

Safety and pharmacokinetics of dose-intensive imatinib mesylate plus temozolomide: Phase 1 trial in adults with malignant glioma

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We determined the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of imatinib mesylate, an inhibitor of the receptor tyrosine kinases platelet-derived growth factor receptor (PDGFR), the proto-oncogene product *c-kit*, and the fusion protein Bcr-Abl, when administered for 8 days in combination with temozolomide (TMZ) to malignant glioma (MG) patients. MG patients who had not failed prior TMZ were eligible to receive TMZ at a dose of 150–200 mg/m² per day on days 4–8 plus imatinib mesylate administered orally on days 1–8 of each 4-week cycle. Patients were stratified based on concurrent administration of CYP3A4-inducing antiepileptic drugs (EIAEDs). The imatinib dose was escalated in successive cohorts of patients independently for each stratum. Imatinib, at doses ranging from 400 mg to 1,200 mg, was administered with TMZ to 65 patients: 52 (80%) with glioblastoma multiforme (GBM) and 13 (20%) with grade III MG. At enrollment, 34 patients (52%) had stable disease, and 33 (48%) had

progressive disease; 30 patients (46%) were on EIAEDs. The MTD of imatinib for patients concurrently receiving or not receiving EIAEDs was 1,000 mg. DLTs were hematologic, gastrointestinal, renal, and hepatic. Pharmacokinetic analyses revealed lowered exposures and enhanced clearance among patients on EIAEDs. Among GBM patients with stable disease at enrollment ($n = 28$), the median progression-free and overall survival times were 41.7 and 56.1 weeks, respectively. Imatinib doses up to 1,000 mg/day for 8 consecutive days are well tolerated when combined with standard TMZ dosing for MG patients. A subsequent phase 2 study is required to further evaluate the efficacy of this regimen for this patient population. *Neuro-Oncology* 10, 330–340, 2008 (Posted to *Neuro-Oncology* [serial online], Doc. D07-00147, March 21, 2008. URL <http://neuro-oncology.dukejournals.org>; DOI: 10.1215/15228517-2008-003)

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The outcome for patients with glioblastoma multiforme (GBM), the most common primary CNS tumor in adults, remains poor. Although recent randomized studies confirm that temozolomide (TMZ),

a DNA methylator derived from imidazotetrazine,¹ in combination with radiotherapy (XRT) improves outcome for GBM patients, the survival benefit of this approach is modest, with a median progression-free survival (PFS) of 7–10 months and a median overall survival (OS) of less than 15 months.^{2,3} Furthermore, subsets of patients, such as those with established unfavorable clinical prognostic factors, including older age, poor performance status, and limited resection, and those with tumors expressing the DNA-repair protein O⁶-alkylguanine-DNA alkyltransferase (AGT), derive marginal, if any, benefit from this regimen compared to XRT alone.^{4–6} Clearly, more effective therapies are critically needed. Two established factors contributing to poor outcome are resistance to cytotoxic therapy and limited drug delivery. Therefore, treatment strategies designed to overcome chemotherapy resistance or improve drug delivery may improve the overall antitumor activity of TMZ for GBM patients as well as the outcome of poor prognostic patient subsets.

Imatinib mesylate (Gleevec, formerly STI-571) has established activity against both hematologic and solid organ cancers due to effective inhibition of selective receptor tyrosine kinases, including the fusion protein Bcr-Abl, proto-oncogene products c-kit and c-fms, and platelet-derived growth factor receptor (PDGFR).⁷ In addition, imatinib has ancillary properties that may augment its antitumor activity when administered with chemotherapy, including abrogating resistance to cytotoxic agents^{8–10} and enhancing chemotherapy delivery by decreasing tumor interstitial pressure.^{11–13} Although single-agent imatinib therapy has limited activity in recurrent malignant glioma (MG) patients,^{14,15} we hypothesized that a dose-intensive imatinib schedule may increase the sensitivity of MGs to TMZ based on the potential mechanisms of action described above. In preclinical studies, we observed enhanced antitumor benefit when imatinib was combined with TMZ in some intracranial MG xenograft models (J.N.R., unpublished observations). The present study was therefore conducted to initiate an evaluation of this approach by determining the maximum tolerated dose (MTD) and safety of dose-intensive imatinib when combined with standard TMZ dosing in MG patients. Dose escalation beginning at the established dose level of imatinib (400 mg/day) was performed independently among patients stratified by concurrent use of CYP3A-inducing antiepileptic drugs (EIAEDs; phenytoin, carbamazepine, phenobarbital, oxcarbazepine, and primidone)^{14,16} based on the increased imatinib metabolism induced by these agents.

Patients and Methods

Protocol Objectives

The primary objective of this study was to define the MTD and dose-limiting toxicity (DLT) of imatinib when administered with TMZ in the treatment of adults with MG. Secondary objectives included to further define the toxicity of this regimen, to evaluate the pharmacokinetics of imatinib when administered with TMZ, and

to evaluate for antitumor activity. Finally, we explored potential correlations between outcome and expression of the *MGMT* gene in the tumor cell in order to determine whether the study regimen had activity in patients with elevated tumor *MGMT* content.

