Gemcitabine uptake in glioblastoma multiforme: potential as a radiosensitizer

J. Sigmond¹, R. J. Honeywell¹, T. J. Postma², C. M. F. Dirven⁴, S. M. de Lange¹, K. van der Born¹, A. C. Laan¹, J. C. A. Baayen³, C. J. Van Groeningen¹, A. M. Bergman⁵, G. Giaccone⁶ & G. J. Peters¹*

¹Department of Medical Oncology; ²Department of Neurology; ³Department of Neurosurgery, VU University Medical Center, Amsterdam; ⁴Department of Neuro-Surgery, Erasmus University, Rotterdam; ⁵Netherlands Cancer Institute, Amsterdam, The Netherlands; ⁶National Cancer Institute, Bethesda, MD, USA

Received 26 February 2008; revised 1 June 2008; accepted 2 June 2008

Glioblastoma multiforme (GBM), the most frequent malignant brain tumor, has a poor prognosis, but is relatively sensitive to radiation. Both gemcitabine and its metabolite difluorodeoxyuridine (dFdU) are potent radiosensitizers. The aim of this phase 0 study was to investigate whether gemcitabine passes the blood–tumor barrier, and is phosphorylated in the tumor by deoxycytidine kinase (dCK) to gemcitabine nucleotides in order to enable radiosensitization, and whether it is deaminated by deoxycytidine deaminase (dCDA) to dFdU. Gemcitabine was administered at 500 or 1000 mg/m² just before surgery to 10 GBM patients, who were biopsied after 1–4 h. Plasma gemcitabine and dFdU levels varied between 0.9 and 9.2 μM and 24.9 and 72.6 μM, respectively. Tumor gemcitabine and dFdU levels varied from 60 to 3580 pmol/g tissue and from 29 to 72 nmol/g tissue, respectively. The gene expression of dCK (β-actin ratio) varied between 0.44 and 2.56. The dCK and dCDA activities varied from 1.06 to 2.32 nmol/h/mg protein and from 1.51 to 5.50 nmol/h/mg protein, respectively. These enzyme levels were sufficient to enable gemcitabine phosphorylation, leading to 130–3083 pmol gemcitabine nucleotides/g tissue. These data demonstrate for the first time that gemcitabine passes the blood–tumor barrier in GBM patients. In tumor samples, both gemcitabine and dFdU concentrations are high enough to enable radiosensitization, which warrants clinical studies using gemcitabine in combination with radiation.

Key words: gemcitabine, glioblastoma multiforme, radiosensitizer

introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults, and patients have a poor prognosis. First-line treatment is surgery and radiation therapy and/or chemotherapy. Until recently, the median survival time was <1 year [1–5]. A recent phase III study has demonstrated the benefit of adjuvant temozolomide, an alkylating agent, administered during and after radiotherapy [3]. Temozolomide administered in combination with radiotherapy resulted in a median survival benefit of 2.5 months while the median survival increased from 12.1 months with radiotherapy alone to 14.6 months with radiotherapy plus temozolomide. The 2-year survival increased to 26%. Concomitant administration of temozolomide and radiation therapy has therefore become a standard therapy after surgery. However, temozolomide is a relatively poor radiosensitizer, compared with other cytotoxic agents [6].

The deoxynucleoside analogue gemcitabine (dFdC, Gemzar, Eli Lilly, IN, USA) is an excellent radiosensitizer both in vivo and in vitro [7, 8]. Gemcitabine is considered for combination therapy with radiation in glioblastomas. Gemcitabine is routinely used in treatment of solid tumors such as non-small-cell lung cancer (NSCLC), breast and ovarian cancer, bladder cancer and pancreatic cancer [9]. When gemcitabine is transported into the cell by specialized nucleoside transporters, it needs phosphorylation by the enzyme deoxycytidine kinase (dCK) to become active [10]. The main mechanism of action is incorporation of its active metabolite gemcitabine triphosphate (dFdCTP) into the DNA competing with the normal nucleotide deoxycytidine triphosphate (dCTP) [11] while gemcitabine diphosphate (dFdCDP) is able to inhibit the enzyme ribonucleotide reductase (RNR) that is involved in DNA synthesis [12]. Furthermore, gemcitabine has several self-potentiating effects such as its enhanced incorporation into DNA by inhibition of RNR that will lead to depletion of deoxyribonucleotides (dNTP) pools including that of dCTP [13]. Gemcitabine can be deaminated by deoxycytidine deaminase (dCDA) to
difluorodeoxuryridine (dFdU). Although dFdU is considered as an inactive metabolite, it can act as a radiosensitizer [14] at concentrations which can easily be reached in plasma [15, 16]. At these concentrations, dFdU has also shown to possess cytotoxic activity [10].

