolled at a single Institution. Characteristics of patients are shown in Table 1. Median age was 57 years (range 43–72) and median KPS was 90 (range 70–100). Seventeen patients (74%) underwent subtotal resection. Fifteen patients (65%) presented with neurological signs and/or symptoms of disease.

Activity

All patients were evaluable for activity of radiosensitizing gemcitabine and completed the chemo-radiotherapy treatment plan. Four patients responded to treatment for an overall response of 17.5% (95% CI 5.2–40.3) (Fig. 1). Fourteen patients (61%) achieved disease stabilization, for

Table 1 Patients charachteristics

Total patients	23
Age in years, median (range)	57 (43–72)
Gender	
Male	11 (48%)
Female	12 (52%)
Karnofsky Performance Status, median (range)	90 (70–100)
Barthel Index, median (range)	80 (60–100)
Mini-Mental Status, median (range)	26 (25–30)
Time to enrollment from diagnosis in weeks, median (range)	4.9 (3–6)
Surgical procedure	
Subtotal resection	17 (74%)
Biopsy	6 (26%)
Neurological signs/symptoms	
Yes	15 (65%)
No	8 (35%)
MGMT promoter methylation status	
Methylated	11 (48%)
Unmethylated	9 (39%)
Unknown	3 (13%)

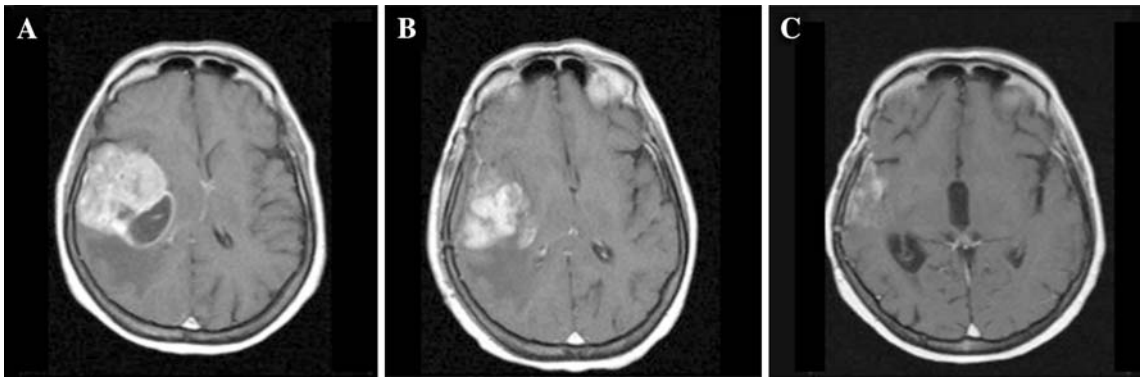

Fig. 1 Long surviving patient after response to gemcitabine plus radiation treatment. The patient had an unmethylated MGMT promoter. **a** Prior to subtotal resection, MRI Spin-Echo T1 axial plane sequence after Gd-DTPA infusion shows a GBM in the fronto-temporal area. **b** After surgery, MRI in T1 after Gd-DTPA infusion in axial plane shows an area of enhancement in relation to residual tumor. **c** MRI performed 40 days after the end of chemo-radiotherapy shows a dramatic reduction in size of the residual tumor in T1 axial plane after Gd-DTPA infusion

a disease control rate (responses + disease stabilizations) of 78.5%. Five patients (21.5%) developed progressive disease. All responding patients had undergone subtotal resection of the tumor and the median duration of response was 18 months (95% CI 6–31).

At completion of chemo-radiotherapy, median KPS was 80 (range 60–100), median Barthel index was 80 (range 50–100) and median MMS was 26 (range 22–30). No statistically significant differences were noted between baseline and final KPS, Barthel index or MMS.

Safety

All patients completed radiotherapy for a total dose of 60 Gy. Median duration of radiotherapy was 6 weeks (range 6–7). All patients were assessable for safety of radiosensitizing gemcitabine. Treatment-related adverse events (all grades, maximum toxicity per patient reported) are summarized in Table 2. Severe hematological adverse events were rare, consisting of grade 3 neutropenia in two patients (8.5%). In both cases neutropenia was afebrile and occurred on day 12 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 most common non-hematological adverse event, which resulted to be of grade 3 in two patients (8.5%), of whom nobody was receiving treatment with antiepileptics. In one of these two patients, chemotherapy was discontinued as per protocol, since no recovery was observed after 2 weeks. No treatment-related grade 4 toxicities were observed. The mean delivered dose-intensity of gemcitabine was 168 mg/m^2/week, corresponding to 96% of the planned dose intensity.