Patient Eligibility

Patients were required to have a histologically confirmed diagnosis of MG (GBM, anaplastic astrocytoma [AA], anaplastic oligodendroglioma [AO], or anaplastic oligoastrocytoma) and to have received prior XRT. Patients were eligible to enroll with either stable or recurrent disease, but patients with prior imatinib therapy, as well as those with either prior progression or prior \geq grade 3 toxicity on TMZ, were excluded. Patients with prior low-grade glioma were eligible as long as histologic transformation to MG before enrollment was confirmed. Patients were also required to be at least 18 years of age, to have a KPS score \geq 60%, to be on a stable corticosteroid dose for \geq 1 week, to have satisfactory hematologic results (hemoglobin $>$ 9 g/dl, absolute neutrophil count $>$ 1,000 cells/ μ l, platelet count $>$ 100,000 cells/ μ l) and biochemical results (serum creatinine, blood urea nitrogen [BUN], aspartate aminotransferase, and bilirubin $<$ 2.0 times the upper limit of normal), to have recovered from all expected toxicity related to previous therapy, and to provide written informed consent. No restriction was placed on the number of prior recurrences or treatments. At least 2 weeks between prior surgical resection, XRT, chemotherapy (6 weeks for nitrosoureas), or investigational agents and enrollment was also required.

Patients were excluded for pregnancy or nursing; lack of effective, appropriate contraception; acute infection requiring intravenous antibiotics; intratumoral hemorrhage (except postoperative grade 1); significant concurrent medical illness or prior malignancy; concurrent warfarin use; \geq grade 2 peripheral edema, pulmonary or pericardial effusions, or ascites; and prior stereotactic radiosurgery or radioimmunotherapy unless there was obvious radiographic disease progression or biopsy-proven recurrent tumor.

Treatment Design

Prior studies confirmed that the metabolism of imatinib is significantly enhanced by concurrent use of EIAEDs. Therefore, patients were accrued independently into two separate strata: patients not taking EIAEDs (stratum A) and patients taking EIAEDs (stratum B). The starting imatinib dose was 400 mg/day for both strata. Subsequent dose levels increased imatinib dosing by 200 mg/day until DLT was observed. Cohorts of three to six patients were treated per dose level. Imatinib was provided by Novartis Pharmaceuticals (Florham Park, NJ, USA) and was given orally with food on days 1–8 of each 28-day cycle. On days 4–8 of each cycle, patients were advised to take their imatinib 2–3 h before their TMZ dose. Daily imatinib doses greater than 600 mg were split equally into two doses; otherwise, dosing was once a day.

TMZ was provided by Schering Plough (Kenilworth,

NJ, USA) and was initially administered in a fasting state at a dose of 200 mg/m² to all patients as a single daily dose for days 4–8 of each cycle. Patients received study therapy until unacceptable toxicity, tumor progression, or consent withdrawal.

Dose Escalation and Statistical Considerations

The dose level was escalated in successive cohorts of three patients as long as DLT did not occur. If one instance of DLT was observed among the initial three evaluable patients treated at a dose level, an additional three patients were treated at that dose level. Dose escalation continued if no episodes of DLT occurred in those additional three patients. If two instances of DLT were observed at a dose level, the MTD was surpassed, and a total of six patients were treated at the previous level to assure its tolerability. The MTD was defined as the highest dose causing DLT in no more than one of six patients treated at that dose level.

DLT was assessed during the first and second cycles of study therapy. Nonhematologic DLTs were defined as \geq grade 3 attributable events, except for alopecia. Nausea, vomiting, and diarrhea that were refractory to standard medical therapy were included as DLTs. Hematologic events were defined as DLTs if they occurred after a TMZ dose reduction to 150 mg/m² per day and included grade 4 neutropenia or thrombocytopenia lasting \geq 5 days. In addition, any toxicity resulting in a \geq 14-day delay to re-treat was considered DLT.

Time to progression and OS were measured from the date cycle 1 began and analyzed by the Kaplan-Meier method including 95% confidence intervals.

Toxicity Evaluation

Toxicity was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0. Patients were evaluated by physical examination before each cycle and as clinically indicated. A complete blood count with differential was obtained weekly, and a serum biochemistry profile including electrolytes, BUN, creatinine, and liver function tests was obtained before each cycle and as clinically indicated. A urinalysis was performed before the first cycle, as was a beta human chorionic gonadotropin test in women with reproductive potential.

Response Evaluation

Study investigators determined response by neurologic examination and contrast-enhanced MRI before the start of every other treatment cycle. A complete response (CR) was defined as disappearance of all enhancing tumor on consecutive MRIs at least 6 weeks apart, with corticosteroid discontinuation and neurologic stability or improvement. A partial response (PR) was defined as \geq 50% reduction in size (product of largest perpendicular diameters) of enhancing tumor with stability or improvement of neurologic status and corticosteroid requirement. Progressive disease (PD) was defined as

\geq 25% increase of enhancing tumor or new lesion. Stable disease (SD) was defined as any assessment not meeting CR, PR, or PD criteria.