The positive interaction between gemcitabine and radiotherapy is likely due to a combination of mechanisms that include deoxycytidine triphosphate (dATP) depletion, cell cycle redistribution, reduction of apoptotic threshold, inhibition of DNA synthesis and reduction of DNA repair [8]. Since the rate of the de novo pathway for synthesis of pyrimidine nucleotides is very low, brain tissue mainly depends on preformed nucleosides for synthesis of nucleotides [17, 18]. Nucleosides can relatively easily pass the blood–brain barrier, and are being taken up by the brain [19, 20]. Hence, preclinical studies showed considerable uptake of gemcitabine in brain [21]. Since the blood–tumor barrier is less restrictive and can even be passed by drugs which normally do not accumulate in brain [22], we anticipated that gemcitabine would be taken up relatively easily by brain tumor tissue.

The aim of this phase 0 study was to determine whether gemcitabine can indeed pass the blood–tumor barrier, and whether it is taken up and metabolized sufficiently in the tumor to enable radiosensitization. For this purpose, we also investigated whether the critical gemcitabine metabolizing enzymes dCK and dCDA are expressed and active in GBM.

**materials and methods**

**patient selection**

Patients were considered eligible for this study on the basis of the following criteria: patients with recurrent GBM who had resection and a tumor load allowing removal of sufficient samples to perform the anticipated analyses. The size was on the basis of earlier studies carried out either in patients or in animal models. Patients were ≥18 years (range from 18 to 70 years), had adequate (hematological) bone marrow function (white blood cells (WBC) ≥3 x 10^9/l, absolute neutrophil count ≥1.5 x 10^9/l, platelet count ≥100 x 10^9/l), adequate renal function (serum creatinine <120 μmol/l or creatinine clearance ≥25 ml/min), adequate liver function (serum bilirubin <25 μmol/l), adequate cardiac function and an interval of 3 months between earlier radiotherapy and surgery was required because of (sub)acute reactions and changes after radiotherapy. Written informed consent was obtained from all patients before entering the study. The study was approved by the ethical review board of the VU University Medical Center.

**study design**

Gemcitabine was administered as a 30-min i.v. infusion 1–4 h before surgery. Ten patients were enrolled between 2001 and 2004. Since radiosensitization of gemcitabine is often observed at lower doses, we studied gemcitabine at 500 mg/m² (n = 5). Ethical safety considerations were another reason to treat the first five patients at 500 mg/m². Because no side-effects were seen, the dose was allowed to escalate to the normal therapeutic dose of 1000 mg/m² (n = 5). Previous studies in our institute aiming to evaluate drug accumulation in tumors demonstrated that five patients would be sufficient to find differences between dose levels. The time points were chosen because in previous studies a plateau level of dFdC dCTP in WBC was observed between 2 and 4 h after drug administration [15, 16], as well as dFdC dCTP in tumor biopsy specimens [23]. Biopsy specimens were taken during surgery to measure the gemcitabine metabolites and critical enzymes involved in the metabolism of gemcitabine (dCK and dCDA). Tumor samples were immediately frozen in liquid nitrogen and stored at −80°C before further evaluation. Blood samples were planned to be taken before and 30 min after infusion of gemcitabine, and at the time of the biopsy.

Biopsies were taken during surgery, immediately frozen in liquid nitrogen and stored at −80°C before further evaluation. Blood samples (9 ml) were drawn in heparinized tubes containing 0.25 mg tetrahydrodouridine (THU) to prevent deamination of gemcitabine and the tubes were immediately placed on ice. Plasma was obtained by centrifugation of the samples (3200 g for 5 min at 4°C) and stored at −20°C until analysis. The buffy coat at the interface between plasma and erythrocytes was used for isolation of mononuclear blood cells, using a Ficoll–Hypaque density gradient (Pharmacia, Uppsala, Sweden) as described previously [15]. After purification the cell pellet was immediately frozen in liquid nitrogen and subsequently stored at −80°C until analysis.