Efficacy

All patients received temozolomide 150–200 mg/m^2 for 5 days every 28 days at the end of chemo-radiotherapy. The median number of cycles of temozolomide administered was 6 (range 4–12). At a median follow-up of 9.7 months (range 2.5–28.6), median PFS was 6.8 months (95% CI 6.1–7.6). The rate of patients who were free from progression at 6, 12 and 18 months was 81.8, 18.2 and 13.6%, respectively. A total of 11 patients received second-line

Table 2 Summary of treatment-related adverse events

	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Hematological toxicity				
Leukopenia	1 (4.5)	1 (4.5)	–	–
Neutropenia	–	1 (4.5)	2 (9)	–
Anemia	1 (4.5)	–	–	–
Non-hematological toxicity				
Mucositis	1 (4.5)	–	–	–
Hypertransaminasemia	2 (9)	3 (13)	2 (9)	–
Fever	1 (4.5)	1 (4.5)	–	–
Diarrhea	1 (4.5)	1 (4.5)	–	–
Nausea/vomiting	1 (4.5)	1 (4.5)	–	–
Fatigue	1 (4.5)	2 (9)	–	–

chemotherapy consisting of fotemustine in eight cases and carmustine in three. Only two patients received third-line chemotherapy. Also, two patients underwent second surgery. Median OS was 10.1 months (95% CI 8.7–11.6) and the rate of patients alive at 6, 12 and 18 months was 86.4, 43.8 and 29.2%, respectively.

MGMT promoter methylation status and activity

Methylguanine methyltransferase promoter methylation status was successfully assessed in tumors from 20 individuals. Figure 2 shows the activity of radiosensitizing gemcitabine according to the MGMT promoter methylation status. Out of the 11 patients with methylated MGMT promoter, three responses (27.5%) and seven disease stabilizations (63.5%) were observed, for a disease control rate of 87%. Among the nine patients with unmethylated MGMT promoter, one response (11%) and six disease stabilizations (66.5%) were reported, for a disease control rate of 77.5%.

Discussion

Clinically, it has been demonstrated that the early introduction of chemotherapy and the simultaneous administration with radiotherapy is crucial for the improvement of treatment outcome of newly diagnosed glioblastoma multiforme [1, 16]. In this disease setting, the delivery of temozolomide, given concurrently and sequentially to radiotherapy, has been shown to significantly improve patients survival compared with radiotherapy alone, thus resulting in a new standard of care [1]. Unfortunately, it has been impossible so far to assess with precision the relative contribution of the concurrent and the adjuvant chemotherapy, respectively [1]. Against this background, the present study was specifically designed to address the activity of gemcitabine, an antimetabolite with established radiosensitizing properties, in combination with radiotherapy for newly diagnosed glioblastoma multiforme. Being the activity of chemo-radiotherapy, the primary objective of the study, the presence of evaluable and/or measurable disease after surgical procedure was mandatory for inclusion in the study. Also, all patients were treated with temozolomide until progression following completion of the concomitant radiotherapy–gemcitabine, given the demonstrated activity of this drug [1].

In the present study, FDR gemcitabine at 175 mg/m^2 given weekly as radiosensitizer for newly diagnosed GBM met the primary activity objective, producing a response rate of 17.5%. Also, responses appeared to be long-lived (median duration 18 months) (Fig. 1) and a remarkable disease control rate of 78.5% was reported. Importantly, the