Dose Modification and Retreatment Criteria

The daily imatinib dose was reduced by 100 mg for patients on stratum A and by 200 mg for patients on stratum B for related \geq grade 3 nonhematologic or grade 4 hematologic toxicity. The daily TMZ dose was also reduced by 50 mg/m² for grade 4 hematologic toxicity to a minimum of 100 mg/m². Retreatment required adequate hematologic and biochemical parameters (defined in eligibility criteria) and resolution of any related \geq grade 3 toxicity to \leq grade 1.

Patients were removed from study for evidence of PD at any time after study initiation, more than two dose reductions due to toxicity, noncompliance, or voluntary withdrawal.

AGT Immunohistochemistry Staining

Immunohistochemistry was performed for AGT expression from archival tumor samples as described previously.^{5,17} Embedded tissue sections were deparaffinized in xylene for 4 h. Sections were washed in absolute alcohol, blocked in 1.85% H₂O₂/methanol, and then rehydrated in distilled water. Antigens were retrieved following heating in AR-10 buffer (Biogenix, San Ramon, CA, USA) for 10 min followed by cooling for 30 min. Slides were washed twice in phosphate-buffered saline, blocked with 5% normal goat serum for 15 min, and then incubated overnight with anti-AGT antibody (mT3.1) or control IgG1. Slides were washed twice in phosphate-buffered saline, incubated with secondary antibody, and resolved using a multilink horseradish peroxidase detection system developed with 3,3'-diaminobenzidine solution. Slides were counterstained with Harris modified hematoxylin. Nuclei of 1,000 tumor cells were quantitated to determine the percentage of positive immunoreactive nuclei; cytoplasmic-only and granular nuclear reactivity were regarded as negative.

Pharmacokinetic Analysis

Although not required for study entry, enrolled patients were encouraged to participate in pharmacokinetic studies. Blood samples were collected from patients on days 1 and 8 of cycle 1 before treatment and at 0.5, 1, 1.5, 2, 4, 6, 8, and 24 h after their morning imatinib dose. Plasma supernatants were separated by centrifugation and immediately frozen (-20°C). Plasma concentrations of imatinib and its metabolite, CGP74588, were determined by high-pressure liquid chromatography/mass spectrometry.¹⁸ Data were used to calculate the maximum plasma imatinib concentration (C_{max}) and time achieved (T_{max}). Noncompartmental analysis^{19,20} was used to calculate the area under the concentration versus time curve from time zero to the last sampling point before the next dose of imatinib ($\text{AUC}_{0-\infty}$). Apparent clearance (Cl_{app}) was calculated as $\text{dose}/\text{AUC}_{0-\infty}$.

Results

Patient Characteristics

Sixty-five patients with MG were enrolled at Duke University Medical Center between May 2004 and August 2006 (Table 1). Forty-three patients (66%) were male. The median age was 53.7 years (range, 28.5–73.3 years). All patients had a KPS score > 60%. Fifty-two patients (80%) had GBM, 11 (17%) had AA, and 2 (3%) had AO. Three patients with GBM had prior low-grade glioma.

Thirty-five patients (54%) were not on EIAEDs (stratum A), and 30 (46%) were on EIAEDs (stratum B). Patient characteristics did not differ substantially between EIAED strata.

Thirteen (20%) patients underwent a gross total resection before enrollment, while 16 (25%) and 15 (23%) enrolled following a subtotal resection or biopsy, respectively. All patients received prior XRT, and two also received stereotactic radiosurgery. Fifty-eight patients (89%) received prior TMZ, and 35 patients (54%) also received chemotherapy other than TMZ. Thirty-three

Table 1. Patient characteristics

Characteristic	Stratum A: Not on EIAEDs	Stratum B: On EIAEDs	All
Number of patients	35	30	65
Median age (range), years	53.3 (29.8–69.9)	53.9 (28.5–73.3)	53.7 (28.5–73.3)
Male (%)	24 (69)	19 (63)	43 (66)
Histology (%)			
GBM	27 (78)	25 (83)	52 (80)
AA	7 (20)	4 (13)	11 (17)
AO	1 (3)	1 (3)	2 (3)
Prior LGG	1 (3)	2 (7)	3 (5)
Tumor status at enrollment (%)			
Stable disease	13 (37)	19 (63)	32 (49)
Progressive disease	22 (63)	11 (37)	33 (51)
KPS score (%)			
90–100	25 (71)	20 (67)	45 (69)
80	6 (17)	6 (20)	12 (18)
70	2 (6)	4 (13)	6 (9)
60	2 (6)	0	2 (3)
Surgery (%)			
GTR	7 (20)	6 (20)	13 (20)
STR	6 (17)	10 (33)	16 (25)
Biopsy	7 (20)	8 (27)	15 (23)
None	15 (43)	6 (20)	21 (32)
Prior XRT (%)	35 (100)	30 (100)	65 (100)
Prior chemotherapy (%)			
None	1 (3)	2 (7)	3 (5)
1 agent	18 (51)	17 (57)	35 (54)
2 agents	10 (29)	18 (27)	18 (28)
≥3 agents	6 (17)	3 (10)	9 (14)
TMZ	31 (89)	27 (90)	58 (89)
Non-TMZ	22 (63)	13 (43)	35 (54)
Prior progressions (%)			
0	13 (37)	19 (63)	32 (49)
1	20 (57)	7 (23)	27 (42)
2	2 (6)	4 (13)	6 (9)
Alive (%)	8 (23)	13 (43)	21 (32)
On study therapy	0	1	1
Off study with SD	2	2	4
Off study with PD	6	10	16
Deceased	27 (77)	17 (57)	44 (68)

Abbreviations: EIAEDs, enzyme-inducing antiepileptic drugs; GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; LGG, low-grade glioma; GTR, gross total resection; STR, stereotactic radiosurgery; XRT, radiotherapy; TMZ, temozolomide; SD, stable disease; PD, progressive disease.