**chemicals**

Gemcitabine (2′,2′-difluorodeoxuryridine, dFdC, Gemzar) was kindly provided by Eli Lilly Research Laboratories (Indianapolis, IN). [3H]-CdA was purchased from Moravek Biochemicals (Brea, CA, USA). All other chemicals were of analytical grade and commercially available, unless specified otherwise.

**gemcitabine and dFdU analysis in plasma**

The plasma concentrations of gemcitabine and dFdU were analyzed as described previously [15, 16]. Briefly, 50 μl of 40% trichloroacetic acid (TCA; 5% final concentration) was added to 350 μl plasma, centrifuged and neutralized. The extract was stored at −20°C. Separation and quantification of gemcitabine and dFdU from the plasma was achieved using a validated isocratic high-performance liquid chromatography (HPLC) assay as described earlier [16].

**gemcitabine phosphate analysis in WBC**

Cellular nucleotides were extracted and analyzed by HPLC as reported previously [15, 16]. Briefly, cells were suspended in 150 μl ice-cold phosphate-buffered solution and extracted as noted for gemcitabine and dFdU. Separation and quantitation of the normal ribonucleotides and dFdC dCTP was achieved with a gradient anion exchange HPLC.

**tissue preparation**

Frozen tissues were pulverized using a micro-dismembrator as earlier described [24] to maintain stability of gemcitabine metabolites and enzymes. The pulverized tissue was resuspended in buffer (50 mM Tris, 1 mM EDTA, 15 mM cytidine monophosphate (CMP), 20 mM sodium fluoride (NaF), 4 mM dithiothreitol, pH 7.6) at 1 g tumor per 3 ml buffer. After sonification, the suspension was divided into two eppendorf vials, one containing THU for measurement of gemcitabine metabolites. The rest of the suspension was used for enzyme assays and RNA isolation. This suspension was centrifuged for 30 min at 10 000 g at 4°C. The pellet was dissolved in RNAzol and stored at −80°C while the supernatant was used for dCK and dCDA assays.

**quantitative LC-PCR and enzymic assays**

RNA was isolated from pulverized tissues using RNAzol (Campro Scientific) and reverse-transcribed to cDNA using random hexamers as described previously [25]. Real-time PCR using a Light-Cycler (LC, Roche Diagnostics) with SYBR-Green detection to enable rapid and sensitive detection of dCK-mRNA expression was carried out as previously described [26]. The expression of dCK was quantified relative to β-actin.

The dCK activity in GBM tumor samples was determined by using a radioactive assay as described earlier [27] with 50 μM [3H]-CdA as a substrate and 25 μl diluted (20 000 g) supernatant (protein range 5.9–20.3 μg/μl) from the pulverized tissues. CDA is a very specific dCK substrate and not a substrate for, e.g., thymidine kinase 2.
The activity of the gemcitabine deactivating enzyme dCDA was measured as described earlier [28] using 500 μM gemcitabine as the substrate. To 100 μl of enzyme containing supernatant (2–14 μg protein/μl), 20 μl of 5 mM gemcitabine and 80 μl of dCDA buffer were added. This reaction mixture was incubated for 15–45 min at 37°C. Substrate (gemcitabine) and product (dFdU) were separated by reversed-phase HPLC as described previously.

tissue analysis of gemcitabine and its metabolites

For plasma analysis of gemcitabine and dFdU, we initially used a standard HPLC method, by precipitation of proteins with TCA as described above. This assay did not provide sufficient sensitivity and selectivity to measure gemcitabine. Therefore, a sensitive liquid chromatographic/atmospheric pressure chemical ionization tandem mass spectrometric method (LC-APCI-MS/MS) was developed to enable the determination of very low gemcitabine concentrations, as described elsewhere [29]. Briefly, the tissue suspension was mixed with 11N3-deoxycytidine (5 μg/ml), which was used as the internal standard. Isopropyl alcohol was added to precipitate tissue proteins. After centrifugation, the supernatant was freeze-dried and reconstituted in ethyl acetate and Milli-Q water, permitting back extraction into water. The aqueous layer was used for analysis with the LC-APCI-MS/MS system (a PerkinElmer series 200 HPLC coupled with a SCIEX API 3000 mass spectrometer). Chromatographic separation on a Spherisorb phenyl-column was achieved with an isocratic formic acid/acetonitrile mobile phase. Ions of gemcitabine ([m/z 264/112]) were detected and quantified with an accuracy, precision and limit of quantification of 99.8%, ±7.9% and 19 nM, respectively. The method was linear from 19 nM up to 2 μM.