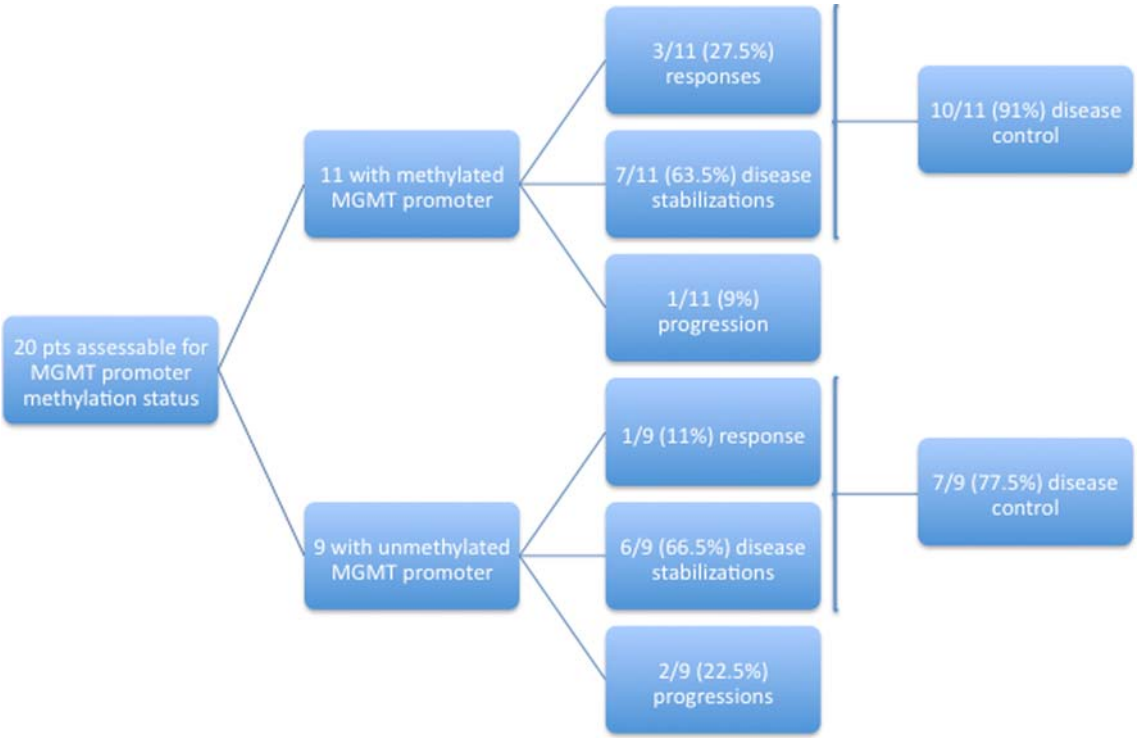

Fig. 2 Activity of chemo-radiotherapy according to MGMT promoter methylation status. Gemcitabine seemed to provide sufficient radiosensitization both in patients with methylated and unmethylated MGMT promoter

results in terms of activity and disease control obtained with radiosensitizing gemcitabine compare favorably with the corresponding values of 15.5 and 57.5%, respectively, obtained with the nitrosurea agent fotemustine given concurrently with radiotherapy [17]. Furthermore, promising values of 6.8 and 10.1 months were observed for PFS and OS, respectively, which are relevant considering that all patients of the study had residual disease after surgery, including six patients with biopsied-only tumors. For this reason it is difficult to compare PFS and OS of the present study with those obtained in similar studies of concomitant radiotherapy–temozolomide with or without adjuvant temozolomide, where 17.5–34.5% of patients had received complete tumor resection prior to study entry [16, 18]. Nevertheless, PFS and OS were secondary objectives in the present study, and are likely to have been influenced by sequential temozolomide, for PFS, and successive therapies administered at disease progression, for overall survival.

The radiotherapy–gemcitabine combination was well tolerated with only four cases of grade 3 adverse events requiring a dose reduction of 25% in three cases and drug discontinuation in one patient (Table 2). Nevertheless, the mean delivered dose-intensity of gemcitabine was not greatly affected, being 96% of the planned dose intensity. Notably, no progressive neurologic deterioration potentially related to enhanced radiosensitization of concurrent gemcitabine was reported. This might have been of concern, since neurologic worsening was found to be the dose-limiting toxicity of the previous phase I study [12]. On the whole, the treatment-related morbidity did not differ significantly from that observed with fotemustine or temozolomide given concurrently with radiotherapy [16–18], although radiosensitizing gemcitabine appeared to perform better in terms of hematological toxicity. Particularly, no suspension of gemcitabine for hematological toxicity was required in the present study, compared with a 14% of fotemustine discontinuation for grade 3 thrombocytopenia observed in a similar report of chemo-radiotherapy [17].

Even though it could be argued that radiosensitization with gemcitabine does not add to the antitumor effect of radiation, robust evidence suggests that this is not the case. In fact, a recent report has shown that gemcitabine has the ability to cross the blood–brain barrier, given that gemcitabine along with its active metabolites has been detected in tumor tissue of recurrent patients receiving gemcitabine monotherapy at the doses of 500 or 1,000 mg/m^2 [19]. Moreover, the same study documented the tumoral presence of dCK at levels that were sufficient to enable gemcitabine phosphorylation into the active metabolites [19]. In addition, a clinical study investigating gemcitabine with whole brain radiotherapy for the treatment of brain metastases from different tumor types reported a remarkable 54.5% of responses to the brain [20]. In light of these data, the present study clinically confirms that gemcitabine could act as potent radiosensitizer in newly diagnosed glioblastoma multiforme.

Importantly, radiosensitizers different from standard temozolomide may be useful for patients in whom little or no benefit is expected from agents such as temozolomide or nitrosureas, which would be the case of individuals with unmethylated MGMT promoter [3, 21, 22]. For this reason, unmethylated patients could be eligible for alternative treatment strategies such as gemcitabine plus radiation. In this regard, it is interesting to see that a disease control rate of 77.5% was observed in patients with unmethylated MGMT promoter, not significantly inferior to the disease control rate of 91% observed in patients with methylated MGMT promoter (Fig. 1), thus suggesting that gemcitabine may act as radiosensitizer irrespective of the status of the MGMT promoter.

In conclusion, this study shows that FDR gemcitbine given concurrently with radiotherapy is clinically active as radiosensitizer for newly diagnosed GBM and might be worthy of being investigated further in future studies of chemo-radiotherapy. The analysis of the MGMT promoter methylation of the tumors suggests that the radiosensitizing effect can be achieved irrespective of the methylation status of the MGMT promoter.

Conflict of interest statement The authors report no conflict of interest.

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