Table 2. Dose-limiting toxicities (DLTs) by dose level and stratum

Stratum/Imatinib Dose	No. Patients Treated	No. Patients with DLT ^a	Type of DLT ^a
Stratum A: not on EIAEDs ^b (n = 35)			
400	4	0	—
600	7	0	—
800	12	1	Transaminase elevation (grade 3)
1,000	6	0	—
1,200	6	2	Neutropenia (grade 3) Hypocalcemia (grade 4), hypophosphatemia (grade 4), hypokalemia (grade 3), and nausea/emesis (grade 3)
Stratum B: on EIAEDs ^b (n = 30)			
400	7	0	—
600	6	0	—
800	5	0	—
1,000	6	0	—
1,200	6	2	Hyperbilirubinemia (grade 3) Creatinine elevation (grade 3)

^aDose-limiting toxicity: grade 3 or greater attributable nonhematologic toxicity excluding nausea, diarrhea, or alopecia or grade 4 neutropenia or thrombocytopenia lasting ≥ 5 days during cycle 1 or 2. All patients were evaluable for D; however, those who did not complete the first two cycles of therapy were replaced because DLT was assessed during the first two cycles of therapy.

^bEIAEDs: enzyme-inducing antiepileptic drugs (phenytoin, phenobarbital, carbamazepine, oxcarbazepine, and primidone).

patients (51%) enrolled at recurrence, and the remainder enrolled with SD. Among recurrent patients, 27 (82%) enrolled at first recurrence, while 6 (18%) enrolled at the time of second recurrence.

As of July 5, 2007, 21 patients (32%) remained alive, and 1 patient (2%) continued to receive treatment on study. Forty-four patients (68%) had died.

Dose-Limiting Toxicity

Table 2 summarizes the frequencies and types of DLTs observed at each dose level per stratum. For stratum A (no EIAEDs), one patient experienced DLT at the 800-mg dose level. Two patients experienced DLT at the 1,200-mg dose level: one patient with grade 3 neutropenia, and another with grade 3 nausea/emesis accompanied by hypocalcemia (grade 4), hypophosphatemia (grade 4), and hypokalemia (grade 3). These toxicities all resolved within 48 h following intravenous hydration and electrolyte supplementation. For stratum B (on EIAEDs), two DLTs occurred at the 1,200-mg dose level: acute renal failure (grade 3) and hyperbilirubinemia (grade 3). Both of these toxicities resolved completely following discontinuation of the study regimen. Based on these results, we determined the MTD of imatinib to be 1,000 mg (500 mg twice daily) for patients on or not on EIAEDs when administered with standard TMZ.

Non-Dose-Limiting Toxicity

Table 3 summarizes grade 2 or greater toxicities that did not meet criteria for DLT. A total of 332 cycles of imatinib plus TMZ were administered: 152 cycles to patients on stratum A and 180 cycles to patients on stratum B.

Overall, the combination of imatinib plus TMZ was well tolerated. Among grade 3 or 4 events, hematologic toxicities (thrombocytopenia, 6%; neutropenia, 3%) were the only adverse events to complicate more than 1% of cycles. The most common nonhematologic toxicities included grade 2 nausea/emesis (5%) and fatigue (5%), although neither of these events occurred commonly at grade 3 or 4 levels (1%). Other nonhematologic adverse events that occurred in greater than 1% of cycles were rash (3%) and infection (2%). Both of these events also occurred rarely at higher grades.

Three patients developed grade 2 hemorrhage. One patient treated at the 400-mg dose level in stratum A

Table 3. Incidence of grade 2 or greater adverse events noted in 1% or more of cycles across all dosing cohorts

Toxicity	Grade 2 [n (% of cycles)]	Grade 3 or 4 [n (% of cycles)]
Nausea/emesis	17 (5)	4 (1)
Fatigue	15 (5)	4 (1)
Thrombocytopenia	11 (3)	19 (6)
Rash	10 (3)	2 (1)
Anemia	8 (2)	1 (<1)
Infection	8 (2)	4 (1)
Neutropenia	1 (<1)	9 (3)
Diarrhea	4 (1)	2 (1)
Anorexia	4 (1)	2 (1)
Hyponatremia	1 (<1)	4 (1)
Hypoalbuminemia	3 (1)	0
Hemorrhage	3 (1)	0
Edema	2 (1)	0
Dyspnea	2 (1)	0

had gastrointestinal bleeding after eight cycles of therapy, but this resolved following removal of known intestinal polyps. This patient had no further hemorrhagic events and completed 18 cycles of therapy. Two patients, both in stratum B, developed a CNS hemorrhage. One patient with recurrent AA who was heavily pretreated, including prior CNS brachytherapy, developed an intratumoral hemorrhage after six cycles at the 800-mg dose level. Further imatinib therapy was discontinued, and the patient had no further CNS bleeding. A second patient developed a CNS hemorrhage after six cycles at the 1,200-mg dose level at the time of tumor progression. This patient refused additional salvage therapy and entered hospice.