For analysis of dFdCTP, we initially used the TCA extract prepared for HPLC analysis of gemcitabine and dFdU. However, in most tissues, dFdCTP was not detectable, possibly because of degradation of the triphosphate during preparation of the tissue despite the presence of nucleotidase/phosphatase inhibitor CMP and NaF. Therefore, we used another approach in which all nucleotides were degraded to the nucleosides, including gemcitabine phosphates (dFdCMP, dFdCDP, dFdCTP) to gemcitabine. For this purpose, 30 μl of the sample extract was transferred to a 0.5 ml polypropylene tube. Subsequently, 3 μl of ammoniumbicarbonate and 3 μl of ammoniumacetate (pH 7.0) were added to each tube, followed by 4 μl of alkaline phosphatase (1 unit, Sigma, Zwijndrecht, NL) and incubated for 1.5 h at 37°C in a 5% CO2 atmosphere. After the incubation, each sample was transferred to an HPLC vial and 4 μl was injected into the LC-MS-MS system. The concentration of gemcitabine nucleotides was calculated by correcting them for the earlier measured gemcitabine concentrations.

results

gemcitabine and dFdU analysis in plasma

Patient characteristics are shown in Table 1. All patients were diagnosed with GBM at initial surgery, and all were subsequently treated with radiotherapy usually followed by adjuvant chemotherapy. Subsequent therapy often consisted of palliative chemotherapy. Almost all patients received dexamethasone and most received an antiepileptic treatment. No differences were observed in the clinical status or previous therapy of the two groups of patients; hence, it is unlikely that differences are related to therapy or co-medication.

Plasma concentrations of gemcitabine and dFdU at the time of the biopsy are illustrated in Table 2. Gemcitabine levels in plasma varied from 0.9 to 9.2 μM and in dFdU from 25 to 72 μM. The gemcitabine plasma concentrations in patients 4 and 6, 30 min after gemcitabine infusion, were 17.7 and 184 μM.

### Table 1. Characteristics of patients with recurrent glioblastoma multiforme, undergoing recraniotomy, aimed at debulking

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gemcitabine dose (mg/m²)</th>
<th>Time (min:h)</th>
<th>Gemcitabine (μmol/l)</th>
<th>dFdU (μmol/l)</th>
<th>dFdCTP (mol/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>2:15</td>
<td>0.9</td>
<td>42.7</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td>0:46</td>
<td>9.2</td>
<td>31.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>0:58</td>
<td>2.2</td>
<td>32.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>3:41</td>
<td>ND</td>
<td>24.8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>1:12</td>
<td>0.8</td>
<td>47.9</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>3:35</td>
<td>ND</td>
<td>45.6</td>
<td>79</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>1:45</td>
<td>3.4</td>
<td>72.6</td>
<td>2.4</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>2:00</td>
<td>ND</td>
<td>64.8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>2:02</td>
<td>ND</td>
<td>64.8</td>
<td>24.5</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time points of taking blood samples after administration are shown individually. Patients 1–5 received 500 mg/m² and patients 6–10 1000 mg/m². Note that dFdU levels are higher in the 1000 mg/m² group. The levels of dFdCTP were not measurable in most patients treated at 500 mg/m² possibly because at this lower dose, accumulation is less and levels were below detection limit possibly due to small size of these samples (<1 million cells), the limited availability and the variation in time points. Re-analyses of plasma samples that were still available by the more sensitive LC-MS-MS showed that gemcitabine nucleotides were present (2–79 pmol/10⁶ cells).
cells) in WBC in patients (Table 2). From other patients no samples were available.

gemcitabine metabolites in GBM biopsies
The biopsy specimen usually consisted of part of the debulked tumor. Usually the samples were large enough for most of the planned assays, but sometimes the samples were very small (only 20 mg, sufficient for PCR, but not for drug and metabolite measurement). Gemcitabine was not detectable in tumor biopsies using HPLC analysis but dFdU was detectable in the tumor tissue (Figure 1B) which varied from 29–60 nmol/g tissue in the 500 and from 49–72 in the 1000 mg/m² group (t-test, not significant). However, analysis by a specific and sensitive LC-MS-MS assay revealed the presence of substantial amounts of gemcitabine in all patients that varied between 60 and 3580 pmol/g tissue (Figure 1A), while in four out of four available GBM biopsies gemcitabine phosphates were also detected (Figure 1C). From the other patients insufficient material was available. The distribution of gemcitabine in tumors could be evaluated in patient 1, from whom sufficient material was available to divide the tumor into cross-sections. On the surface of the tumor, the gemcitabine concentration varied between 260 and 510 pmol/g tissue while a concentration of 300 pmol/g was found inside the tumor. From the other patients, insufficient material was available to evaluate gemcitabine and dFdU. In patient 1, we also obtained tumor fluid and cerebrospinal fluid liquor which contained 1.0 and 0.9 μM gemcitabine, respectively, and 11.3 and 11.1 μM dFdU, respectively.

gemcitabine metabolic enzymes: dCK and dCDA
In order to determine whether gliomas have a sufficient expression of dCK, we measured both gene expression and activity. The gene expression of dCK was low, but in a relatively small range (Table 3). dCK activity in the tumors was also low compared with, e.g., NSCLC xenografts [20] and varied from 1.06 to 2.32 nmol/h/mg protein. The activity of gemcitabine deactivating enzyme dCDA varied between 1.51 and 5.50 nmol/h/mg protein, which is ~100-fold lower than that in liver, a tissue with a high expression of dCDA (Peters et al., unpublished data).

discussion
Our study clearly demonstrates that gemcitabine passes the blood–tumor barrier in relapsed GBM, as demonstrated by the presence of gemcitabine metabolites in GBM biopsies. Both gemcitabine and dFdU are present in the tumor while additional analysis of the tumor samples using highly selective and sensitive LC-MS-MS methodology showed that gemcitabine nucleotides are also present. Although dFdU might have entered the brain tumor directly from the systemic circulation, this is impossible for the gemcitabine nucleotides because they are formed intracellularly, cannot efflux from the cells and cannot pass the blood–tumor barrier [10].

We did not observe differences in plasma and tissue gemcitabine concentrations between the two dose levels given. However, differences might be masked by the variation in time points of tumor sampling after gemcitabine infusion. However, for the dFdU tumor concentrations, a significant difference between the 500 and 1000 mg/m² group was observed possibly because after 2 h a dose-dependent plateau in plasma concentrations is often observed [16, 22, 28] and dFdU can be transported from the blood to the tumor.

Figure 1. (A) Gemcitabine accumulation measured in glioblastoma biopsies (by LC-MS-MS). Time points of obtaining the tumor samples after administration of gemcitabine are individually shown on top of the graph. (B) Gemcitabine metabolite difluoro(deoxy)uridine accumulation measured in glioblastoma biopsies (by high-performance liquid chromatography). (C) Gemcitabine phosphates in glioblastoma biopsies measured by LC-MS-MS. Time points of taking tumor samples after administration are individually shown on top of the graph. Patients 1–5 received 500 mg/m² and patients 6–8 1000 mg/m². Not from all patients enough material was available for all assays.
Since pretreatment dCK levels predict in vivo gemcitabine sensitivity [26, 27], we investigated dCK on the level of gene expression and enzyme activity. Unexpectedly, we observed a clear difference in dCK expression between the 500 and 1000 mg/m² group. However, this was not reflected in the small range of dCK activity of 1.06–2.32 nmol/h/mg protein which was in the same range as in insensitive xenografts [27]. Similarly, dCK levels were in a very small range in a previous study comparing various brain tumors [30]. The low dCK activity might explain why no response to gemcitabine was found in patients with GBM in previous clinical trials [31, 32]. Furthermore, the activities of dCDA in the biopsies were very low compared with, e.g., liver (100-fold lower) (data not shown). Earlier studies showed a low level of dCDA in brain tumors as well [29], with a low dCDA/dCK ratio. Therefore, the dCk and dCDA activities are favorable for gemcitabine phosphorylation, which could indeed be demonstrated by our study.