Pharmacokinetic Analyses

Samples for pharmacokinetic analyses were available from 12 patients not taking EIAEDs (stratum A) and 8 patients taking EIAEDs (stratum B) (Table 4). Within each group, there was great variability in pharmacokinetic values for patients treated with the same dose of imatinib. There was no difference in C_{max} or T_{max} between patients taking EIAEDs and those not taking EIAEDs. Within each group of patients, there was no difference in C_{max} or T_{max} on days 1 and 8. Although the daily dosing schedule of imatinib precluded adequate sampling to assess terminal half-life ($T_{1/2}$) with suitable precision, the $T_{1/2}$ estimated for patients taking EIAEDs

Table 4. Pharmacokinetic parameters by dose level and stratum

Daily Dose/Parameter	Imatinib			
	Day 1		Day 8	
	Stratum A	Stratum B	Stratum A	Stratum B
400 mg				
No. patients	2	4	2	4
C_{max} (range), $\mu\text{g/ml}$	3.05 (4.09–2.01)	3.86 (1.79–6.25)	3.13 (2.44–3.81)	3.72 (1.79–6.13)
T_{max} (range), h	3 (1.0–4.0)	2.13 (2.0–4.0)	4.00 (4.00)	1.86 (1.5–2.0)
$T_{1/2}$ (range), h	11.6 (11.2–12)	5.05 (3.7–6.9)	9.80 (4.6–15.0)	7.65 (3.8–10.9)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	49.4 (30.7–68.1)	27.1 (5.9–45.9)	31.70 (12.7–50.6)	37.40 (17.1–65.1)
Cl_{app} (range), ml/min	157.5 (98–217)	452.25 (145–1,136)	201.50 (132–271)	223.25 (102–390)
600 mg				
No. patients	2	1	3	1
C_{max} (range), $\mu\text{g/ml}$	3.52 (3.01–4.04)	2.69 (NA)	34.08 (3.78–4.29)	2.88 (NA)
T_{max} (range), h	4 (4)	4 (NA)	4.00 (2.0–6.0)	2.00 (NA)
$T_{1/2}$ (range), h	11.2 (11.1–11.2)	6.1 (NA)	15.40 (4.3–27.7)	4.20 (NA)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	56.7 (54.8–58.5)	25.6 (NA)	83.00 (64.3–109.2)	19.30 (NA)
Cl_{app} (range), ml/min	177 (171–183)	390 (NA)	127.00 (92–156)	518.00 (NA)
800 mg				
No. patients	2	0	2	0
C_{max} (range), $\mu\text{g/ml}$	3.21 (2.45–3.96)	NA	4.92 (4.38–5.45)	NA
T_{max} (range), h	2 (2)	NA	4 (4)	NA
$T_{1/2}$ (range), h	7.25 (5.1–9.4)	NA	23.8 (6.9–40.7)	NA
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	40.5 (24.1–56.9)	NA	153.5 (142.4–164.5)	NA
Cl_{app} (range), ml/min	197 (117–277)	NA	44 (41–47)	NA
1,000 mg				
No. patients	1	1	2	1
C_{max} (range), $\mu\text{g/ml}$	3.05 (NA)	2.36 (NA)	4.75 (3.64–5.86)	2.93 (NA)
T_{max} (range), h	4 (NA)	4 (NA)	1.25 (1.0–1.5)	4 (NA)
$T_{1/2}$ (range), h	6.7 (NA)	5.1 (NA)	23.6 (17.3–30.0)	4.1 (NA)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	42.3 (NA)	20.4 (NA)	97.3 (72.4–122.2)	74.4 (NA)
Cl_{app} (range), ml/min	197 (NA)	409 (NA)	398.5 (115–682)	521 (NA)
1,200 mg				
No. patients	2	2	2	1
C_{max} (range), $\mu\text{g/ml}$	2.14 (1.83–2.45)	2.26 (1.94–2.55)	4.82 (4.67–4.96)	3.11 (NA)
T_{max} (range), h	3 (2.0–4.0)	4 (2.0–6.0)	4 (4)	4 (NA)
$T_{1/2}$ (range), h	11.4 (5.4–17.7)	4.15 (3.9–4.4)	83.3 (18.6–148)	9.7 (NA)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	34.8 (27.0–42.5)	16.7 (14.7–18.7)	63.7 (62.2–65.2)	89.6 (NA)
Cl_{app} (range), ml/min	303 (236–370)	607 (534–680)	157 (153–161)	112 (NA) (continued)

Abbreviations: C_{max} , maximum plasma concentration; T_{max} , time to achieve maximum plasma concentration; $T_{1/2}$, terminal half-life; $AUC_{0-\infty}$, area under the concentration versus time curve from time zero to the last sampling point before the next dose of imatinib; Cl_{app} , apparent clearance, calculated as dose/ $AUC_{0-\infty}$.

was much shorter than that estimated for patients not taking EIAEDs. On days 1 and 8, Cl_{app} in patients taking EIAEDs was less than that in patients not taking EIAEDs. As with imatinib, there was no difference in C_{max} or T_{max} of CGP74588 between patients taking EIAEDs and those not taking EIAEDs. Also as with imatinib, there was great variability in the pharmacokinetic values for CGP74588 in both groups of patients.

Outcome

The median follow-up was 85.4 weeks (range, 71.9–105.0 weeks) for all patients, 103.7 weeks (range, 49.7–111.7 weeks) for those on stratum A, and 85.7 weeks (range,

72.3–105.3 weeks) for those on stratum B. The median OS, median PFS, and 6-month PFS rate for all patients and subsets based on histology, EIAED stratum, activity of underlying disease at enrollment, and whether treated with TMZ before enrollment are summarized in Table 5. Among GBM patients, the median 6-month PFS rate was greater for patients who enrolled with SD compared to those with progression ($p = 0.0024$), but did not differ based on EIAED use or prior TMZ exposure. Outcome comparisons were not performed for subsets of grade III MG patients due to the small number of such patients.

Sixty-four patients were evaluable for response. One patient became noncompliant with study therapy after 2 weeks and was deemed nonevaluable for response.

Table 4. Pharmacokinetic parameters by dose level and stratum (continued)

Daily Dose/Parameter	CGP74588			
	Day 1		Day 8	
	Stratum A	Stratum B	Stratum A	Stratum B
400 mg				
No. patients	2	4	2	4
C_{max} (range), $\mu\text{g/ml}$	0.47 (0.31–0.62)	1.03 (0.69–1.60)	0.88 (0.44–1.33)	1.46 (0.48–2.48)
T_{max} (range), h	2.75 (1.5–4.0)	2.13 (1.0–4.0)	2.75 (1.5–4.0)	3.38 (2.0–6.0)
$T_{1/2}$ (range), h	13.8 (13.3–14.3)	8.48 (3.7–12.8)	11.20 (4.2–18.2)	16.30 (8.9–23.1)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	6.3 (4.6–8.0)	8.05 (2.7–14.1)	7.90 (2.5–13.2)	23.30 (9.9–39.8)
Cl_{app} (range), ml/min	NA	NA	NA	NA
600 mg				
No. patients	3	1	3	1
C_{max} (range), $\mu\text{g/ml}$	0.91 (0.6–1.2)	2.68 (NA)	1.10 (0.9–1.6)	1.30 (NA)
T_{max} (range), h	4.7 (4.0–6.0)	4 (NA)	3.80 (1.5–6.0)	2.00 (NA)
$T_{1/2}$ (range), h	26.7 (14.7–48.4)	12.3 (NA)	21.70 (13.3–32.0)	15.20 (NA)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	12.4 (9.4–15.7)	9.5 (NA)	21.00 (16.1–28.3)	7.60 (NA)
Cl_{app} (range), ml/min	NA	NA	NA	NA
800 mg				
No. patients	2	0	2	0
C_{max} (range), $\mu\text{g/ml}$	0.63 (0.37–0.89)	NA	1.69 (1.29–2.10)	NA
T_{max} (range), h	2 (2)	NA	3 (2.0–4.0)	NA
$T_{1/2}$ (range), h	6 (4.0–8.0)	NA	50.1 (6.5–93.7)	NA
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	6.8 (2.9–10.7)	NA	23.5 (6.1–40.8)	NA
Cl_{app} (range), ml/min	NA	NA	NA	NA
1,000 mg				
No. patients	1	2	1	
C_{max} (range), $\mu\text{g/ml}$	0.39 (0.24–0.54)	0.97 (NA)	1.18 (0.61–1.74)	1.55 (NA)
T_{max} (range), h	4 (4)	4 (NA)	1.3 (1.0–1.5)	4 (NA)
$T_{1/2}$ (range), h	8.3 (7.9–8.7)	5.9 (NA)	10.2 (8.6–11.8)	4 (NA)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	5.6 (2.6–8.6)	8.3 (NA)	19.7 (18.4–21.0)	47.6 (NA)
Cl_{app} (range), ml/min	NA	NA	NA	NA
1,200 mg				
No. patients	2	2	2	1
C_{max} (range), $\mu\text{g/ml}$	0.26 (0.19–0.32)	1.26 (0.58–1.94)	0.71 (0.27–1.14)	3.11 (NA)
T_{max} (range), h	3 (2.0–4.0)	3 (2.0–4.0)	2.75 (1.5–4.0)	4 (NA)
$T_{1/2}$ (range), h	4.9 (3.0–6.7)	6.4 (4.2–8.6)	50.8 (16.4–85.2)	11.8 (NA)
$AUC_{0-\infty}$ (range), $\mu\text{g/ml}\cdot\text{h}$	2.4 (2.1–2.7)	5.7 (4.7–7.2)	10.1 (3.7–16.4)	40 (NA)
Cl_{app} (range), ml/min	NA	NA	NA	NA