Because gemcitabine is a very potent radiosensitizer [8, 33] and the most effective standard postoperative therapy of GBM consists of adjuvant radiation–temozolomide, gemcitabine–radiation therapy might also be more effective than radiotherapy alone. Penetration of gemcitabine into the tumor is dependent on blood–tumor barrier passange, and radiosensitization depends on the length of interval between drug treatment and radiation, and on the tumor concentration of gemcitabine and of its active metabolites. Radiosensitization by gemcitabine in vitro requires very low drug concentrations [7, 8], at which antitumor activity of gemcitabine as a single drug is unlikely. In cell lines, a low gemcitabine concentration given 0 to 32 hours before radiotherapy (0–12 Gy) enhanced the effect of radiation with at a dose enhancement ratio of 0.85 to 2.82 [8]. Also in spheroids of Gli6 glioblastoma cells, exposure to 100 nM gemcitabine increased the specific growth delay (SGD), defined as the growth delay of treated cultures divided by the doubling time of control culture [34]. The SGD of gemcitabine and radiation combined was larger (2.98) when compared with radiation (0.74 at 5 Gy) or gemcitabine alone (1.42) [4]. In the GBM biopsies, comparable gemcitabine concentrations can be reached and seem to be retained for some time, since they were measured between 0.46 and 3.41 h after infusion of gemcitabine.

Previously, we demonstrated that gemcitabine sensitivity of xenografts is related to pretreatment dCK activity and gene expression [26, 27]. Both Gregoire et al. [35] and Pauwels et al. [36] demonstrated that a certain level of dCK expression is required for radiosensitization by gemcitabine. Apparently, GBM has significant dCK activity, since we can detect phosphorylated gemcitabine in these tumors. This level was sufficient to enhance radiosensitization in models [36].

Also gemcitabine’s deaminated product dFdU has radiosensitizing properties [14]. In ECV304, human bladder carcinoma cells and NSCLC cell line H292, radiotherapy-induced growth delay was enhanced by dFdU at concentrations between 10 and 50 μM. The concentrations in the GBM biopsy specimens are in this range, which has the potential to radiosensitize cells with a dose modifying factor of >1. dFdU appears very rapidly in the plasma and remains elevated in the plasma for a prolonged period (>24 h) of time at levels known to enhance growth inhibition in combination with radiotherapy. The origin of dFdU found in the tumor is not clear; either gemcitabine taken up by the tumor is deaminated by dCDA or dFdU is transported from the plasma into the tumor.

The present study showed that gemcitabine is not only taken up by the tumor but also phosphorylated. Also in rat model systems, gemcitabine uptake into a brain tumor was demonstrated, but either metabolites were not evaluated [37] or both gemcitabine and dFdU were found at levels comparable to blood and other tissues [21]. However, since rats do not have CDA, occurrence of dFdU was very low. Gemcitabine has the potential to be used as a radiosensitizer. However, since temozolomide plus radiation is now considered as standard treatment of most GBM patients, future applications might be limited to tumors in which temozolomide is not expected to be active, i.e. tumors in which the temozolomide DNA adduct is repaired by alkylguanine transferase. Since temozolomide itself is not a very potent radiosensitizer compared with gemcitabine [6], tumors expected to be insensitive to temozolomide might benefit from treatment with another radiosensitizer. This would be the case in tumors in which the MGMT promoter is not methylated [38]. These patients do not show a survival advantage for temozolomide, and might be eligible for an alternative treatment such as gemcitabine plus radiation.

In conclusion, to our knowledge, this is the first study to demonstrate that gemcitabine passes through the blood–tumor barrier in GBM patients. Both plasma and tumor levels of gemcitabine and its metabolite dFdU are high enough to enable radiosensitization. Clinical studies into the efficacy of the combination of gemcitabine and radiation are warranted, especially in patients who are not expected to benefit from radiation with temozolomide.

**funding**

Educational Grant of Eli Lilly & Company, Indianapolis, IN, USA.
Annals of Oncology

original article

references