Table 5. Outcome

Population	<i>n</i>	Median Overall Survival (95% CI), weeks	Median Progression-Free Survival (95% CI), weeks	Six-Month Progression-Free Survival Probability (95% CI)
All patients	65	47.6 (37.6, 72.0)	26.6 (10.9, 36.4)	52.3% (39.6%, 63.6%)
GBM patients	53	45.1 (36.1, 59.1)	26.6 (9.9, 36.4)	52.8% (38.6%, 65.2%)
Grade III MG patients	12	72.0 (37.7, ∞) ^a	27.8 (17.3, 45.9)	50.0% (20.8%, 73.6%)
GBM patients not on EIAEDs	28	40.1 (30.1, 56.1)	18.6 (8.7, 29.7)	50.0% (30.6%, 66.6%)
GBM patients on EIAEDs	25	49.9 (37.3, ∞) ^a	29.7 (8.4, 48.9)	56.0% (34.8%, 72.7%)
GBM patients with SD at enrollment	28	56.1 (38.3, ∞) ^a	41.7 (26.1, 51.1)	67.9% (47.3%, 81.8%)
GBM patients with PD at enrollment	25	35.3 (29.7, 45.1)	10.0 (7.9, 27.3)	36.0% (18.2%, 54.2%)
GBM patients with prior TMZ	48	49.9 (36.1, 91.0)	26.9 (9.9, 40.1)	40.0% (5.2%, 75.3%)
GBM patients without prior TMZ	5	26.6 (6.6, 44.1)	11.1 (4.4, 37.1)	54.2% (39.2%, 67.0%)

Abbreviations: 95% CI, 95% confidence interval; GBM, glioblastoma multiforme; MG, malignant glioma; EIAEDs, CYP3A-inducing antiepileptic drugs; SD, stable disease; PD, progressive disease; TMZ, temozolomide.

^aSample size was too small to calculate the upper confidence limit of the median.

Overall, eight patients (12%) achieved a radiographic response (CR, *n* = 1; PR, *n* = 7): six GBM patients (12% of all GBM patients) and two grade III MG patients (15% of all grade III MG patients). Fig. 1 shows an MR image from a representative patient with recurrent GBM who achieved a PR. The only CR occurred in a patient with an AA who enrolled with SD following two cycles of post-XRT TMZ. Among patients achieving a PR, five enrolled with SD, and two enrolled at first progression. Overall, 31 (48%) additional patients achieved a best radiographic response of SD, including 22 with GBM (42% of GBM patients) and 9 with grade III MG (69% of grade III MG patients).

Archival tumor tissue was available for AGT immunohistochemistry from 28 patients (43%). Although no

correlation between AGT expression and duration of study treatment was observed (data not shown), three patients with AGT levels of 60%–80% completed seven or more cycles of study therapy.

Discussion

The current standard of care for newly diagnosed GBM patients, TMZ administered daily during XRT followed by six monthly TMZ cycles, provides a modest OS benefit compared with XRT alone² but nominal benefit to patients with established negative clinical prognostic factors or tumors expressing the DNA repair protein AGT.^{4,6} Thus, although the addition of TMZ to XRT

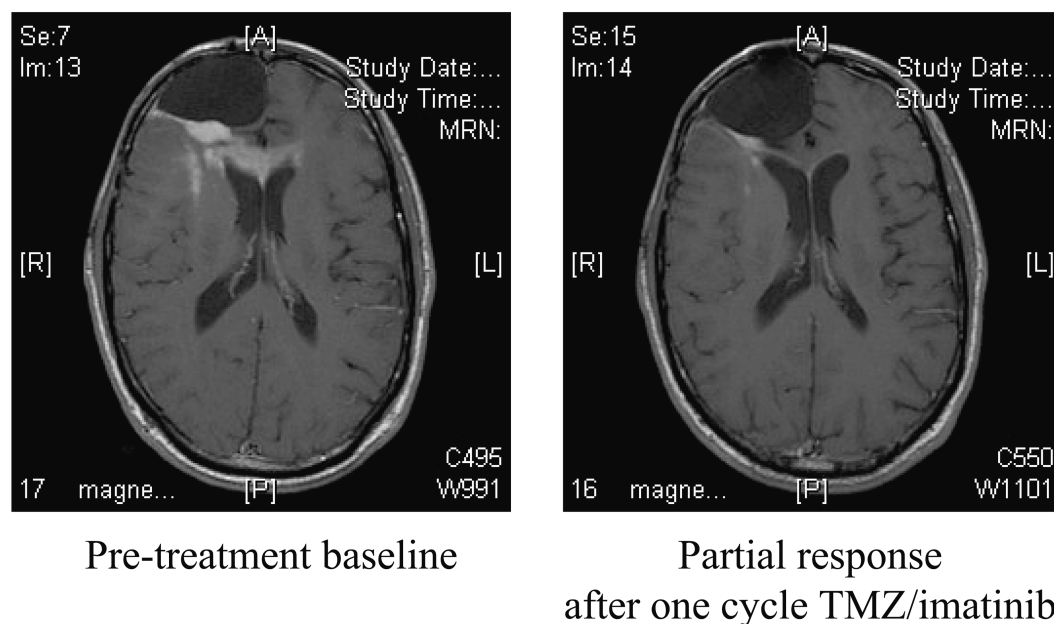


Fig. 1. Representative radiographic partial response to temozolomide (TMZ) plus imatinib in a patient with recurrent GBM (T1-weighted MRI after gadolinium administration).

is an important advance, outcome remains far from satisfactory. Therefore, therapeutic approaches to enhance the antitumor activity of TMZ represent a logical and attractive strategy to further improve outcome for newly diagnosed GBM patients. Increasing the dose intensity of TMZ is one strategy to improve its overall antitumor activity²¹ that is currently being evaluated in a large randomized, multinational study being conducted by the Radiation Therapy Oncology Group, European Organization for Research and Treatment of Cancer, and National Cancer Institute of Canada. An alternative strategy to enhance the antitumor activity of TMZ is to combine it with an agent that exerts a complementary mechanism of cytotoxicity such as a topoisomerase inhibitor.²²

Imatinib exhibits several actions that may potentiate the antitumor activity of TMZ. First, imatinib inhibits PDGFR, a key tyrosine kinase that mediates mitogenic and survival signaling pathways as well as angiogenesis in GBM.²³⁻²⁷ Combining growth factor inhibitors with cytotoxic agents can enhance apoptosis, leading to a greater antitumor effect than achieved by either agent alone.²⁸ Second, imatinib may enhance the antitumor activity of TMZ by inhibiting PDGFR-mediated angiogenesis and stem cell activation.²⁹⁻³² Third, imatinib can enhance chemotherapy delivery by decreasing tumor interstitial pressure, leading to increased capillary-to-interstitium transport *in vivo*.¹¹⁻¹³ Finally, imatinib also diminishes tumor cell DNA repair following chemotherapy or XRT by reducing expression of Rad51, a critical component of the DNA double-strand break pathway.^{8,9}

In the present study, we administered imatinib in a dose-intensive schedule designed to enhance the antitumor activity of TMZ. Specifically, imatinib was administered daily for 3 days before TMZ, and then concurrently with TMZ over 5 days, during each 28-day treatment cycle. The dose of imatinib was escalated in successive cohorts of patients, and we defined the MTD to be 1,000 mg/day for all patients, regardless of EIAED status. The rationale for determining the MTD of imatinib separately for cohorts of patients on and not on EIAEDs is based on the profound ability of EIAEDs to decrease exposures of CYP3A-metabolized agents, such as imatinib.¹⁴⁻¹⁶ The pharmacokinetic estimates and variability seen in this study are consistent with those reported in previous pharmacokinetic studies of imatinib. As expected, patients receiving EIAEDs had faster clearance and smaller $T_{1/2}$ values of imatinib than did those whose hepatic cytochrome P450 enzymes were not induced. The failure to observe any consistent differences between imatinib and CGP74588 pharmacokinetics on days 1 and 8 indicates no effect of TMZ on imatinib pharmacokinetics.

Overall, daily doses up to 1,000 mg of imatinib for 8 days were well tolerated when administered with 5-day cycles of TMZ each month. DLTs varied and included

hepatic, hematologic, and renal events, including severe electrolyte disturbance. Significant non-DLTs were not common among patients on either stratum and essentially reflected the toxicity profile typically observed following successive cycles of TMZ, except for occasional patients who developed grade 2 peripheral edema, dyspnea, or hemorrhage. In the present study, two patients (3%) developed CNS hemorrhage after multiple cycles of therapy. Although this rate is similar to that observed among recurrent MG patients,¹⁴ increased rates of CNS hemorrhage have been reported with higher, continuous dosing schedules of imatinib¹⁴ and may be related to inhibition of PDGFR signaling on tumor vascular pericytes.

Recently, imatinib plus hydroxyurea, a ribonucleotide reductase inhibitor, was shown to have activity when administered on a continuous daily schedule for recurrent MG patients.^{16,33-35} In the present study, we demonstrate that imatinib administered in a dose-intensive imatinib schedule is well tolerated in combination with standard TMZ. We also observed radiographic responses and durable antitumor activity in some patients treated with this regimen. These benefits were not likely attributable to imatinib given its limited, single-agent effect in recurrent MG patients.^{14,15} Due to the limitations of the phase 1 design of this study, it is not possible to determine whether dose-intensive imatinib contributed to the outcome of our patients compared with that expected with TMZ alone. Sixty-eight percent of patients who enrolled with SD remained progression-free for at least 6 months. Furthermore, the 6-month PFS among patients enrolling with PD was 36%; this value compares favorably with that reported for recurrent GBM patients treated with TMZ (21%).³⁶ In addition, we noted unexpectedly durable antitumor benefit in three patients despite significantly elevated AGT levels, suggesting that imatinib may abrogate the deleterious effect of AGT to improve TMZ responsiveness in some patients. Further study to validate our observations and better understand the potential mechanism of interaction between dose-intensive imatinib and TMZ is warranted. A future phase 2 clinical trial randomizing patients to either dose-intensive imatinib plus TMZ or TMZ alone will help define the therapeutic benefit of this approach, and should also explore whether dose-intensive imatinib improves TMZ response among patients with poor-risk clinical factors or elevated tumor AGT content.

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